against 6-AN when the vitamin is given 12-24 hours prior to antimetabolite treatment may reflect the rapid metabolism of NAM and its decrease to normal levels with 8-24 hours after injection(6).

Summary. Pregnant rats were given single injections of nicotinamide (NAM) (8-100 mg/kg body wt) before or after a teratogenic dose of the vitamin antimetabolite 6-aminonicotinamide (6-AN) late in gestation. NAM effectively competed with 6-AN in preventing cleft palate when given 2 hours before, simultaneously, or up to 2 hours after antimetabolite treatment. However, vitamin replacement therapy was unable to prevent palatal abnormalities or restrict the action of 6-AN to specific time periods when given 12-24 hours before or 2-72 hours after 6-AN administration.

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Response of the Isolated Dogfish Gastric Mucosa to Histamine. (31879)

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To simplify an aspect of gastric physiology, many have embraced a proposal that histamine is the final common mediator of acid secretion(1,2,3). At least one scientist has expressed his reservations(4). To date most work has been conducted on the whole animal. Such studies cannot be interpreted stoichiometrically because of our uncertain knowledge of the kinetics of disposition of the several agents that excite secretion of hydrochloric acid.

Davidson *et al* found that the concentration of gastrin required to increase acid secretion by the isolated bullfrog gastric mucosa is less by several orders of magnitude that that of histamine (5). Many years ago Babkin and coworkers failed to evoke acid secretion with histamine in the intact skate (6). I am reporting that though the isolated gastric mucosa of another elasmobranch, the spiny dogfish, does respond to histamine, the interstitial concentration of histamine eliciting maximal secretion of acid is so much that it is unlikely that an extracellular release of histamine mediates cholinergic excitation of acid secretion in this species.

Materials and methods. Dogfish *(Squalus acanthias)* were caught on a trawl line in Frenchman's Bay and held in live cars. In less than 10 minutes after removal of a fish from the sea its mucosa was mounted in a plastic chamber. The mucosa was separated as a flat sheet by dissection on a cold block. When placed in the chamber, the sheet of mucosa became two portions, each having an area of 2.9 or 3.5 cm^2 and whose surfaces were bathed by 20 ml of saline. Serosal surfaces were bathed by a solution having Na^+ 257, K^+ 10, Ca^{++} 10, Mg^{++} 4, Cl^- 240, HCO_3^- 30, $PO_4^{\equiv}$ 3, SO_4^+ 4 mEq/1, and 28 mM/1

TABLE I. Secretory Response of Isolated Gastric Mucosae Evoked by Carbachol, Histamine, and Gastrin. H^+ μEq cm^{-2} hr^{-1}

Agent	μM	45-min periods: First (Before treatment)	Second (Before treatment)	Third (After treatment)	Fourth (After treatment)	Fifth (After treatment)	Mucosa wet wt (g cm^{-2})	n
Dogfish								
Control		2.14 ± .36	1.64 ± .20	1.54 ± .20	1.52 ± .19	1.44 ± .17	.270 ± .011	26
Carbachol	0.1	1.74 ± .42	1.41 ± .32	1.76 ± .26	2.05 ± .26	1.94 ± .26	.214 ± .018	6
"	1	1.78 ± .23	1.44 ± .13	2.49 ± .16	3.22 ± .18	3.19 ± .19	.252 ± .011	17
"	10	1.89 ± .25	1.34 ± .11	2.87 ± .19	3.48 ± .23	3.18 ± .18	.254 ± .009	26
"	100	2.21 ± .59	1.83 ± .33	2.94 ± .49	3.14 ± .69	2.95 ± .56	.234 ± .026	4
Histamine	2	2.79 ± .97	1.96 ± .32	2.81 ± .28	2.75 ± .42	2.62 ± .38	.318 ± .029	3
"	20	2.31 ± .38	1.71 ± .38	2.31 ± .27	2.48 ± .28	2.44 ± .28	.291 ± .022	11
"	200	1.77 ± .19	1.42 ± .31	2.28 ± .18	2.92 ± .19	3.00 ± .16	.267 ± .020	8
"	2000	1.57 ± .16	1.23 ± .15	2.69 ± .24	3.24 ± .21	3.49 ± .28	.243 ± .012	12
Hog gastrin II	0.005	1.17 ± .11	.50 ± .10	.61 ± .07	.80 ± .24	.90 ± .22	.235 ± .026	4
Idem	0.05	1.19 ± .13	.67 ± .07	.88 ± .06	1.00 ± .11	1.03 ± .13	.239 ± .015	8
Histamine*	200	1.62 ± .52	1.75 ± .23	1.87 ± .26	1.92 ± .23	1.83 ± .24	.262 ± .014	11
Bullfrog								
Control		.14	.08	.23	.49	.27	.154	2
Carbachol	1	.87 ± .37	.88 ± .38	1.50 ± .16	1.75 ± .27	1.47 ± .31	.220 ± .031	4
Histamine	20	.93 ± .41	1.07 ± .43	1.71 ± .20	2.02 ± .19	2.10 ± .17	.209 ± .025	4
Hog gastrin II	0.05	.17	.14	1.46	1.86	1.77	.147	2

$\bar{X}$ ± SE; * For this series histamine was added just prior to the end of first period.

glucose; they were gassed by 5% CO_2 95% O_2. A similar solution gassed by 100% O_2 was used to bathe the mucosal surfaces in which HCO_3^- and $PO_4^{\equiv}$ were replaced by equivalent Cl^-. Solutions were changed several times during an initial stabilization period of 20-40 minutes. Subsequently the fluid bathing the mucosal surface was replaced at 45 minute intervals. Duplicate 6 ml aliquots of the removed solution were titrated to pH 7 with 50 mM NaOH. Just prior to the end of the second period, 50λ of the appropriate concentrated drug solution was added to the serosal compartment. Experiments were conducted at a mean ambient temperature of 20 C, but with a rise of 2-3 C over several hours. To provide a basis for comparison, H^+ secretion by isolated bullfrog gastric mucosae was studied in a similar fashion except that bathing solutions previously described(7) were used and experiments were conducted at 25 C. Pure hog gastrin II was graciously supplied by Professor R.A. Gregory, Imperial Chemical Industries Ltd., lot no. 53943.

Results. Isolated gastric mucosae of the dogfish, like those of teleosts, amphibia, reptiles, and mammals, spontaneously secrete acid at a slow rate of 1-2 μEq cm^{-2} hr^{-1}. Both histamine and the parasympathomimetic agent carbachol, (2-hydroxyethyltrimethyl ammonium carbamate), can increase the rate by 100-150%. However for the dogfish the optimal concentration of carbachol was 10 μM while 2000 μM of histamine were needed for the maximal effect, Table I. In the series where H^+ secretion was followed for four 45 minute periods, the greatest response was attained during the third period, or about 120 minutes after addition of drug. The highest concentration of porcine gastrin used, 0.05 μM, did not elicit a marked response from the dogfish gastric mucosa though this concentration is sufficient for the isolated bullfrog gastric mucosa(5).

Where appropriate, paired assays were evaluated with Student's *t* test, Table II. The response to 2000 μM histamine was significantly greater than the response to 200 μM. Though 100 μM carbachol has not been shown to be less effective than 10 μM carbachol, the decreased H^+ secretion encountered in the third 45 minute period provides a clue that 100 μM is excessive.

Drs. V.H. Cohn and K.S. Kim, Department of Pharmacology, George Washington University, were kind enough to determine the

TABLE II. Secretory Response of Gastric Mucosae Analyzed by Paired Differences.

Mucosa A	μM	Mucosa B	μM	Paired differences: $\Delta\bar{x}$	SE	H^+ μEq cm^{-2} hr^{-1} P	n
Dogfish							
Carbachol	10	Control		+1.30	.27	<.005	7
"	10	Carbachol	1	+ .11	.21	>.50	7
"	10	"	100	+ .54	.27	<.20	4
"	1	"	.1	+1.03	.34	<.05	6
"	1	Gastrin	.05	+1.70	.35	<.025	4
Histamine	200	Control		+ .59	.18	<.025	8
"	2000	Histamine	200	+ .52	.17	<.025	8
"	2000	Carbachol	10	+ .43	.20	<.10	8
Bullfrog							
Histamine	20	Carbachol	1	+ .28	.18	<.30	4

The difference, $\Delta\bar{x}$, for paired portions of a mucosa was computed from [($\bar{x}$ of A post-treatment periods—$\bar{x}$ of A pre-treatment periods)—($\bar{X}$ of B post-treatment periods—$\bar{x}$ of B pre-treatment periods)]. P based upon Student's distribution of t.

histamine content of dogfish gastric mucosa. After sacrifice, mucosae from 2 fish were homogenized in 4 M perchloric acid. Bioassay yielded values of 2.5 and 8.7 μg/g; comparable to those reported for the bullfrog gastric mucosa(5).

Discussion. Studies such as this and that of Davidson et al(5) require that if histamine is the transmitter for gastrin and cholinergic excitation, one of three conditions must obtain: a) one molecule of excitant releases many molecules of histamine, b) excitation is achieved by the intracellular release of histamine, or c) modes of transmission are substantially different in different vertebrates. Though I do not know the lethal limit for histamine in the dogfish, a stipulation that the interstitial concentration of histamine must attain a value of 2 mM for optimal effect would place a considerable demand upon the mucosal stores and synthesis of histamine. If the intervention of histamine is intracellular then much of the indirect reasoning in favor of a role for histamine should be set aside. But two observations remain: histamine is present in the gastric mucosa and, under some circumstances, excitation of gastric secretion is accompanied by a reduction of the mucosal content. The finding of histamine in gastric juice deserves further comment. We have previously pointed out that bases do find their way into gastric juice(8). The determination of gastric juice histamine might be viewed as an approach to estimating the extracellular concentration of histamine. It is also sensible that the initiation of acid secretion creates a sink into which histamine will flow.

The delay of 120 minutes in reaching the maximal response of the dogfish gastric mucosa to histamine is evidence that the relative refractoriness is not due to the local destruction of histamine. The delay can be considered as suggestive of an intracellular locus of action for histamine. Unfortunately we were not able to test this implication by determining if a higher extracellular pH would speed the onset of action and lower the threshold for histamine.

The present study does not clarify the comparative insensitivity of the dogfish gastric mucosa to hog gastrin. This could perhaps be explained by the relative insensitivity to extracellular histamine but is perhaps more likely due to a species differentiation of the polypeptide gastrin, presuming that a gastrin hormone is liberated by the antrum of elasmobranchs.

Summary. The dogfish gastric mucosa is relatively refractory to histamine. An interstitial concentration of 2 mM is required to insure maximal secretion of acid. This concentration is high enough to suggest that if cholinergic excitation is mediated by the release of histamine, histamine is released within the oxyntic cells of this species. The dogfish mucosa is much less responsive to hog gastrin than is the bullfrog gastric mucosa.

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Diuretic Action of Glycerol in Dogs.* (31880)

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Diuretic drugs that inhibit the renal tubular reabsorption of sodium are often ineffective in edematous patients with hyponatremia. Drugs that could preferentially inhibit the renal tubular reabsorption of water would therefore be useful. Osmotic diuretics such as mannitol and urea cause relatively greater urinary excretion of water than of sodium (1,2) but have certain practical disadvantages clinically.

Glycerol has been given orally to treat glaucoma(3-6) and cerebral edema(6,7). Oral and intravenous glycerol have been noted to cause a diuresis(6-9), the characteristics of which have not previously been reported. Experiments were performed in dogs to characterize the nature and magnitude of this effect of glycerol, prior to its trial as a diuretic in humans.

Methods. Subjects. Twelve female mongrel dogs weighing between 11 and 19 kg were anesthetized with iv pentobarbital 30 mg per kg and then maintained with small supplementary doses. Infusions were given through a polyethylene catheter advanced into the inferior vena cava. Urine was collected from an indwelling catheter; collection periods were initiated and terminated by the instillation and aspiration of air. Heparinized blood was obtained at the midpoint of each clearance period from an indwelling jugular needle.

Glomerular filtration rate (GFR) was usually estimated by the clearance of inulin; the clearance of exogenous creatinine was estimated in those experiments in which fructose was given. Equilibrating infusions were given for between 80 and 150 minutes prior to the control periods in order to assure stability of sodium excretion ($U_{Na}V$). Urine flow (V) was either stable or decreasing slightly during the 2-3 ten minute control periods. Four dogs received constant sustaining infusions of 154 mM NaCl and 4 others were given infusions of 38-51 mM NaCl with 1.5-3.3% glucose or fructose.

Meralluride (0.75 ml) was given intravenously to 3 dogs prior to glycerol, and to 2 others during glycerol diuresis. Another dog received pitressin before glycerol, and one other during glycerol diuresis. The pitressin-treated dogs and 2 of those that received meralluride were given sustaining infusions of 77 mM NaCl with 4-5% mannitol.

Glycerol was added to the sustaining infusions in concentrations of 16-25% (v/v) and was given at a rate of 0.67-1.0 ml/min (0.7-1.3 mOsm/k/min) for 40-60 minutes. Urine collections were restarted 3 to 18 minutes after starting glycerol. Neither hemolysis nor glycosuria were observed.

Analytical. Inulin and creatinine were measured by methods previously reported(10). Sodium and potassium concentrations were measured with an Autoanalyzer, chloride concentrations with a Cotlove chloridometer, and osmolalities with a Fiske osmometer.

Plasma volumes were estimated with T-

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