main storage site for the organochlorine pesticides in the winter flounder, it must be noted that in the early post-injection period the liver, comprising less than 1% of body weight, contains over 20% of the pesticide. Even as late as 1 week it still accounts for almost 6% of the labeled DDT. Its capacity to store or bind DDT is therefore high, and it is potentially an important site for metabolism and excretion of this pesticide.

According to the previous discussion excretion plays little role in the early handling of injected pesticide. This may also be observed directly from the radioactivity in urine, bile, and the seawater in which the fish was maintained. During the first 24 hours after injection less than 0.5% of the recovered drug was present in the urine. Even at 1 week the total urinary excretion accounted for only 2% of the drug. Biliary excretion was more extensive (1.3% at 24 hr and 10% by 1 wk). However, these figures represent pesticide present in gall bladder bile, not in bile which has traversed the digestive tract. Since preliminary in vivo experiments show that DDT is well absorbed in the flounder gut, net biliary excretion may be much less. Significant excretion via the gills was not detected. The water of the experimental containers showed less than 1% of the recovered drug. However, several fish maintained in free-flowing aquaria showed marked decrease in total recovery, raising the possibility of increased gill excretion under these circumstances.

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1970 #34

DDA: AN INHIBITOR OF CHLORPHENOL RED TRANSPORT BY FLOUNDER KIDNEY TUBULES IN VITRO

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DDA (bis[p-chlorophenyl] acetic acid) is a major polar metabolite of DDT and constitutes the principle form found in the urine of mammals (Ann. Rev. Pharm. 5:27-52, 1965). Since, structurally, DDA is an organic acid, it might be predicted that it would be secreted via the well-known renal transport system for organic acids such as chlorphenol red. This system is particularly well-developed in flounder kidney tubules and the isolated renal tubule preparation was developed by Forster, who recognized its potential for screening possible inhibitors of organic acid transport (Science 108:65-67, 1948).

The kidneys of small (50-150 g) winter flounder, Pseudopleuronectes americanus, were teased into fragments (about 1 mg) and incubated for up to 2 hr at 17-20° C in depression slides containing the appropriate concentrations of chlorphenol red (CPR) and DDA in Forster's medium. In each depression there was 0.1 ml medium and about 5 mg tissue. The medium was changed at 10 min intervals to maintain constant concentrations of the organic acids.

The first series of experiments was conducted by incubating the teased tubules with unlabeled CPR (10^{-5} M) alone and with CPR plus 10^{-3} M (280 ppm) or 10^{-4} M (28 ppm) DDA. Results were determined by visually rating dye uptake into tubular lumens. With tubules from three fish, 10^{-3} M DDA completely inhibited dye uptake at all times examined (30, 60, 90 and 120 min). In the presence of 10^{-4} M DDA, dye uptake was not detectably different from control uptake at

30 min; however, after 30 min no further accumulation of dye was observed, while control tubules continued to accumulate CPR. In addition, tubules from two fish were incubated with 10^{-} M CPR plus either 1.4 x 10^{-4} M (50 ppm) DDT in 1% N, N-dimethylformamide (NNDF) in Forster's medium or 1% NNDF alone. Neither NNDF alone nor NNDF with DDT inhibited dye uptake at any time.

To further quantitate the degree of inhibition, isotope experiments were conducted using 10^{-7} to 10^{-5} M ^{3}H-CPR (Bull. MDIBL 9:30-31, 1969) with 10^{-8} to 10^{-4} M unlabeled DDA. At t end of 2 hours the tissue fragments were removed, blotted, weighed, dissolved in 1 ml of Solu ene (Packard Instrument Co.), and counted in 10 ml of toluene scintillation fluid (5 g/liter PP 250 mg/liter POPOP) on a Nuclear Chicago scintillation counter. Aliquots of medium were treated similarly. Tissue to medium concentration ratios and percent inhibition were calculat Holding ^{3}H-CPR constant at 10^{-7} M, inhibition ranged from 18 to 53% and tended to increase higher DDA concentrations (30% inhibition at 10^{-8} M DDA, 18% at 10^{-7} M, 32% at 10^{-6} M, 39% at 10^{-5} M, and 53% at 10^{-4} M). Inhibition also increased when ^{3}H-CPR concentrations were d creased while holding DDA constant at 10^{-4} M (34% inhibition at 10^{-5} M CPR, 48% at 10^{-6} M, and 53 and 70% at 10^{-7} M). Finally, net efflux of ^{3}H-CPR was examined using tissue pre-incu bated for 2 hours in 10^{-7} M ^{3}H-CPR, rinsed three times, and incubated for one hour in Forst alone, 10^{-4} M DDA, or 10^{-4} M CPR. In all cases somewhat less than half the counts were released from tissue to medium.

These results show that DDA inhibits renal tubular transport of the organic acid, CPR. F thermore, the degree of inhibition increases as DDA concentration rises and falls as CPR con centration rises. In addition, the parent pesticide, DDT, which lacks the carboxyl group, does not inhibit organic acid transport. Finally, DDA does not damage the cells so that CPR leaks out rapidly. The explanation most consistent with these results is that DDA is a competitive i hibitor of the organic acid transport system.

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1970 #35

PROTEIN SYNTHESIS, DNA SYNTHESIS AND CLEAVAGE DELAY IN Echinarachnius parm ZYGOTES: THE EFFECT OF ULTRAVIOLET RADIATION

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These studies were undertaken to further clarify the effect of an imposed ultraviolet (UV lesion into gametes and zygotes of E. parma on the "division related" protein synthesis durin the early synchronous cleavages. We have previously shown (Bull. MDIBL 9:54, 1969) that thi protein synthesis is delayed in irradiated zygotes and that this phenomenon is photoreversibl This reversibility indicates that the observed delay is DNA related.

Sperm were irradiated with 2.47 x 10^{3} ergs/cm^{2} at 254 nm which is about 80% lethal whe photoreactivation is not used. These irradiated sperm were used to fertilize non-exposed egg and the subsequent extension of cycloheximide sensitivity was used as a measure of synthesis delay. Cleavage delay and DNA synthesis were determined. In order to determine the effect o photoreversibility on DNA synthesis zygotes were radiated at 60 min post fertilization with s