1971 #13

DISTRIBUTION OF ^{14}C-DDT IN THE LOBSTER AFTER ADMINISTRATION VIA INTRAVASCULAR OR ORAL ROUTES OR AFTER EXPOSURE TO DDT-CONTAINING SEA WATER

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We had previously reported (Bull MDIBL 10, 23, 1970) a persistence of ^{14}C-DDT in the hepatopancreas of the lobster for up to 7 days after injection into the pericardial sinus. It was of interest therefore to investigate the duration of this persistence and to quantify the half-life of DDT in this organ. Other studies were conducted to measure ^{14}C-DDT tissue levels in lobsters after feeding DDT-contaminated food or after exposure to DDT-containing water. Lobsters weighing about 400-500 g were procured locally (Lunts and Small Lobster Bar, Trenton, Maine). One group of animals received 0.1 mg/kg of ^{14}C-DDT (Amersham/Searle, ring labeled) in 50% ethanol by injection into the pericardial sinus. After administration of the pesticide, animals were placed in a standard lobster crate, were submerged in Laboratory Cove at about 30 feet of water and were fed pieces of mackerel two times a week. After the time intervals shown in Table 1, the animals were dissected, and further

Table 1

% DOSE OF ^{14}C-DDT REMAINING IN LOBSTER AFTER PERICARDIAL INJECTION

Tissue ***	Days After Injection			
	7	14	20	34
Hepatopancreas	88.4 ± 17.2	79.5 ± 21.2	69.7 ± 13.9*	63.4 ± 6.4*
Egg mass	0.5**	0.9 ± 0.4	1.3 ± 1.5	1.07 ± 0.70
Gonad	.13 ± .05	<.1	<.1	<.1
Tail Muscle	0.25 ± .14	1.28 ± .77*	1.70 ± .88	1.1 ± 0.6*
Gill	0.40 ± .23	0.38 ± .17	0.30 ± .09	0.36 ± .10
Intestine	0.14 ± .09	0.10 ± 0.01	<.1	<.1
Stomach	0.34 ± .25	0.30 ± .12	1.17 ± 1.59	0.30 ± .12

* Significantly different ($P < 0.05$)

** Only one animal

*** Tissue levels for heart, green gland, and brain were all <0.1% at all times

handling was as described elsewhere in this Bulletin (Janicki, Guarino, and Kinter, 1972). TLC of hepatopancreas extracts revealed that more than 90% of the radioactivity was DDT itself.

The dose given to animals was the same as we described previously and hence the actual radioactivity appearing in organs was essentially the same as reported for 7 days after receiving DDT

(Bull MDIBL 10, 23, 1970). In this communication, data have been converted to percentage of the administered dose remaining in a given organ at a given time. The persistence of DDT in the hepatopancreas is again borne out in this study. Plotting the hepatopancreas values, semilogarithmically *vs.* time gave an estimate of the $t_{1/2}$ of DDT in this organ of 46 days. The egg masses accumulated DDT and values in this tissue did not significantly decay for the interval of this experiment. As the Table shows there appeared to be some redistributions of the amount of radioactivity in tail muscle where from 2 to 5 week values were significantly higher than those at one week.

To demonstrate the possible relevance of results obtained by injection into the pericardial sinus to more realistic sites of entry, uptake of DDT from water and food was measured. For the latter experiments, six lobsters were placed in 10 L of aerated sea water containing 0.1 ppm of ^{14}C-DDT (total of 1.1×10^6 c/m) for 6 hrs. At the end of this time the animals were returned to tanks with running sea water, for 7 days prior to sacrifice. To determine the fate of DDT after absorption by the oral route, pieces of mackerel liver were placed on a microscope slide and ^{14}C-DDT in benzene was added to give a dose of 0.1 mg/kg (about 3×16^6 c/m) on each piece. After evaporation of the solvent, these pieces of liver were placed near the mouth parts of lobsters that had been previously screened with untreated liver for their willingness to take food in this manner (only about 50% did). The microscope slides were counted for residual radioactivity and the administered dose was corrected for the actual calculations used in Table 2.

Table 2

^{14}C-DDT LEVELS IN THE LOBSTER SEVEN DAYS AFTER EITHER EXPOSURE FROM DDT-CONTAINING WATER OR FOOD*

	From Water	From Food
Plasma	27 ± 12	62 ± 37
Hepatopancreas	7365 ± 1210	48104 ± 22698
Green Gland	334 ± 62	2900 ± 228
Heart	73 ± 56	961 ± 295
Egg mass	303 ± 280	3235 ± 1298
Intestine	382 ± 205	3927 ± 1576
Gonad	168**	1588**
% of Absorbed Dose in Hepatopancreas	97.0 ± 0.5	95.7 ± 2.2

* mean counts/min/g or ml ± SD for 3-6 animals

** single value only

After feeding the animals, they were returned to free flowing tanks as described above. After 7 days they were sacrificed and tissues were prepared for radioassay as described for the injection

experiments. It is seen in Table 2 that absorption from both water and food did, indeed, occur; and furthermore, the tissue distribution is essentially the same as after injection into the pericardial sinus. As expected, there was quite a range in the tissue values from these modes of administration, but nontheless, the amounts of radioactivity residing in the hepatopancreas from either water or food is more than 95% of the absorbed dose.

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THE ROLE OF NA-K-ATPase IN THE RENAL REABSORPTION OF SODIUM IN *Squalus acanthias*

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Previous studies have demonstrated that Na-K-ATPase plays an important role in net sodium transport across several epithelial membranes. Increased specific activity of this enzyme in the gill of lower vertebrates and in the kidney of mammals has been shown to parallel rises in active sodium transport under a variety of experimental and environmental conditions. Conversely, in the dog, inhibition of enzymatic activity in the kidney with ouabain is associated with a marked decrease in the tubular reabsorption of sodium and a natriuresis. In the present experiments the relationship of Na-K-ATPase to renal sodium metabolism was studied in the elasmobranch, since in this unique species the tubular transport of sodium is of lesser importance in osmoregulation.

Studies were performed in unanesthetized dogfish, maintained at 13°C in running seawater. All injections were administered through an intraaortic catheter with the tip placed proximal to the kidneys. Measurements of renal function were performed during control periods lasting 2 to 16 hours, and for four hours following the injection of ouabain.

In preliminary experiments it was determined that a dose of ouabain exceeding 75 μg/kg resulted in death within 4 hours. Attempts to increase the amount of this agent reaching renal tissue, by infusing solutions into the tail vein, did not alter these results. In 9 normal fish the renal specific activity of Na-K-ATPase was 7.30 ± 0.34 (mean ± S.E.) and of Mg-ATPase was 17.29 ± 3.53 Pi μM/mg protein per hour. One hour following the injection of ouabain, in a dose which varied from 100 to 250 μg/kg, the mean Na-K-ATPase level was 2.81 ± 0.18 (a reduction of 61.6 ± 2.5 per cent) and the Mg/ATPase level was 17.29 ± 3.5 Pi μM/mg protein per hour. There was no apparent relationship between dose and the degree of enzyme inhibition.

In 4 fish measurements of GFR, urine volume, sodium and potassium excretion were made before and during four hours following the injection of ouabain (average dose - 57 μg/kg). The specific activity of Na-K-ATPase was measured at the termination of the experiment in each fish and compared to control fish studied on the same day. As shown in Table 1 the level of Na-K-ATPase was reduced to a mean of 64.0 ± 9.3 per cent in the experimental fish, a value similar to that obtained after giving larger doses. Ouabain caused a slight increase in GFR which rose from 1.26 ± 0.20 to 1.89 ± 0.16 ml/kg · hr and in V which rose from 0.52 ± 0.10 to 0.64 ± 0.05 ml/kg · hr. Both the fraction and