

responding diffusional water fluxes were  $52.6 \pm 1.57$  and  $52.3 \pm 1.35$  ml h<sup>-1</sup>.(100g)<sup>-1</sup> respectively for skates in SW and 50% SW, assuming water spaces of 82% (SW) and 91% (50% SW) of body weight (Goldstein and Forster, Am. J. Physiol. 220:742, 1971). Hypophysectomy did not produce significant alteration in the turnover of internal water in skates adapted to either SW or 50% SW (Table 1).

Previous values (units as above) obtained for water turnover in the dogfish, *Scyliorhinus caniculus* and in two Batoids, *Raja montagu* and *Torpedo marmorata* were  $156.6 \pm 13.08$  (n=14),  $167 \pm 11.1$  (n=4) and  $97 \pm 8.5$  (n=4). The values in *S. caniculus* and *R. montagu* are much higher than those found in *R. erinacea* and *radiata*. It may be noted that the experiments with the former species were conducted at a somewhat higher temperature  $16^\circ \pm 1^\circ\text{C}$  than those with the latter. The difference in values for turnover of water among these elasmobranchs suggests that those which can adapt to environmental dilution (*R. erinacea* and *radiata* and *T. marmorata*) possess a diffusional water permeability much lower than the dogfish, *S. caniculus*, which is incapable of resisting large variations in environmental salinity. Changes in environmental salinity produce large movements of water across the branchial epithelium. Elasmobranchs having a low water permeability may be able to adapt more easily to changes in environmental salinity than those having high water permeability. It would be interesting to measure the diffusional water permeability in Elasmobranchs which live permanently in fresh water such as *Potamotrygon*.

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#### METABOLISM OF <sup>14</sup>C-DDT BY THE WINTER FLOUNDER, *Pseudopleuronectes americanus*

J.B. Pritchard, A.M. Guarino, and W.B. Kinter; Mt. Desert Island Biol. Lab., Salisbury Cove, Maine; National Cancer Institute, Bethesda, Maryland; and SUNY Upstate Medical Center, Syracuse, New York

In response to concern over pollution of the marine environment, we are in the process of examining the handling of the persistent organochlorine pesticide, DDT, by a marine teleost, the winter flounder. Last summer we followed the tissue distribution of radioactivity at intervals from 15 minutes to 1 week after intravenous injection of ring-labeled <sup>14</sup>C-DDT (Bull. MDIBL 10:64-67, 1970). We have now extended these studies to examine the metabolism of the injected <sup>14</sup>C-DDT.

Ring-labeled <sup>14</sup>C-DDT was injected and tissue and body fluid samples were taken as described in last year's report (reference above). Two to four fish were sacrificed at intervals of 15 minutes, 1 hour, 4 hours, 8 hours, 24 hours, 48 hours, and 1 week following injection. Tissues were chosen for analysis on the basis of previously demonstrated high content of labeled pesticide and/or vital function (liver, kidney, muscle, gonad, and brain). Plasma, bile, and urine were analysed to reveal the nature of pesticide in transit either within or from the body. All samples were extracted immediately since it had been shown in preliminary experiments that storage of intact tissue, even frozen, permits some breakdown of DDT over extended periods of time. For example, fresh flounder liver contained 90-95% DDT while liver frozen for 9 months had 60-90% DDD. On the other hand, the

extracts were stable for at least several months at refrigerator temperatures.

Tissues were extracted according to a modification of the method of Fredeen and Saha (J. Fish. Res. Bd. Can. 28:105-109, 1971). Each sample was homogenized in 2 ml acetonitrile per gram tissue. Sufficient perchloric acid was added to bring the homogenate to pH 2. Acidification had a dual function. First, it precipitated tissue protein, and second it maximized the recovery of DDA in acetonitrile by converting it to the non-ionized form. Total recovery of label following single extraction was greater than 90%. Thin layer chromatography showed no difference in the composition of this initial extract as compared to subsequent extracts. Thus, only single extraction was used in the work described below. While acetonitrile was an excellent solvent for extraction of tissues, its miscibility with water made it impractical for use with body fluid samples. These samples (urine, bile, and plasma) were acidified as above and extracted three times with twice their volume of heptane. Recovery was not as high as with acetonitrile (ranged from 50-80%), but it was again demonstrated that residual pesticide was identical in composition with that of the primary extract. If extracts (either acetonitrile or heptane) contained sufficient radioactivity, they could be chromatographed directly. Otherwise, they were evaporated to dryness under a stream of air at room temperature and taken up in 0.1 ml of heptane for chromatography.

Identification of metabolites was made by thin layer chromatography on silica gel plates with two solvent systems originally developed by Guarino. The first of these systems, 100% heptane, gave markedly different Rf's for the major DDT metabolites (Rf's of 0.0 for DDA, 0.6 for DDD, 0.8 for DDT, and 0.9 for DDE). The second system, heptane (5): ethanol (1), was particularly useful for confirming the presence of DDA and other polar metabolites (Rf's of 0.2 for DDA and 0.8 for DDD, DDT and DDE). Each experimental radioactive sample was co-chromatographed with standards of DDT and all of the above metabolites. The location of each compound was determined visually after spraying the plate with silver nitrate and exposing it to ultraviolet light. DDT and metabolites were removed from the plate and counted by liquid scintillation spectrometry. All radioactivity co-chromatographed with one of the standards except that some of the counts co-chromatographing with DDA in the 100% heptane system were observed to run at Rf's greater than DDA in the heptane: ethanol system. These relatively water-soluble compounds have been summed with DDA and reported as polar metabolites.

At all times in all tissues and fluids, except urine, there was a paucity of metabolites (Table 1). For example, the liver which in many organisms is an important site of DDT metabolism, contained from 91-95% unaltered DDT at all times. In general, DDE was present in extremely small quantities, if at all (0-2%). DDD accounted for 1-10% of the label and polar metabolites the remaining (1-5%). Only urine contrasted with this pattern (Table 1). It showed a preponderance of one or more polar metabolites (40-80%). DDT was the other major urinary component, accounting for 5-30% of the urinary radioactivity. Materials co-chromatographing with DDD and DDE were also present (5-20%). It has not yet been possible to fully identify the polar metabolites. About one-fifth of this radioactivity co-chromatographed with DDA itself. The remaining four-fifths, judged by its behavior in the present solvent systems, is less polar than DDA.

Clearly the flounder has a limited ability to metabolize DDT. No tissue or fluid except urine contains more than a few percent metabolites, and as already shown (Bull. MDIBL, 10:64-67, 1970) only 2% of the injected dose is excreted via the urine in one week. Limited metabolism has two major consequences. First, the flounder is essentially incapable of detoxifying DDT. Over 95% of

Table 1  
 REPRESENTATIVE DATA SHOWING RELATIVE CONTENT (%) OF DDT AND METABOLITES  
 IN TISSUES AND BODY FLUIDS (Values at each time after DDT  
 injection are means for 2-4 flounder)

	1 hr	4 hr	24 hr	1 wk
Liver*				
DDT	94.8	94.6	92.1	93.6
Polar	0.5	2.0	2.2	2.1
DDD	4.0	2.2	3.3	2.1
DDE	0.7	1.2	2.5	2.1
Plasma*				
DDT	91.2	87.4	96.0	96.9
Polar	4.3	5.2	2.7	1.6
DDD	2.9	3.0	1.4	0.0
DDE	1.6	4.4	0.0	1.6
Urine				
DDT	---**	17.8	27.5	14.8
Polar	--	50.0	58.2	69.8
DDD	--	10.7	7.2	8.9
DDE	--	21.4	7.2	6.7

\* Kidney, brain, gonad, and bile were similar to liver and plasma.

\*\*Counts too low to determine per cent composition at 1 hr.

the pesticide present in the fish is DDT itself or DDD (usually almost as toxic as DDT). Second, the lack of metabolism which prevents the production of water soluble, more easily excreted products such as DDA and the high lipid-solubility of the parent DDT combine to yield extremely slow elimination of DDT from flounder. In conclusion, *P. americanus* retains a large fraction of the DDT presented to it (bioconcentration) and is unable to reduce the toxicity of the pesticide retained. Thus, since the flounder has no major lipid depot and holds its pesticide load primarily in muscle, its flesh is potentially dangerous for terminal consumers including man.

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#### THE RATE OF MOVEMENT OF THE CLEAVAGE STIMULUS IN CLEAVING SAND DOLLAR EGGS (*Echinarachnius parma*)

Raymond Rappaport; Department of Biological Sciences, Union College, Schenectady, New York

Cleavage in animal eggs now appears to be accomplished by the active constriction of a circular band located in the equatorial surface and cortex. The band has distinctive ultrastructural as well as physical properties and its organization is accomplished by the mitotic apparatus. This particular activity of the mitotic apparatus may be considered stimulatory but the nature of the stimulus and the means employed in its transportation within the cell are unknown. These experiments were designed to yield information concerning the stimulus transport system by determining the rate of