DDT Inhibition of Active Chlorophenol Red Transport in Goldfish (Carassius Auratus) Renal Tubules

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Though DDT was developed as an insecticide over thirty years ago, the underlying mechanisms of toxicity, even in insects, are still poorly understood. Reviewing the literature, KAGAN (1969) concluded that DDT interaction with lipds and proteins leads to infringement of numerous cell membrane processes. The present report documents inhibitory effects of DDT [1,1,1, -trichloro -2, 2-bis (p-chlorophenyl) ethane] and several metabolites on a well known membrane-dependent process: epithelial cell transport of organic anions such as phenol red (phenolsulfonephthalein) and PAH (p-aminohippurate). This transport system is present in the proximal portion of renal tubules of most vertebrates and promotes the urinary excretion of many complex anions by active transport across one or more cell membranes into tubular urine (FORSTER 1967) (WEINER 1971). In fact, the chemical structure of DDA [bis(p-chlorophenyl) acetate] suggested that this anionic metabolite might act as a competitive inhibitor (PRITCHARD and KINTER 1970).

METHOD

Using isolated fish kidney tubules and a transported anionic dye, FORSTER (1948) devised a visual rating procedure for screening potential inhibitors of the organic anion system; we have further simplified the basic procedure by employing depression slides and have added computor analysis to obtain more quantitative data.Goldfish (2-6 inches) were obtained from a local tropical fish supplier and maintained at 15 C on a daily diet of Strike (Agway, Inc.) for at least a week. After decapitation of a fish, the saddle-shaped kidney was excised and placed in a Syracuse dish with 5 ml of a modified (KINTER and CLINE 1961) Forster's saline medium (100 mM NaCl, 2.5 mM KCl, 1.5 mM $CaCl_2$, 1.0 mM $MgCl_2$, 0.5 mM NaH_2PO_4, and 12.5 mM $NaHCO_3$) at 15 C. With fine tweezers the kidney was teased into loose masses of tubules approximating 0.5 mg and 2-6 such masses were placed in each depression of a multiple ceramic-ring slide (No. A-175, Clay-Adams). Using small tipped transfer pipets, adhering medium was drawn off and about 0.1 ml (4 small drops) of medium containing 2 x 10^{-5} M chlorophenol red (3′, 3′′-dichlorophenolsulfonephthalein) was added. Under such conditions, diffusion of oxygen from room air to tubular cells is adequate to sustain maximal chlorophenol red transport (KINTER 1966). Typically, sufficient tissue for 30 depressions was obtained from a single fish and a given concentration of inhibitor was tested in 2-4 depres-

Bulletin of Environmental Contamination & Toxicology,
Vol. 10, No. 3 © 1973 by Springer-Verlag New York Inc.

sions. Polar inhibitors were dissolved directly in dye-containing Forster's medium while non-polar inhibitors were solublized or, at concentrations above 10^{-5}, held in fine suspension with the miscible organic solvent, DMF (N, N-dimethyleformamide), present at a final concentration of 1% on a volume basis. The DDT and related compounds were 97% or higher purity p,p´-isomers (Aldrich Chemical Co.).

Incubation was carried out for up to 3 hr in a moist chamber at 18-20 C. Every 15-30 min depression slides were removed, the media drawn off and replenished, and the teased masses in each depression evaluated for dye uptake with a compound microscope set for bright-field observation at 100x magnification. With kidney tubules from teleosts such as goldfish and flounder, chlorophenol red from incubation medium is concentrated in luminal fluid so that tubular lumens appear dark red compared to cells and medium (FORSTER 1967). We selected 2 x 10^{-5} M dye in medium to give maximal luminal uptake with minimal background coloration and, as previously reported for goldfish (KINTER and CLINE 1961), only about 10% of the tubules in a given mass concentrated dye. An arbitrary visual rating on a 1 to 5 ascending color-intensity scale was made for the functioning tubules in each depression and, after averaging the ratings for comparable depressions, a scattergram of dye uptake versus incubation time could be plotted (Fig. 1A). To facilitate data analysis, however, a least squares curve-fitting computer program was employed to generate dye uptake curves (Figs. 1B and 1C). Each curve represents data from at least 5 fish (30 for controls) and has a standard error of less than 15%. Control data with both Forster's saline and 1% DMF (Fig. 1B) were obtained with all 30 fish.

RESULTS

To validate the method, dose-response data were obtained for two well known competitive inhibitors, PAH and Diodrast (iodopyracet), and the metabolic uncoupler, DNP (2, 4-dinitrophenol). A half-inhibited chlorophenol red uptake curve generated in the presence of 10^{-4} M PAH is shown in Fig. 1C. The percent inhibition relative to control uptake at 90 min was calculated for each concentration of inhibitor successfully tested (Table I). In spite of nearly complete, i.e., 88%, inhibition with 10^{-3} M DNP, marked tissue deterioration was observed only with 10^{-2} M DNP. At a given dose, PAH was consistently the least, Diodrast the intermediate, and DNP the most effective inhibitor of dye uptake. These results confirm the previously reported sequence of effectiveness against chlorophenol red uptake in goldfish tubules (KINTER and CLINE 1961) and the effectiveness of DNP, itself an organic anion, probably relates to dual action as both a transport competitor and an oxidative uncoupler (FORSTER 1967)(WEINER 1971).

Testing of the non-polar DDT,DDD, and DDE required a water-miscible, organic solvent which, itself, did not inhibit dye uptake. As shown in Fig. 1B , DMF was, if anything slightly

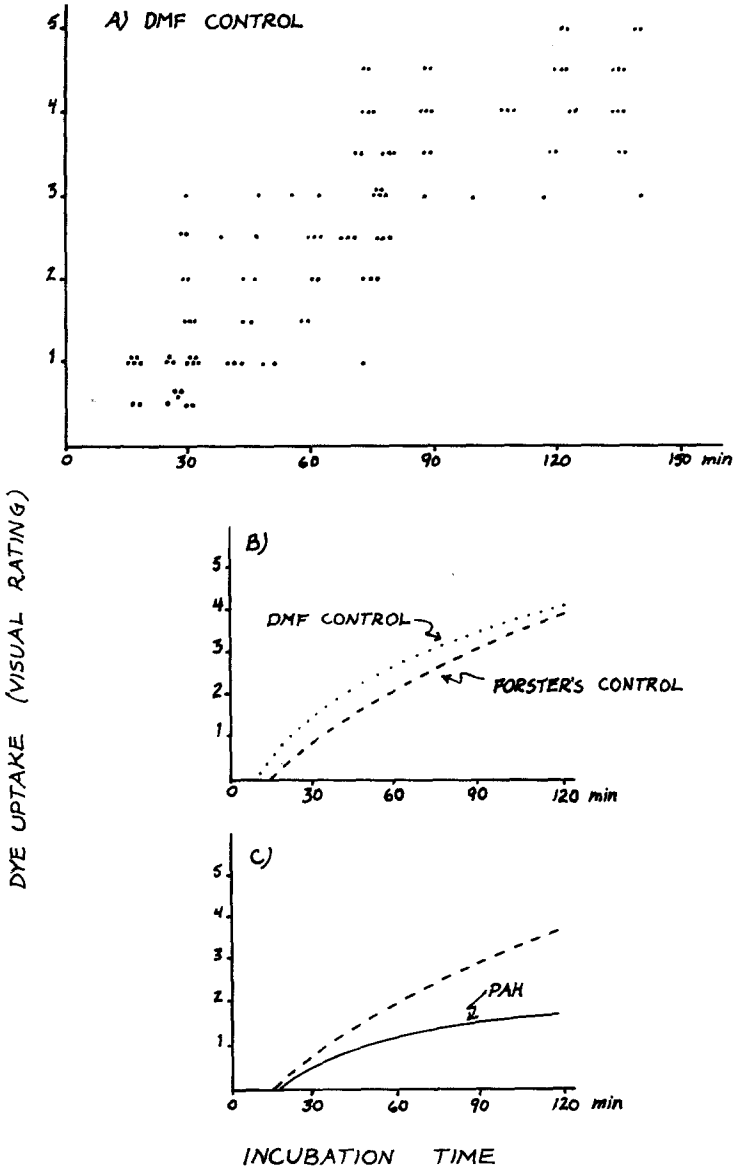

FIG. 1. Chlorophenol red uptake in luminal fluid of goldfish tubules: A) scattergram of individual ratings for 30 fish during incubation with 2 x 10^{-5} M dye in Forster's medium plus 1% DMF; B) computer generated uptake curves for above control data and that without DMF; C) inhibited dye uptake with 10^{-4} M PAH in Forster's medium.

TABLE 1.

Inhibition of chlorophenol red uptake by selected concentrations of known inhibitors and of DDT and its metabolites.

	% INHIBITION AT 90 MINUTES*		
INHIBITOR	10^{-3} M	10^{-4} M	10^{-5} M
PAH	73	50	27
Diodrast	80	57	40
DNP	88	63	43
DDT	71	31	23
DDD	--	29	14
DDE	--	49	14
DDA	77**	52†	33

* Calculated with respect to appropriate control; DMF control 15% higher than Forster's at 90 min. ** At 30 min. †At 60 min.

stimulatory, i.e.,by about 15%. In passing, it is of interest that 1% DMSO (dimethylsulfoxide), which is commonly used with mammalian preparations, caused rapid deterioration of goldfish tubules. Convincing dose-response data were obtained with DDT (Fig. 2); also its non-polar metabolites, DDD and DDE, were clearly inhibitory at 10^{-4} M, though not tested at a higher concentration due to precipitation (Table 1). The polar metabolite DDA, appeared to be an even more effective inhibitor than DDT (Fig. 2 and Table 1). Dose-response comparison, however, is complicated by the fact that DDT required solublization and that tissue deterioration limited the periods of data collection to 60 and 30 min, respectively, with 10^{-4} and 10^{-3} DDA. Deterioration was not observed with non-polar compounds. Lastly, it is apparent from individual uptake curves (e.g., Figs. 1C and 2) that DDT and its metabolites acted rapidly like the known competitors and uncoupler, i.e., pre-exposure was not necessary for inhibition under present in vitro conditions. This observation plus the volume of the frequently refreshed medium (Method) argues against any role of metabolic conversion,e.g., to DDA. At least in whole flounder (PRITCHARD 1972) significant conversion to polar metabolites required hours.

DISCUSSION

The present evidence that DDT and non-polar metabolites inhibit concentration of chlorophenol red in the luminal fluid of goldfish tubules points to several possible mechanisms of action which may underly the general toxicity of organochlorine compounds. First, increased permeability of the membrane junctions between tubular cells would enhance back-diffusion of transported

dye from luminal fluid (TRUMP and BULGER 1971). In fact, DDT interacts with at least one membrane phospholipid, lecithin (TINSLEY et al. 1971), and might disrupt junctional complexes. Second, decreased oxidative phosphorylation in the mitochondria would limit the energy for active dye transport; the extensive evidence linking these systems is cited in recent reviews (FORSTER 1967) (WEINER 1971). Moreover, in several fish tissues, DDT or related organochlorines such as endrin inhibit two of the membrane-bound enzymes involved, i.e., mitochondrial Mg activated ATPase (CUTKOMP et al.1971b) and succinoxidase (YARBROUGH and

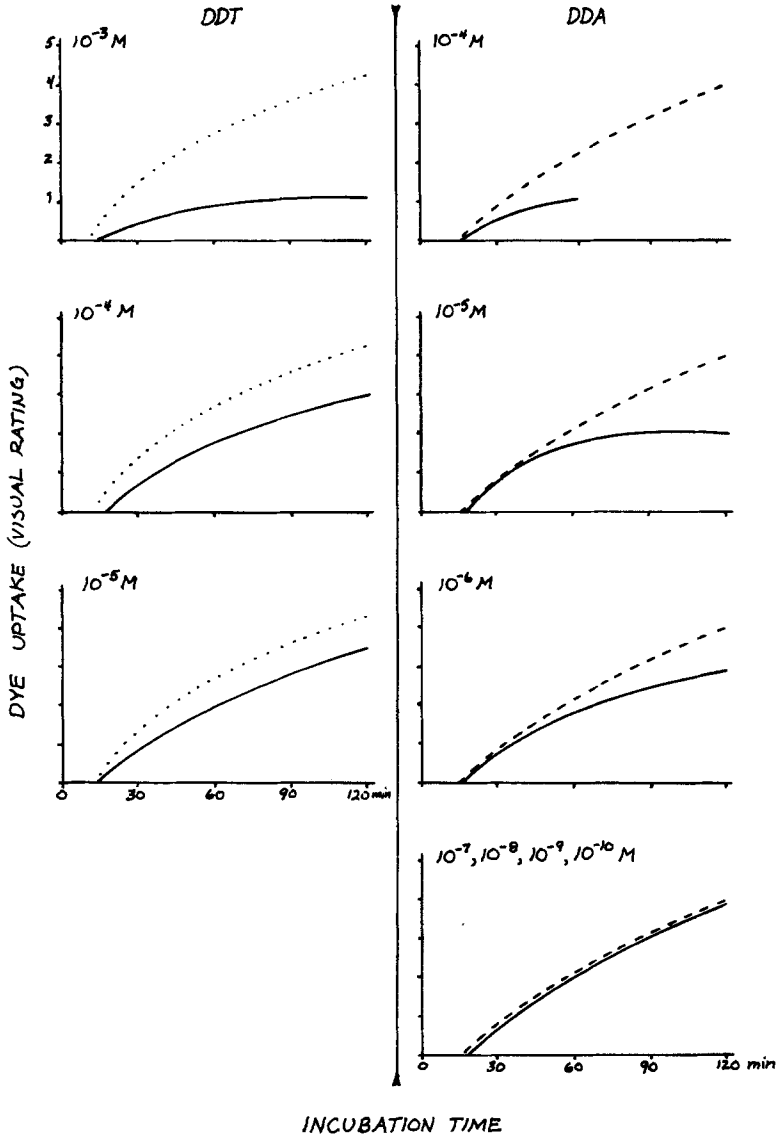

Fig. 2. Inhibition of chlorophenol red uptake by several concentrations of DDT (DMF control) and DDA (Forster's control).

WELLS 1971). Third, decreased sodium pump activity in the tubular cells would probably depress active dye transport; these systems appear to be linked in some manner involving Na-K transport ATPase (FORSTER 1967) (WEINER 1971). Again, in several fish tissues, DDT and related organochlorines inhibit the membrane-bound Na-K ATPase (CUTKOMP et al. 1971a) as well as the sodium pump (KINTER et al. 1972).

Although no real basis now exists for choosing among the above three mechanisms of action in renal tubules, similar membrane-based mechanisms probably underly the general toxicity of DDT-like compounds in other tissues,e.g., in fish gill and intestine (KINTER et al. 1972). DDA, however, may exert a special dual action, like DNP (Results), in fish kidney. For flounder tubules there is kinetic evidence that DDA, itself an organic anion, competitively inhibits chlorophenol red transport (PRITCHARD and KINTER 1970) and for bluegill brain evidence that it inhibits Na-K ATPase (CUTKOMP et al. 1971a). Certainly, active transport resulting in high intratubular concentrations of a sodium pump inhibitor could account for the tissue deterioration we observed with 10^{-4} and 10^{-3} M DDA.

ACKNOWLEDGMENT

This work was funded in part by the Union College Department of Biological Sciences and Public Health Services Grant AM 15973. We wish to thank Dr. Willard Roth and Dr. Frank Lambert (Union College) for personal support and Dr. William B. Kinter (Mount Desert Island Biological Laboratory) for assistance with the manuscript.

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