1971 #4

BRAIN-BARRIER SYSTEMS TO INULIN IN *Raja erinacea* AND *Hemitripterus americanus*

Helen F. Cserr, J. Fenstermacher, and D.P. Rall. Brown University, Providence, Rhode Island; National Cancer Institute, Bethesda, Maryland; and National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina

We have previously reported investigations of brain-barrier systems in the two lowest vertebrate classes, *Agnatha* (hagfish) and *Chondrichthyes* (dogfish and nurse shark) (Bull. MDIBL 9:7-8, 1969). Results indicated that barrier mechanisms for excluding inulin from brain and CSF are not as effective in several lower vertebrate species as in adult mammals. We have now extended these investigations to include the little skate *(Raja erinacea)* and red sculpin *(Hemitripterus americanus)*.

Carboxyl-^{14}C-inulin was given as a single i.m. injection. CSF, plasma and two regions of brain were sampled 20 hours after isotope administration and assayed for radioactivity. Results are expressed as distribution ratios between brain and plasma (R_{Br}) and between CSF and plasma (R_{CSF}). Units of R_{Br} and R_{CSF} are gm tissue/ml plasma and ml CSF/ml plasma, respectively.

Results summarized in Table 1 indicate that barriers excluding inulin from brain and CSF are as effective in the little skate and red sculpin as in the rat. Low values for R_{Br} and R_{CSF} observed for the skate are not typical of all *Chondrichthyes*, however. Comparison of values for the three species of *Chondrichthyes* listed in Table 1 indicates considerable variation within this class in the extent of inulin penetration into brain and CSF.

Conditions underlying observed species differences may be related to such factors as membrane permeability, variable compartment size, or (for brain only) cellular uptake of inulin.

Table 1

SPECIES DIFFERENCES IN BRAIN-BARRIER SYSTEMS TO CARBOXYL-^{14}C-INULIN
(# of observations)

Class	Species	R_{Br}*		R_{CSF}
		telencephalon	medulla oblongata	
Mammalia	Rattus Sp.**	.013	- - -	.034
Osteichthyes	Hemitripterus americanus	.019 (6)	.028 (6)	.026 (4)
Chondrichthyes	Raja erinacea	.019 (10)	.020 (10)	.033 (6)
"	Squalus acanthias	.044 (10)	.020 (18)	.080 (14)
"	Ginglymostoma cirratum	.078 (6)	.091 (9)	.292 (5)
Agnatha	Myxine glutinosa***	.14 (14)	- - -	- - -

* R_{Br} corrected for residual blood within brain in all species except Myxine.

** Values for Rattus from Ferguson and Woodbury (Exp. Brain Res. 7:181-194, 1969).

*** Myxine data for whole brain.

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