in their livers since: (1) it is very large, ca. 10% of the body weight, and (2) most (ca. 60%) of the wet weight of the liver is fat. We have administered the following maximum doses in mg/kg (in parenthesis) of xenobiotics (in EtOH: emulfor:saline as indicated above) with no significant mortality within 72 hr to groups of 2-6 fish per dose: DDT (4), PCB (Arochlor 1254) (50), ethyl-hexylphthalate (100), 2,4,5-T (15), 2,4-D (20), chlordane (10), heptachlorepoxide (5), lindane (5), malathion (10), phenol (in saline) (50), SLS (in saline) (10), and octane (10). Therefore, the spiny dogfish was able to survive doses of many xenobiotics which are quite toxic to terrestrial mammals.

ACTION OF HISTAMINE$_2$-INHIBITORS ON ACID SECRETION BY THE ISOLATED GASTRIC MUCOSA OF *Squalus acanthias*

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Since the discovery of histamine, its possible physiological role in regulation of acid secretion by the stomach has been the subject of heated controversy. Just as arguments against such a role became more compelling, a new class of histamine inhibitors, H_2-inhibitors, were introduced some four years ago. These inhibitors in vitro block H^+ secretion of amphibian and mammalian gastric mucosae not only when such secretion is elicited by histamine itself but also when stimulated by cholinergic agents such as carabachol as well as gastrin or its analogue pentagastrin.

The relative insensitivity of the isolated dogfish gastric mucosa to all three classes of chemo-transmitters prompted study of the inhibitors in *Squalus acanthias*. In this species, even considering this insensitivity to chemotransmitters, we find that H_2 inhibitors metiamide or cimetidine (2 mM) do not block transmission. At higher concentrations of the several transmitters a small but significant augmentation by cimetidine occurred. The effect, if any, on spontaneous secretion as well as with lower concentrations of excitants was obscured by the secretory rate's relatively large variance even though experiments were paired.

Experiments were conducted as previously (Hogben, Proc. Soc. Exp. Biol. Med. 124:890-893, 1967) except that (a) the secretory rate was obtained by "stat" titration in situ to a pH of 6.75, (b) mucosae were initially isolated and dissected in the HCO_3^--buffered saline, (c) mucosae were mounted after being impaled on pins embedded in the chamber wall about the 2 apertures, and (d) the ratio of surface area to volume of bathing solution was 2.95 cm^2:30 ml. No electrical measurements were made. Chambers, similar to those used previously, allow a section of mucosa to be placed over two adjacent apertures. Upon apposition of the two halves of the chamber we now have two adjacent segments which can be studied in tandem. For the present study, one segment serving as a control was not exposed to inhibitor during the third hour while the other member of the pair was exposed to inhibitor during the third hour. After about one-half hour for adjustment, the rate of spontaneous H^+ secretion was followed over 0-60'. Each of the adjacent segments of mucosa was exposed to a chemo-excitant beginning at 50' and its secretion measured during the interval 60'-120'. Until 110' each of the 2 segments had been treated alike. At 110' one member of the pair was treated with an H_2-inhibitor and the secretory rate of both segments was measured over the final hour, 120'-180'. In about half of the experiments, measurement continued for a 4th hour and in a few instances for a fifth hour. While the reduction of n of each subset precluded useful statistical analysis of the latter 2 hours, no unexpected changes were encountered. In one set of experiments, Spontaneous

Secretion, the protocol differed somewhat. The spontaneous secretory rate was measured for 90'. At 80', 2 mM metiamide was added to the "treated" segment and titration continued from 90' to 180'. Several experiments were continued beyond 180' without unexpected results.

The experience without intervention of an H_2-inhibitor is given in Table 1, SECRETORY RATES OF CONTROL MUCOSAL SEGMENT. For each excitant, the rates for the first hour are those of spontaneous secretion, followed by two successive hours of stimulation. The responses to histamine and carbachol are about twice those we have found formerly. It is not known which, if any, minor procedural changes might be responsible. The failure to respond to 25 μM pentagastrin is conclusive and in sharp contrast

TABLE 1

Secretory Rates of Control Mucosal Segment: H^+ $\mu Eq.hr^{-1}cm^{-2}$

Period	Histamine	Carbachol	Pentagastrin	Spontaneous*
	250 μm	25 μm	25 μm	
1	1.87 ± .68	1.95 ± .64	1.31 ± .89	1.32 ± 1.01
2	3.85 ± .77	5.45 ± .62	0.92 ± .67	1.05 ± .84
3	4.18 ± .91	5.26 ± .66	0.61 ± .54	
n	15	14	6	7

Means and 95% confidence limits. *See text.

to *Rana catesbiana* whose gastric mucosa responds reproducibly to 0.1 μM pentagastrin. It has been estimated that the dogfish gastric mucosa is 200-250 times less sensitive to histamine and carbachol (op. cit. supra.). However, for each of the two species more satisfactory values for the concentrations inducing an inhibition of 50% are needed. Thus it would be premature to conclude that the dogfish gastric mucosa is resistant to pentagastrin and is also relatively more sensitive than to histamine or carbachol.

To sharpen the statistical analysis, for each member of the paired segments we first obtained the difference in secretory rate between the third and second hour. The corresponding value for the control segment was then subtracted from that of the treated segment to provide the best estimate of the action, if any, of an H_2-inhibitor; the "adjusted difference." The values of the adjusted differences are given in Table 2, EFFECT ON STIMULATED AND SPONTANEOUS SECRETION.

The most important finding is that none of the secretory rates were reduced by an H_2-inhibitor even though the concentration used, 2 mM, is 10-fold greater than needed to inhibit secretion by the amphibian or mammalian gastric mucosa. Cimetidine in concert with either histamine or carbachol as well as the combination of metiamide and pentagastrin enhanced acid secretion. However the degree of enhancement was at best marginal for the cimetidine combinations, 9% and 6%, and striking only with the ineffective stimulus pentagastrin, 123%.

When secretion had been stimulated by 25 μM histamine, the metiamide response was a mean adjusted difference of +0.40 $\mu eq.hr^{-1}cm^{-2}$ ± 0.87 (8) and the cimetidine response +0.06 ± 1.26 (4).

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