TABLE 2

Effect on Stimulated and Spontaneous Secretion
(adjusted difference due to $H_2$-inhibitors; $H^+\mu Eq.hr^{-1}cm^{-2}$)

| Agent | Histamine | Carbachol | Pentagastrin | Spontaneous |
|---|---|---|---|---|
| | 250 µM | 25 µM | 25 µM | |
| Metiamide | +0.13 ±.24(9) | +0.16 ±.23(8) | +0.76 ±.66(6) | +0.40 ±.60(7) |
| Cimetidine | +0.40 ±.24(6) | +0.34 ±.30(6) | | |

Means and 95% confidence limits; (*n*)

After 2.5 µM carbachol, the comparable responses were, respectively, -0.04 ± 0.27 (9) and +0.18 ± 0.42 (4). Carbachol 5 µM followed by metiamide yielded a mean adjusted difference of -0.16 ± 0.89 (7).

ORGANIC ACID TRANSPORT: UTILITY OF IN VITRO KINETIC MEASUREMENTS TO PREDICT EFFECTIVENESS OF INHIBITORS IN VIVO.

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Although several studies have addressed the kinetics of in vitro organic acid transport in mammals (Am. J. Physiol., 196:86-92, 1959; 208:391-396, 1965; 220:95-99, 1971) and in fish (Am. J. Physiol., 211:1152-1164, 1966), none have attempted to evaluate the applicability of these affinity estimates in vivo. Such a comparison of affinities in vitro and in vivo was the primary objective of the studies reported below. A secondary objective was the comparison of kinetic parameters estimated from relatively simple isotopic uptake experiments using isolated flounder tubules with those obtained by Kinter using elegant microspectrophotometric techniques (Am. J. Physiol., 211:1152-1164, 1966).

In the in vitro experiments, isolated renal tubules of the winter flounder (*Pseudopleuronectes americanus*) were prepared according to Forster (Science, 108:65-67, 1948). Approximately 10 mg of finely teased tubules were transferred to 1 ml of Forster's medium (15°C) containing substrate ($^{14}$C-p-aminohippurate [$^{14}$C-PAH]) and inhibitor, if any. PAH concentrations from 10 to 500 µM were used. Inhibitor concentrations were 10 µM chlorophenol red (CPR), 1 µM bromcresol green (BCG), 10 µM, 2,2-bis(p-chlorophenyl) acetic acid (DDA), 10 µM iodipamide (IA), and 10 µM probenecid (PROB). PAH accumulation was linear for at least the first 30 min of incubation. Therefore, allowing a margin of safety, a 20 min incubation time was chosen for all kinetic experiments. Tissue uptake of $^{14}$C-PAH was assessed by standard liquid scintillation techniques.

In vivo tubular transport of $^{14}$C-PAH was assessed by standard clearance techniques (Am. J. Physiol., 231:603-607, 1976) using $^{3}$H-polyethylene glycol ($^{3}$H-PEG) as glomerular marker. Doses were 1 µmol/kg for $^{14}$PAH (2 µCi) and 250 mg/kg for $^{3}$H-PEG (5 µCi). Resulting plasma concentrations averaged

1.51 (± 0.16 SE) μM PAH and 0.59 (± 0.02) mg/ml PEG. Inhibitor doses were 10, 1, and 0.1 μmol/kg as indicated in the text below.

It is acknowledged at the outset that the application of Michaelis-Menten kinetic analysis to a complex tissue such as the renal tubule is imperfect at best. Nevertheless, it should be noted that most of these potential errors, such as underestimation of the initial rate, operate to alter the calculated maximum velocity ($V_{max}$) whereas the affinity constant ($K_m$) is much more resistant to such problems. Thus previous workers have been able to obtain much useful information on affinity constants using similar techniques (Am. J. Physiol., 208:391-396, 1965; 220:95-99, 1970). The Lineweaver-Burk plot of control $^{14}$C-PAH accumulation is shown in Figure 1 and the affinities ($K_i$) of several inhibitors are given in Table 1. By way of comparison, Kinter's (Am. J. Physiol., 211: 1152-1164, 1966) microspectrophotometric measurements gave a $K_m$ of 11 μM for CPR vs 14.5 here. On the other hand, his $K_i$ for PAH was estimated at 45-72 μM vs 177 μM here. Mammalian PAH affinities are considerably lower than either affinity estimate for the flounder. For example, PAH $K_m$ were 0.54 mM (Am. J. Physiol., 208:391-396, 1976) and 0.59 mM (Am. J. Physiol., 220:95-99, 1971) in the rabbit and 0.87 mM in the rat (unpublished).

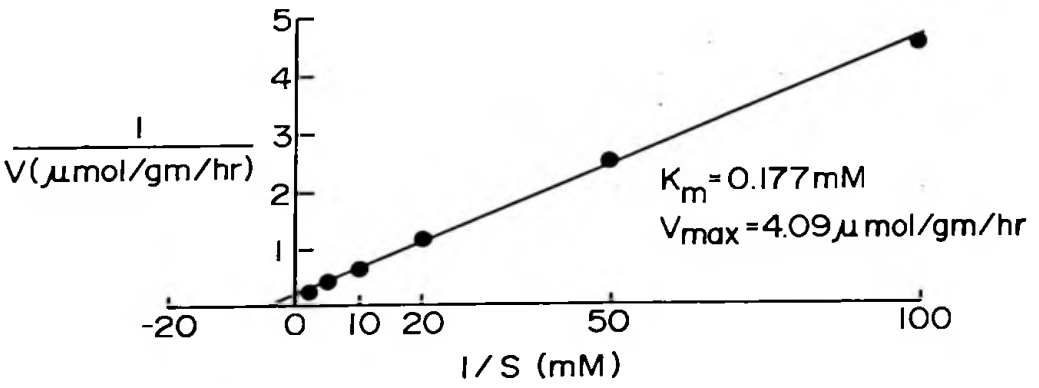


Figure 1. Lineweaver-Burk plot of $^{14}$C-PAH accumulation (V) by isolated flounder kidney tubules in vitro as a function of medium PAH concentration (S). Points shown are the means of measurements in 25 fish. Velocities are in μmol/g wet weight/hr.

Using the $K_i$ values from Table 1, it is possible to rank these inhibitors on the basis of their affinities. If we assign a value of 1.00 to the affinity of BCG (the most potent inhibitor), the other affinities are then 0.120 for PROB, 0.065 for DDA, 0.045 for CPR, and 0.036 for IA. As an internal check on the validity of these estimates, tubules were incubated with 10 μM $^{14}$C-PAH and the $K_i$ concentration of inhibitor (Table 2). Despite widely differing chemical concentrations, BCG, CPR, and PROB produced nearly identical inhibition of PAH transport. DDA inhibition was particularly variable and averaged significantly less than that caused by BCG, CPR, and PROB.

Clearance experiments using DDA, CPR, and BCG as inhibitors of $^{14}$C-PAH clearance in vivo are summarized in Table 3. These results must be taken as preliminary since they reflect only 48 clearance periods in 4 fish. However, the agreement with the in vitro data is striking. BCG at

TABLE 1

Kinetic Constants for PAH Uptake by Isolated Flounder Tubules*

| Inhibitor | (N) | [Inhibitor] | $K_m$ | $K_m$ | $K_i$ |
|---|---|---|---|---|---|
| | | (μM) | (μM) | (μM) | (μM) |
| Bromcresol green | (6) | 1 | 137 | 348 | 0.65 |
| Probenecid | (4) | 10 | 176 | 501 | 5.4 |
| DDA | (4) | 10 | 155 | 310 | 10.0 |
| Chlorophenol red | (8) | 10 | 114 | 193 | 14.5 |
| Iodipamide | (6) | 10 | 99 | 154 | 18.0 |

*$K_i$, the inhibition constant, was calculated from the equation $K_m' = K_m (1 + [i]/K_i)$ where $K_m'$ is the $K_m$ for PAH uptake in the presence of the specified inhibitor concentration [i].

TABLE 2

Uptake of PAH in the Presence of the $K_i$ Concentration of Several Inhibitors*

| Inhibitor | $K_i$ | % Control Rate | (SE) |
|---|---|---|---|
| | (μM) | | |
| Bromcresol green | 0.65 | 23 | ( 6) |
| Probenecid | 5.4 | 28 | ( 8) |
| DDA | 10.0 | 43 | (11) |
| Chlorophenol red | 14.5 | 23 | ( 6) |

*Each inhibitor was run with a paired control in 5 fish. The rate of uptake in the presence of each inhibitor was significantly reduced from control values ($P < 0.05$). Of the individual inhibitors, only DDA differed significantly ($P < 0.05$) from the others. $^{14}$C-PAH concentration in the medium was 10 μM in all experiments.

one-tenth the dose produced similar inhibition to DDA and CPR, just as it did in vitro. On the the other hand, CPR and DDA demonstrated similar affinity in vitro and in vivo.

In summary, it would appear that estimation of affinities through simple in vitro experiments could well predict in vivo transport inhibition. Clearly a broader series of inhibitors with widely differing affinity and more extensive in vivo evaluation are still needed to support this conclusion, but results thus far are encouraging. Certainly such a simple in vitro method has considerable potential, particularly in evaluating the effects of environmental contaminants and their metabolites.

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TABLE 3

Preliminary Assessment of the Effects of DDA, CPR, and BDG on the Clearance of $^{14}$C-PAH by the Winter Flounder*

| Inhibitor | Plasma [Inhibitor] | $C_{PAH}$ | % Inhibition |
|---|---|---|---|
| | μM | | |
| DDA** | 0 | 200.1 | |
| | 1 | 16.4 | 91.8 |
| | 2 | 8.2 | 95.9 |
| | 10 | 2.6 | 98.7 |
| CPR** | 0 | 171.9 | |
| | 1 | 29.7 | 82.7 |
| | 10 | 5.0 | 97.1 |
| BCG** | 0 | 113.4 | |
| | 0.1 | 18.0 | 84.1 |
| | 1.0 | 0.8 | 99.3 |

*Abbreviations: DDA = 2,2-bis@p-chlorophenyl) acetic acid; CPR = chlorophenol red; BCG = bromcresol green.

**DNA results reflect 12 control and 12 experimental clearance periods in 2 fish. CPR and BCG results are from single fish with 6 control and 6 experimental clearance periods in each fish.

## CYTOTOXICITY OF PLASMA AND TISSUE HOMOGENATES OF SHARKS ON MURINE LYMPHOBLASTS IN CULTURE

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There is an increasing interest at the National Cancer Institute and elsewhere in finding antineoplastic substances from the sea (Li et al., Cancer Chemother. Rep. 4(2):97, 1974). Snodgrass et al. (JNCI 56:981, 1976) have demonstrated that significant inhibition of the growth of Lewis Lung Carcinoma is produced in mice by parenteral administration of dogfish serum. In a previous bulletin (MDIBL 15, in press 1976), we have documented the megalocytic (increase in the mean cell volume) and cytotoxic effects of spiny dogfish plasma on a subline of Leukemia 5178Y cells and L1210 cells in vitro. Cytofluorimetric analysis of the DNA distribution of L5178Y/AR cells cultured in the presence of spiny dogfish plasma established that cell cycle progression was arrested at the $G_1$ phase. Preliminary attempts to characterize the factors causative of the megalocytic effect suggested that it was a nondialyzable macromolecule.

In the present report, we have compared the megalocytosis and cytotoxicity produced by plasma from different species of sharks with that of plasma from dogfish. We also have studied the