between cells to the lumen and accumulation by luminal transport. Additional support for this conclusion is provided by 1) the lack of net reabsorption in vivo and 2) the inability of glucose and phlorizin to alter luminal uptake while effectively inhibiting in vitro uptake (Kleinzeller et al. Bull. MDIBL 13:67-69, 1973).

Two conclusions may now be drawn. First, each sugar is handled differently at both faces of the tubular cell. Second, the handling of either α-m-G or 2-d-G by the luminal membrane is markedly different from the handling of the same sugar at the opposite pole of the cell (i.e. the peritubular membrane).

1974 #29

RENAL HANDLING OF ^{14}C-DDA IN VIVO BY THE WINTER FLOUNDER *Pseudopleuronectes americanus*

JOHN B. PRITCHARD, MARGARET A. CAUTHEN, MARION A. KINTER, PAMELA GOLDSTEIN AND ANTHONY M. GUARINO. MEDICAL UNIVERSITY OF SOUTH CAROLINA, CHARLESTON, SOUTH CAROLINA; AND LABORATORY OF TOXICOLOGY, NATIONAL CANCER INSTITUTE, NATIONAL INSTITUTES OF HEALTH, BETHESDA, MARYLAND

Recent in vitro studies in two species of fish and on mammal have provided strong evidence for active transport of the polar DDT metabolite, DDA [2,2-bis-(p-chlorophenyl) acetic acid], by the kidney (Pritchard, et al., Bull. MDIBL 14 (in press); Pritchard, Pollution and Physiology of Marine Organisms, F.J. Vernberg, Ed., Academic Press (in press); and unpublished observations). Such in vitro techniques focus primarily on transport in a secretory direction at the peritubular membrane of the renal cell. They must be coupled with in vivo clearance studies to assess the contribution of secretory transport to net excretion, thus to evaluate the role of organic

acid transport in DDA excretion. In addition such clearance studies provide

an opportunity to test the effectiveness of drugs in altering DDA excretion.

Clearance experiments were performed essentially as described by Maack

and Kinter (Amer. J. Physiol. 216:1034-1043, 1969). The urinary bladders of

winter flounder (*Pseudopleuronectes americanus*) weighing 200-400 gm were

catheterized with a flexible 3.5 French umbilical artery catheter (0.12 ml

internal volume) at least 12 hours before the experiment. One hour prior to

initiation of the first collection period, 250 mg/kg inulin (containing

tracer quantities of ^{3}H-inulin) and 2.5 μMoles ^{14}C-DDA/kg were injected into

the pectoral musculature. Large quantities of unlabeled inulin were used to

minimize the problems inherent with ^{3}H-inulin as a measure of glomerular

filtration rate (Williams et al., Bull. MDIBL 13:128-131, 1973). Urine was

collected in 100 μl micropipettes. The rate of urine production was deter-

mined by timing the production of the last 50 μl of each sample. This

technique permitted many clearance periods to be obtained even at the slow

rates of urine production characteristic of winter flounder. Blood samples

of 0.3 ml were taken at T_0 and 30 minute intervals thereafter by puncture of

the caudal vain. Inhibitors were given i.v. at 25 μMoles/kg. Resulting

plasma concentrations were approximately 2×10^{-5}M DDA, 0.5 mg/ml inulin,

and 2×10^{-4}M inhibitors. Plasma binding of DDA was measured by ultrafiltra-

tion after Guarino, et al. (Cancer Chemotherapy Repts., 57, 125-140, 1973).

In calculating the clearances, plasma values were corrected for both binding

and delay in collection of the urine due to passage through the catheter.

The distribution of ^{14}C-DDA was measured in additional fish injected i.v.

as in the clearance experiments above. Tissue samples were taken four hours

after injection.

TABLE 1

PLASMA BINDING OF DDA[a].

INHIBITOR	% FREE
IN VITRO[c]	
--	4.10 ± 0.08 (4)[e]
PROB[b]	4.24 ± 0.21 (2)
CPR[b]	9.10 ± 0.10 (2)
IN VIVO[d]	
--	2.99 ± 0.26 (5)
PROB	2.88 ± 0.17 (3)
CPR	3.14 ± 0.73 (2)

a) $[DDA] = 2 \times 10^{-5}M$

b) $[INHIBITOR] = 1 \times 10^{-4}M$ PROB = Probenecid
      CPR = Chlorophenal red

c) IN VITRO = Plasma, DDA, and inhibitor (if any) were
      mixed in vitro prior to analysis by ultrafiltration.

d) IN VIVO = DDA and inhibitor (if any) were injected i.m.
      as in clearance experiments. Plasma samples were
      obtained 4 hours later by venipuncture.

e) Expressed as mean ± SEM (N).
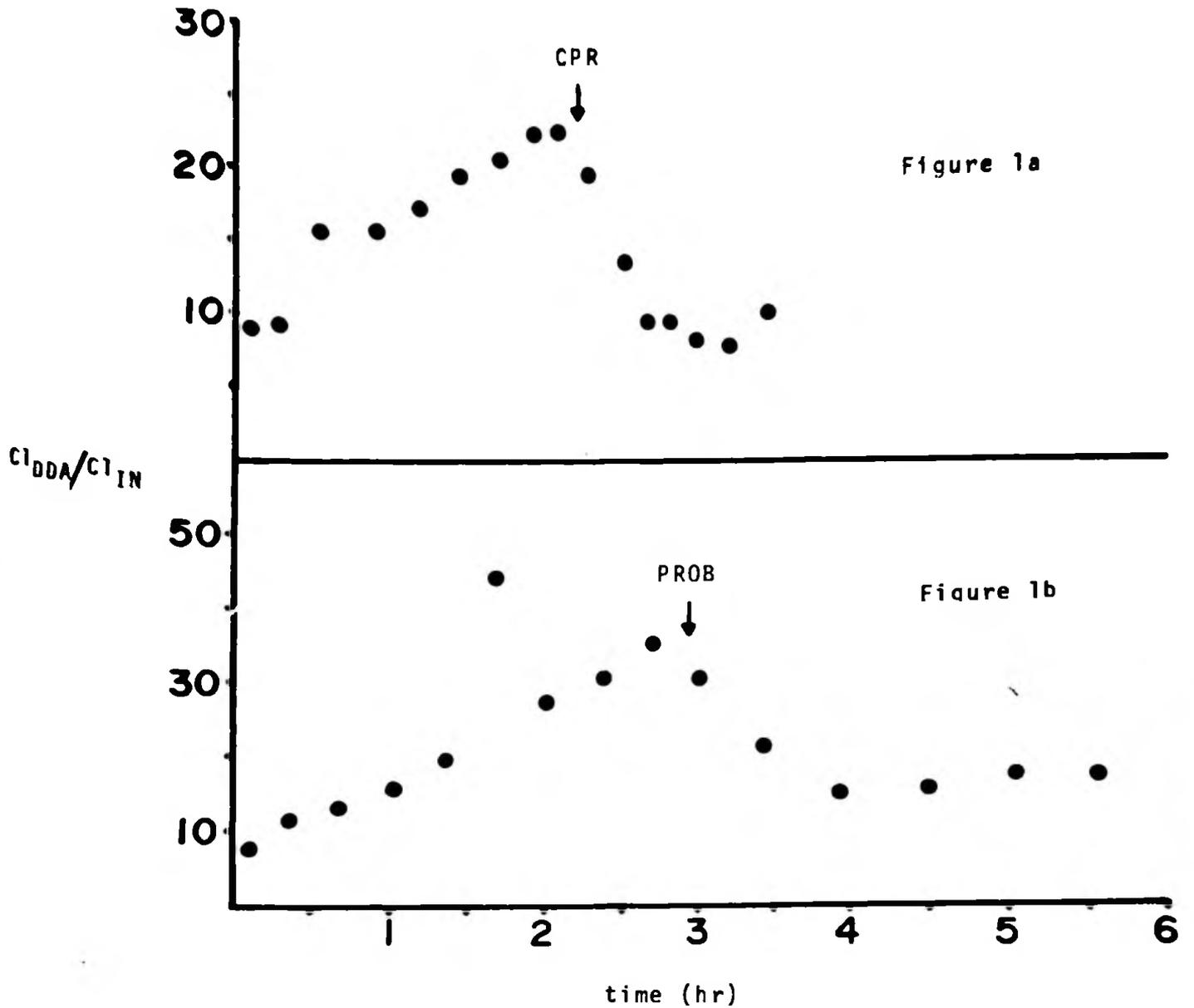
Figure 1   Clearance DDA/Clearance Inulin plotted vs the midpoint
            of each clearance period. Chlorophenol red (CPR)
            or Probenecid (PROB) were injected intravenously at
            the times indicated by the arrows. $[DDA]p = 2 \times 10^{-5}M$.
            $[INULIN]p = 0.5$ mg/ml.

97

Since the parent pesticide, DDT, is heavily bound to plasma macromole-
cules (Dvorchik and Maren, Comp. Biochem. Physiol., 42A, 205-211, 1972;
Pritchard, et al., Environ. Health Perspect., 4:45-54, 1973), it was likely
that DDA too would show significant plasma binding. Table 1 shows that DDA
was indeed heavily bound. Only four percent was free when DDA and plasma
were mixed in vitro; and even less, three percent, was free after in vivo
presentation. Since other organic acids were to be used in the clearance
experiments their effect on plasma DDA binding was also examined. Neither
chlorophenol red (CPR) nor probenecid (PROB) significantly altered DDA
binding in vivo, although CPR did increase free DDA from four percent to
nine percent in vitro. Therefore three percent of plasma DDA was considered
to be free in calculating DDA clearances.

The results of typical clearance experiments are shown in Figure 1.
During control periods the clearance of DDA (Cl_{DDA}) exceeded simultaneously
measured inulin clearance (Cl_{IN}) by 10-40 fold. Even though plasma DDA and
inulin were stable or slowly falling during clearance measurements the clear-
ance ratio (Cl_{DDA}/Cl_{IN}) continued to rise for at least two hours. In three
of the six fish, the rate of rise slowed between two and three hours suggest-
ing that the clearance of DDA had attained nearly maximal levels (Figure 1a).
In the remaining fish no such flatting of the curve was seen (Figure 1b).
Both CPR and PROB were effective in reducing Cl_{DDA} to less than 50 percent
of the peak values seen immediately prior to injection of the inhibitor.

The third aspect of this study was a comparison of the distribution of
DDT and DDA, and an assessment of the influence of CPR and PROB on DDA
distribution. As shown in Table 2 there are two major differences in the
distribution of DDT and DDA in the flounder. First DDT tissue to plasma
ratios were much greater than DDA ratios, reflecting the greater solubility

TABLE 2

Distribution of DDT and DDA. Expressed as
Tissue/Plasma Ratios at 4 hrs after Injection

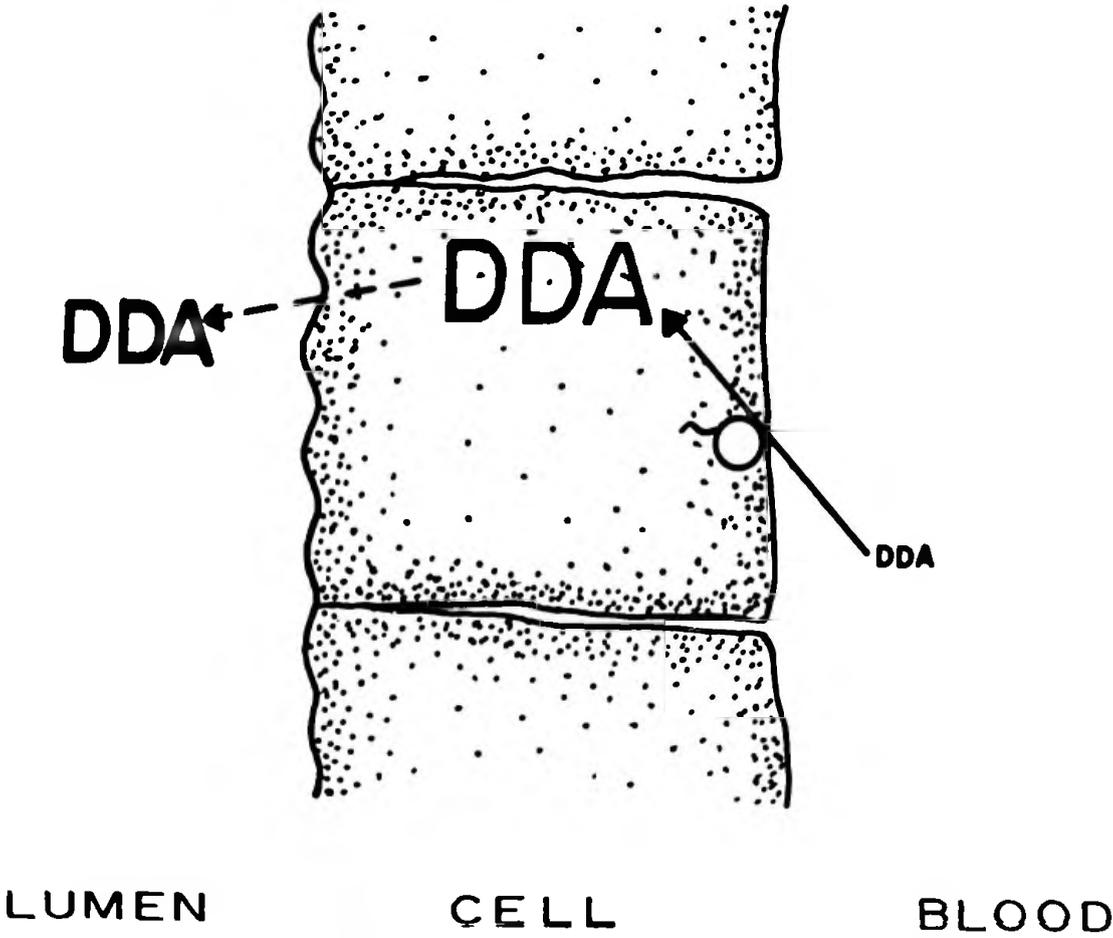|	DDT*	DDA$^+$	DDA$^+$(&CPR)	DDA$^+$(&PROB)
Urine	0.1	6.3	0.5**	0.8**
Bile	1.9	1.8	1.5	1.7
Brain	2.0	0.3	0.4	0.5
Gonad	0.9	0.2	0.3	0.2
Spleen	2.7	0.2	0.2	0.2
Heart	2.1	0.5	0.7	0.6
Gut	2.1	0.4	0.3	0.4
Gill	1.1	0.3	0.4	0.4
Liver	6.3	1.2	0.9	0.8
Kidney	4.6	1.5	0.9***	0.6***
Muscle	0.3	0.1	0.1	0.1

* 4 hrs post i.v. injection (N=4)

+ 4 hrs post i.m. injection (N=4)

** $p < 0.05$ vs DDA T/P urine

*** $p < 0.1$ vs DDA T/P kidney

FIGURE 2

MODEL FOR DDA SECRETION

LUMEN   CELL   BLOOD

of the more polar DDA in plasma. In addition DDA distribution was more uniform; only liver and kidney, which are both capable of organic acid transport (Guarino et al., Bull. MDIBL 43-45, 1972), showed higher DDA content. Second, DDA was much more effectively excreted via the urine (60 times DDT), reflecting both the greater water solubility of DDA and its structure which permits excretion via the organic acid transport system. CPR and PROB had no significant effect on the content of most tissues. Only urine, which dropped to approximately ten percent of its control value and kidney, which fell approximately 50 percent, showed significant changes. Liver T/P fell 25 to 30 percent, but the change was not statistically significant. Thus the effect of these organic acids is specific for kidney where secretory transport of DDA on the organic acid system apparently underlies the high tissue and urine content.

A model consistent with these results and with in vitro studies including autoradiography (Pritchard et al., Bull. MDIBL 14, [In Press], 1974) is shown in Figure 2. Energy dependent accumulation occurs at the peritubular membrane producing high DDA concentrations within the epithelial cell. Movement to the lumen is passive. Such a model may explain the increase in the clearance of DDA seen with time in these studies. This increase may reflect the time course of elevation of intracellular DDA, and thus the increase in a gradient for passive movement to the lumen.

In conclusion DDA is heavily bound in plasma. When appropriate corrections for plasma binding are made the clearance of DDA greatly exceeds GFR, thus exhibits secretion. Further support for this conclusion is provided by the marked inhibition of Cl_{DDA} and kidney DDA content by the organic acids CPR and PROB. Therefore in vivo techniques provide additional evidence for

transport of DDA on the organic acid system and for the feasibility of

modifying net DDA excretion through manipulation of this transport system.

1974 #30

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

JOHN B. PRITCHARD, KARL J. KARNAKY, JR., HAROLD H. CHURCH, AND WILLIAM B. KINTER. MEDICAL UNIVERSITY OF SOUTH CAROLINA, CHARLESTON, SOUTH CAROLINA; AND MOUNT DESERT ISLAND BIOLOGICAL LABORATORY, SALSBURY COVE, MAINE

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[2,2bis(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 accumula-
tion 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-