1970 #8

DOES DDT INHIBIT CARBONIC ANHYDRASE?

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Birds of prey have been found to lay thin shelled eggs, a phenomenon which has been stated to be due to inhibition of carbonic anhydrase by DDT (Peakall, Science, 168:592, 1970, and Bitman, Cecil, and Fries, Science, 168:594, 1970). Keller (Naturwissenschaften, 39:109, 1952) had observed inhibition of bovine carbonic anhydrase by 50 μg/ml of DDT, in vitro, while Wistrand (cited by Maren, Physiol. Rev., 47:595, 1967) found no inhibition of dog enzyme, in amounts claimed by Keller. Anderson and March (Can. J. Zool. 34:68, 1956) were unable to detect any inhibition by DDT on insect carbonic anhydrase either in vivo or in vitro at concentrations up to 3,550 μg/ml. We examined again, in vitro, the question of whether DDT inhibits this enzyme. The matter is of much theoretical and practical importance, since carbonic anhydrase inhibitors clearly reduce the rate of calcium deposition in shell, both in birds and invertebrates (reviewed by Maren, Physiol. Rev., 47:595, 1967.

Carbonic anhydrase activity was analyzed by the method of Maren, Ash, and Bailey (Johns Hopkins Hosp. Bull., 95:244, 1956) which measures the catalytic hydration rate of CO_2. Master solutions of p,p'-DDT and p,p'-DDE were prepared in absolute ethanol or in N,N-dimethyl formamide (DMF). The final concentration of DDT and DDE in the reaction vessel was 50-85 μg/ml in 16% ethanol or 5% DMF. Concentrations greater than 50 μg/ml in the reaction mixture resulted in some precipitation of drug. Solutions were incubated with drug and enzyme (human red cell) up to three days at room temperature. No inhibition was observed.

The effect of DDT on semi-purified bovine carbonic anhydrase was examined using the method of Maetz (Bull. Soc. Chim. Biol. 38:447, 1956) which had also been used by Keller (see above). This method measures the catalytic dehydration rate of carbonic acid.

A number of solvents were used; DMF (2.5% in final solution) yielded the most reliable data. Inhibition progressed from 37 to 88% when the concentration of DDT increased from 500 to 2000 μg/ml. In these experiments there was also some precipitation of drug in the reaction mixture. However, the degree of inhibition observed at 500 μg/ml is relatively small and suggests that DDT may not inhibit carbonic anhydrase effectively at the usual tissue concentrations following ecological exposure.

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1970 #9

THE FATE OF ^{14}C-2,2-BIS (p-CHLOROPHENYL)-1,1,1-TRICHLOROETHANE (p,p'-DDT) IN *Squalus acanthias*

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We studied the pharmacology of p,p'-DDT in the dogfish, *Squalus acanthias*, with particular

attention to the movement of the drug through the body. Ring labeled ^{14}C-p,p'-DDT, obtained from New England Nuclear, was utilized. All data are reported in terms of p,p'-DDT.

The general protocol was as follows: Fish were placed either in a box that contained 50 liters of sea water which was constantly renewed, or in live cars at the dock. The fish were administered 60 μg/kg of ^{14}C-p,p'-DDT and in one case 6 mg/kg (as a mixture of hot and cold p,p'-DDT). Tissue distribution utilized entire organs, with the exception of muscle where a representative sample was removed. Total drug in the muscle and in the plasma was estimated using the data of Burger (Bull. MDIBL 7:5, 1967).

Figure 1 shows the rapid decay of drug from the plasma for the first 60 minutes. Table 1 shows the decay from the plasma and accumulation of drug in various tissues over a 48-hour

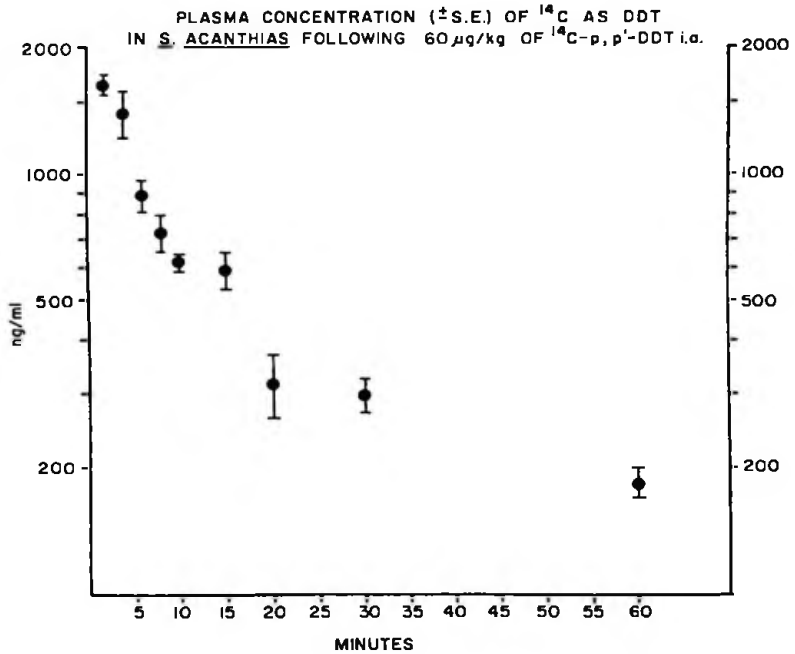

Figure 1. Drug was injected at zero time in ethanol (1-2 ml) over a 10 second period. The syringe was then rinsed 2-3 times with the circulating blood over a 30-second period.

period. When 100 times this dose was given, the 1-hour distribution was roughly the same. When the concentration in the liver at 4-48 hours (about 600 ng/gm, Table 1) is multiplied by the liver weight (about 100 gm/kg fish) we find that essentially all the administered dose is in the liver. Twelve additional fish were studied from 72 hours to 2 weeks, with little or no change in liver concentration. No drug was found in gill effluate or urine.

Table 2 shows the distribution of drug at 4 and 24 hours following an oral dose. Van Slyke and White (J. Biol. Chem. 9:209, 1911) showed that absorption of beef from the stomach and intestine of S. acanthias took about 3 days, with the stomach emptying time being about 6 hours. Thus, the oral route may be significant in the accumulation of DDT. Since the drug is held up in the stomach for about 6 hours, the low recovery of drug may well be due to regurgitation. Further studies will be done to clarify this point.

Table 1

DISTRIBUTION OF RADIOACTIVITY (AS NANOGRAMS DDT/gm TISSUE)[a] IN S. acanthias 10 MIN., 1, 4 AND 48 HOURS FOLLOWING A DOSE OF 60 μg/kg OF ^{14}C-p,p'-DDT

Tissue	ng/Gm			
	10 min	1 hr	4 hr	48 hr
Plasma	616 ± 27	184 ± 28	33 ± 5	6
Liver	183 ± 41	284	579	590 ± 26
Muscle	3 ± 2	36	49	20 ± 4
Kidney	740 ± 73	232	590	155 ± 57
Gonads	74 ± 8	72	147	86 ± 1
Intestine[b]	146 ± 20	84	123	34 ± 1
Bile[c]	0.13 ± 0.07	4 ± 0.2	10 ± 2	38 ± 2
RBC[d]	0[e]	0[f]	0	-
n (plasma)	10	8	5	2
n (bile)	3	3	3	5
n (others)	3	2	2	3

[a] All values are means ± S.E. except when n = 2, then no S.E. is reported.

[b] Duodenum, valvular intestine, and rectum.

[c] From gall bladder.

[d] Concentration calculated from whole blood and plasma data.

[e] 5 min.

[f] 30 min.

Table 2

PERCENT DOSE IN TISSUES OF S. acanthias 4 AND 24 HOURS FOLLOWING AN ORAL DOSE OF 60 μg/kg ^{14}C-p,p'DDT

Tissue	% Dose	
	4 Hrs	24 Hrs
Liver	2	12
Muscle	3.3	9
Others[a]	0.7	1
% Recovered	6	22
Stomach (and contents)	34	17
Intestine (and contents)[b]	12	7
% Recovered	46	24

In the two experiments given, analysis of the ambient water at 1 hour showed 7 and 17% of the dose.

[a] Heart, brain, plasma, bile, gonads and kidney.

[b] Duodenum, valvular intestine and rectum.

The rather surprising finding that none of the very lipid soluble drug is excreted by the gill is explicable on the basis of its extremely low water solubility (1 ng/ml), with free drug being reduced even more by plasma binding, now under investigation. Thus, virtually no free drug is available for either gill or renal excretion.

The liver of this species, which contains approximately 50% fat, is an obvious depot for drug, and is found to sequester it quantitatively.

Preliminary studies showed that there is marked toxicity at 10 mg/kg i.v., of a delayed type. Fish became rigid and paralyzed. Additional studies are planned to investigate the toxicity in more depth.

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1970 #10

THE DISTRIBUTION OF VARIOUS POLAR MATERIALS BETWEEN BLOOD AND TISSUE IN Squalus acanthias

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The tissue distribution of nine intravenously administered polar materials were studied in Squalus acanthias. By choosing materials of various sizes and lipid solubilities, information can be obtained concerning the size of the vascular and extracellular space (ECS) and the permeability of the capillaries and cells for the various tissues. The materials used, with their molecular weights (MW) and approximate olive oil:water partition coefficients (PC) noted in parentheses, were: ^{14}C-dextran (MW $\cong$ 60,000; PC < .00003), ^{14}C- and ^{3}H-inulin (MW $\cong$ 5,000; PC < .00003), ^{3}H- and ^{14}C-sucrose (MW = 342; PC = .00003), ^{3}H-mannitol (MW = 182; PC = .00003), ^{14}C-thiourea (MW = 76; PC = .002), ^{3}H-ethylene glycol (MW = 62; PC = .00049), ^{14}C-urea (MW = 60; PC = .00015), $^{36}Cl^{-}$ (MW = 36; PC = ?), and ^{3}H-water (MW = 18; PC = .0007). Skeletal muscle, heart, intestine, rectal gland, pancreas, and liver were the tissues examined. In addition, pericardial fluid (PCFO and bile were sampled.

A constant or nearly constant blood level of the radioactive material was achieved in unanesthetized, freely swimming dogfish by a combination of intravenous and intramuscular injections. Plasma samples were taken at regular intervals to monitor this. Animals were sacrificed from 10 minutes to 24 hours after the initial intravenous injection, and samples of the various tissues and fluids were rapidly obtained. Tissue samples (20-40 mg) were digested in Nuclear Chicago solubilizer to which liquid scintillation fluid was then added. All samples were analyzed for radioactivity by liquid scintillation spectrometry with appropriate corrections for quenching. All spaces are calculated by dividing the sample's radioactivity per milligram by the final plasma sample's radioactivity per microliter x 100. Determinations of total tissue or fluid water were made by comparison of wet and dry weights. Binding of the labeled materials to plasma proteins was checked by ultrafiltration and was found to be negligible.

Skeletal muscle. The ratio of tissue water to plasma water was 0.77 for this tissue. The dextran space (plasma space) equaled 1%. The distribution spaces at 20 hours were 8% for inulin. 11% for sucrose, 10% for mannitol, and 12% for chloride. The chloride space reached this level at 30 minutes and remained constant thereafter. Longer periods of time 4-20 hours) were re-