Recent anatomical investigations have revealed that the ultrastructure of the shark blood-brain barrier differs from that of rodents and goldfish (Brightman, Reese, Olsson and Klatzo. Progr. Neuropath. 1:146-161, 1971). Whether these ultrastructural variations are associated with different permeability characteristics is not known. Ultrastructure of the blood-brain barrier in skates and hagfish has not been examined.

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

Barry H. Dvorchik, Department of Pharmacology and Therapeutics, University of Florida College of Medicine, Gainesville, Florida

Studies on the pharmacology of p,p'-DDT in the dogfish, *Squalus acanthias* were initiated last year (Dvorchik and Woodworth, Bull. MDIBL, 10:12, 1970 and Dvorchik and Maren, Comp. Biochem. Physiol., in press, 1971) with attention given to the movement of drug through the body following intra-arterial administration. The present study was designed to obtain information on (a) binding to plasma proteins, (b) hepatic metabolism, and (c) toxicity of p,p'-DDT in *S. acanthias*. Ring labeled ^{14}C-p,p'-DDT (Amersham/Searle or New England Nuclear) and nonradioactive p,p'-DDT (Aldrich, 99% pure) were utilized.

Fish (males, 1-3 kg) were placed either in live cars at the dock or in a circular, plastic lined swimming pool (8 ft. x 3 ft.) which contained 200 liters of sea water renewed at the rate of 36 liters/min. Drug was administered intra-arterially, in ethanol. All drug solutions were prepared in such dilution that each fish received 1 ml of ethanol/kg body weight, a dose which was innocuous to the fish.

The binding of p,p'-DDT to plasma proteins was determined by equilibrating a known amount of radioactive DDT with freshly obtained shark plasma. An aliquot was then removed and fractioned successively by density gradient ultracentrifugation according to the procedure of Hatch and Lees (Adv. Lipid Res., 6, 1968). Aliquots of plasma and undialyzed fractions were taken for analysis of total radioactivity, protein (Lowry et al., J. Biol. Chem., 190:513, 1951), and lipid content (J. Biol. Chem., 190:513, 1951).

The metabolism of ^{14}C-p,p'-DDT by the liver of the dogfish was investigated by killing fish at various times after administration of 60 μg/kg ^{14}C-p,p'DDT, removing the liver and separating the radioactivity from the liver oil (Giuffrida et al., J. Assoc. Off. Anal. Chem., 49:634, 1966). After evaporation to dryness the residue was taken back into solution by the addition of 200 μl of n-hexane and an aliquot taken for thin-layer chromatography. The sample was spotted on the right side of a 20 x 5 cm silica gel plate. A standard solution containing p,p'-DDD,DDT, and DDE was spotted on the left. The plate was developed in n-hexane to a 15 cm front. After being air dried, the left side was sprayed with methyl yellow spray reagent prepared according to Krzeminski and Landman (J. Chromatog., 10:515, 1963). The right half corresponding to the standard spots on the left was scraped off, the remainder of the right side of the plate was then scraped in 1 cm segments. All

scrapings were placed in scintillation vials and counted.

Toxicity studies utilized radioactive and nonradioactive p,p'-DDT. After the administration of graded doses the fish were placed in live cars (5 per live car). They were checked daily; the brain removed at death and analyzed for total radioactivity.

The distribution of ^{14}C-p,p'-DDT, protein, and lipid in the fractions obtained from the ultracentrifugation of plasma is given in Table 1. The drug was found to be associated with all fractions; highest in lipid-chylomicron and very low density lipoprotein.

TABLE 1. DISTRIBUTION OF ^{14}C-p,p'-DDT[a], PROTEIN, AND LIPID (± S.E.) IN THE PLASMA OF SQUALUS ACANTHIAS.

	Total Percent Recovered	PERCENT OF TOTAL IN LIPOPROTEIN FRACTIONS		
		Fraction[b] 1	Fraction[c] 2	Fraction[d] 3
^{14}C-p,p'-DDT[a]	65 ± 5.5	59.1 ± 4.0	25.8 ± 4.7	15.0 ± 2.4
(n)	(10)	(10)	(10)	(10)
PROTEIN	68.5 ± 10.0	4.9 ± 1.4	1.7 ± 0.5	94.0 ± 1.0
(n)	(4)	(4)	(4)	(4)
LIPID	79.2 ± 10.9	45.2 ± 4.0	34.1 ± 3.9	20.7 ± 3.2
(n)	(6)	(6)	(6)	(6)

[a] As ^{14}C

[b] Chylomicron and very low density lipoprotein

[c] Low density lipoprotein

[d] High density lipoprotein, very high density lipoprotein and all other constituents of plasma with a density greater than 1.21

The best estimate of the water solubility of p,p'-DDT appears to be 1.2 ng/ml (Bowman et al., J. Agr. Food Chem., 8:406, 1960). The total recovery of radioactivity after ultracentrifugation was 65% or 2.3 μg. From the volume of each fraction and the solubility of 1.2 ng/ml, only 24 ng of the total 2300 ng recovered could be present in the unbound form, or in other words, the drug appears to be at least 99% bound.

Tissue distribution studies carried out over a 17-day period show that there is no significant change in the percent dose in the liver from 48 hours to 17 days following 60 μg/kg DDT i.a. (85 ± 12% vs. 77 ± 7%). The molecular makeup of the radioactivity found in the liver of 2 dogfish 6 and 17 days after 60 μg/kg ^{14}C-p,p'-DDT is shown in Table 2. About 95% of the radioactivity was due to the parent compound with about 2-3% being p,p'-DDE. About 1% of the radioactivity is unknown at this time. The fact that there is only slight hepatic metabolism of p,p'-DDT by the dogfish over a 17-day period is explicable on the basis of the lipid content of the liver and the rate of hepatic metabolism. Only that drug which is capable of reaching the hepatic cells will be metabolized. The oil found in the liver of *S. acanthias* comprises about 65% liver weight. This large

TABLE 2. METABOLISM OF p,p'-DDT BY S. ACANTHIAS FOLLOWING ADMINISTRATION OF 60 µg/kg i.a.[a]

	PERCENT OF TOTAL RADIOACTIVITY	
	6 days	17 days
p,p'-DDT	91	94
p,p'-DDD	3	2.6
p,p'-DDE	5	1.2
Unknown[b]	1	0.9
Unknown[c]	-	0.5

[a] n = 2

[b] Radioactivity at origin ± 1 cm

[c] Radioactivity above 5.5 cm

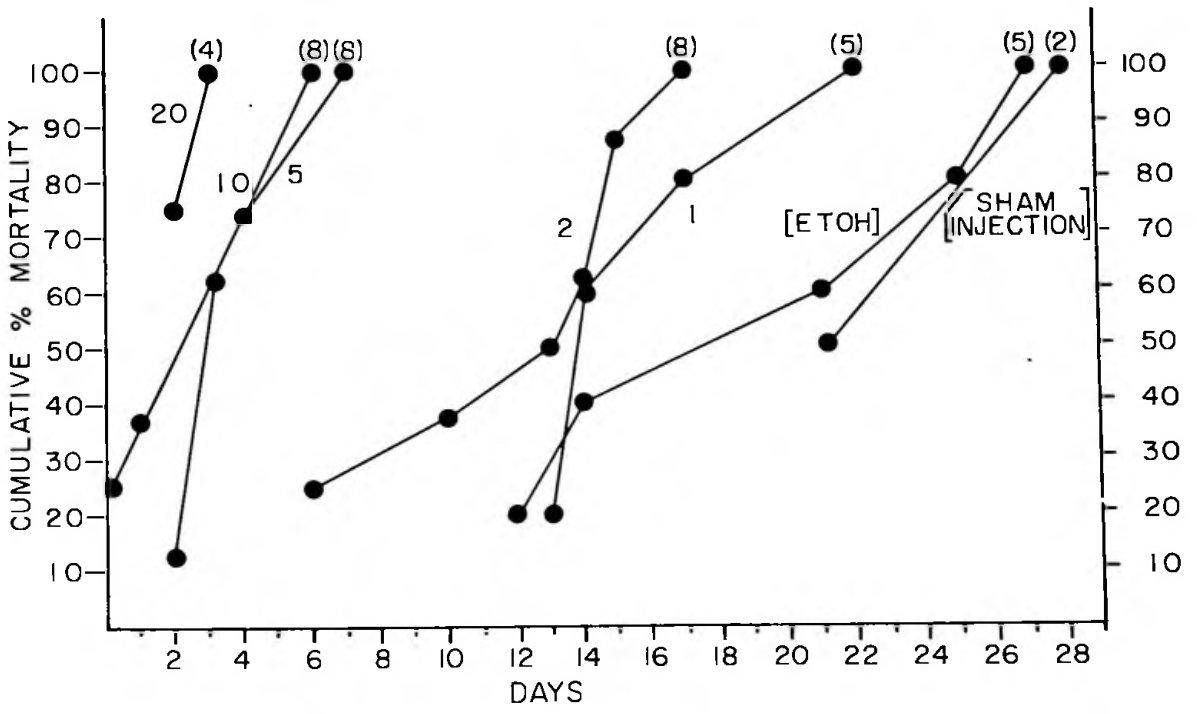

FIGURE 1: CUMULATIVE PERCENT MORTALITY IN *S. ACANTHIAS* ADMINISTERED 20, 10, 5, 2 and 1 mg/kg p,p'-DDT i.a.

( ) = N

amount of lipid material is capable of quantitatively sequestering the DDT present in the liver. Preliminary experiments showed that 97% of the radioactivity in the liver is associated with the oil; 3% with the cells. This 3% is a maximum value as all the oil may not have been removed from the cellular fraction. Thus only a small amount, if any, of the drug present in the liver is capable of reaching the hepatic cells. This, in conjunction with the slow rate of hepatic metabolism associated with *S. acanthias*, severely limits metabolism of DDT.

The toxicity of p,p'-DDT to the dogfish is shown in Figure 1. The maximum non-lethal dose is about 2 mg/kg. Fish which were given the higher doses (20, 10, and 5 mg/kg) of DDT became rigid about 24 hours before death. The fish given 10 mg/kg which died within 6 hours became almost completely rigid within 1 hour after administration. It appears that the concentration of drug found at death in the brains of the fish (Table 3) is far lower than that found in rats, even

TABLE 3. CONCENTRATION OF ^{14}C (± S.E.) AS DDT IN THE BRAIN OF S. ACANTHIAS FOLLOWING I.A. ADMINISTRATION OF p,p'-DDT

DOSE[a] (mg/kg)	N	CONCENTRATION (µg drug/g brain)
20	4	2.45 ± 0.71
10	6	1.87 ± 0.77
5	8	0.35 ± 0.04
2	3	0.18 ± 0.02

[a] As a mixture of radioactive and nonradioactive p,p'-DDT

following a non-lethal dose. Thus Henderson and Woolley (J. Pharmacol. Exp. Ther., 170:173, 1969) found that 100 mg/kg per os given to rats yielded about 20 µg/g brain, with hyperexcitability and mild tremors being the only effect of the drug. It appears that the shark brain is far more sensitive to DDT than is its mammalian counterpart.

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