

transport of DDA on the organic acid system and for the feasibility of modifying net DDA excretion through manipulation of this transport system.

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ACCUMULATION OF ^3H -DDA BY FLOUNDER, *Pseudopleuronectes americanus*, KIDNEY TUBULES IN VITRO

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The persistent chlorinated hydrocarbon pesticide DDT [1,1,1-trichloro-2,2-bis (p-chlorophenyl)ethane] and its metabolites are accumulated by living organisms to levels far exceeding those of their immediate environment. In flounder, as in most species, a major factor contributing to bioconcentration is the extremely limited excretion of these compounds (Pritchard et al., Environ. Health Perspect. 4, 45-54, 1973). In an attempt to elucidate the mechanistic basis for such limited excretion we have previously examined in vitro the renal handling of the major urinary metabolite of DDT, its polar derivative DDA [2,2-bis(p-chlorophenyl)acetic acid]. This metabolite behaved as if it were a substrate for the organic acid transport system in the kidney. Specifically DDA competitively inhibited renal tubular accumulation of chlorophenol red (CPR) in the winter flounder (Pritchard and Kinter, Bull., MDIBL, 10, 67-68, 1970). Furthermore in both the southern flounder and the rat DDA was itself accumulated in renal tissue by two means, one which was energy-dependent and inhibited by CPR or p-amino-hippuric acid (PAH) -- the organic acid transport system -- and a second which behaved like non-

Table 1

Effects of cyanide (CN) and organic acids (CPR and PAH) on renal tubular accumulation of ^{14}C -DDA in vitro. [Inhibitors] = 10^{-3}M . [^{14}C -DDA] = $1.75 \times 10^{-5}\text{M}$. The data are expressed as mean tissue/medium ratios ($\overline{\text{T/M}}$) \pm the standard error of the mean after 60 minutes incubation. N=4.

Inhibitor	0	CN	PAH	CN & PAH
$\overline{\text{T/M}}$	14.11	7.20 ^a	9.58 ^{a,b}	7.37 ^a
	± 1.60	± 0.47	± 0.74	± 0.48

a) $p < 0.05$ vs 0b) $p < 0.1$ vs CN; vs CN & PAH

Inhibitor	0	CN	CPR	CN & CPR
$\overline{\text{T/M}}$	16.03	8.59 ^c	9.35 ^c	6.88 ^{c,d}
	± 0.62	± 0.40	± 0.36	± 0.17

c) $p < 0.001$ vs 0d) $p < 0.01$ vs CN; vs CPR

PAH = p-aminohippuric acid

CPR = chlorophenol red

specific tissue binding (Pritchard, In: Pollution and Physiology of Marine Organisms, F.J. Vernberg, Ed., Academic Press, in press, and unpublished observations). The tissue accumulation studies reported below are two-fold. First we have confirmed the dual nature of DDA uptake in a third species, the winter flounder (*Pseudopleuronectes americanus*). Second we are in the process of visualizing the distribution of ^3H -DDA within renal tissue through autoradiography (preliminary results presented).

Small masses of renal tissue were prepared and incubated with radio-labeled DDA essentially as described by Forster (Science, 108, 65-67, 1948). Briefly, 100 - 300 gm winter flounder were killed by decapitation; the kidney was removed and transferred to oxygenated Forster's saline at pH 7.8 where small masses (1 - 2 mg) were teased from its caudal portion. Three to five such masses were incubated in 1 ml of medium at 15° C for 60 minutes. Each vial was gassed with 100% O_2 and gently agitated throughout the incubation. ^{14}C - or ^3H -DDA concentrations of 2×10^{-5} and 2×10^{-4} M were used. At the conclusion of the incubation period, tissue masses to be analyzed by standard liquid scintillation techniques were removed, blotted, and weighed; tissue for autoradiography was quickly frozen in liquid propane, freeze dried, embedded in plastic, sectioned, coated with liquid emulsion, exposed, and processed according to the method of Stirling et al. (J. Clin. Invest. 51, 438-451, 1972).

As shown in Table 1 after 60 minutes incubation in 2×10^{-5} M ^{14}C -DDA, the tubular masses contained approximately 15 times as much DDA as the surrounding medium. In the absence of metabolic energy (plus 10^{-3} M cyanide) the tissue to medium concentration ratio (T/M) was nearly halved. Thus, as in the southern flounder and the rat, total uptake had two components; one dependent upon metabolic energy and a second (apparently nonspecific binding)

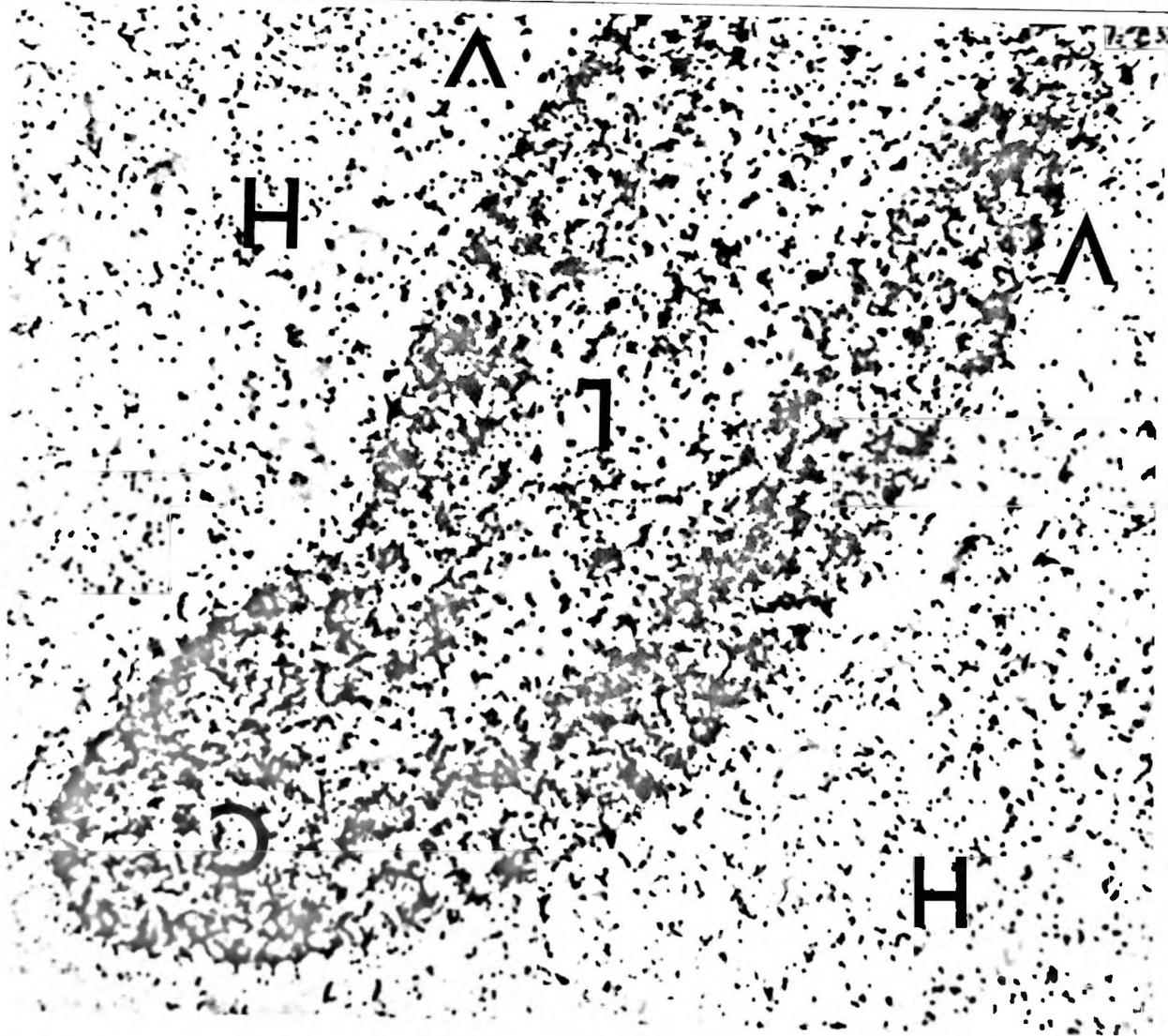


Figure 1: Autoradiograph of a kidney mass incubated 60 min in 2×10^{-6} M ^3H -DDA (120 $\mu\text{Ci}/\text{ml}$) and exposed 9 days; T/M was 10.0 for counted mass from same incubation vial. The greatest accumulation of autoradiograins, i.e., black dots, is over the cells (C) of the obliquely cut proximal tubule. The lumen (L) also shows a higher grain density than that of medium in vascular spaces (V) immediately adjacent to the tubule. The surrounding hematopoietic tissue (H) also shows some accumulation of ^3H -DDA. Present phase-contrast photomicrograph emphasizes the autoradiograph; for clearer morphology of underlying flounder kidney tissue see Kinter (Fortschritte der Zoologie, in press, 1975). 1600X.

which was not. Of the energy-dependent fraction over 60 percent was inhibited by 10^{-3} M PAH or CPR (Table 1). In keeping with the hypothesis that the uptake component insensitive to cyanide represents nonspecific binding PAH, which is only slightly bound to flounder plasma proteins (Maack et al., Bull. MDIBL, 9, 29-30, 1969), did not alter this component of DDA accumulation (Table 1). CPR, which is more heavily bound in flounder plasma, did significantly reduce this component, just as in the southern flounder and the rat. Finally much of the tissue DDA was readily exchangeable with the medium. Efflux from tissue pre-loaded for 60 minutes with ^{14}C -DDA and transferred to a large volume of DDA-free medium (plus 10^{-3} M CN to prevent re-accumulation) lost almost 90 percent of the label in one hour. Two components were seen: a rapid loss presumably from the extracellular compartment ($t_{1/2} = 1 - 2$ min) and a slower exit ($t_{1/2} = 20.0 \text{ SE} \pm 2.5 \text{ min}$, $n = 4$ fish) probably representing loss from cellular compartments.

Preliminary autoradiography (Figure 1) shows extensive accumulation of ^3H -DDA within the tubular cells to levels markedly in excess of the medium. Luminal concentrations also exceed medium concentration and approach tubular cell levels. In contrast comparable autoradiographs with ^3H -CPR and -PAH in winter flounder tubules (Kinter, Fortschritte der Zoologie, in press, 1975) showed luminal concentrations greatly exceeding those in the tubular cells. Finally in intact winter flounder (Pritchard et al., Bull. MDIBL, 14, in press) urinary DDA concentrations exceeded free plasma levels and equaled or slightly exceeded renal tissue levels.

Overall these results are consistent with active DDA secretion into tubular urine via the organic acid transport system, simultaneous with energy independent binding to cellular components.

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