with a significantly increased plasma cholesterol ($p < 0.0005$ in the castrated rats and $p < 0.005$ in the noncastrated rats). The administration of testosterone to castrated male rats resulted in lower levels of plasma cholesterol, but the difference was not significant.

Our results are consistent with other recent reports in other experimental animals. Kudzma et al.[9] administered diethylstilbesterol to 5-day-old chicks of undetermined sex and found an increase in plasma cholesterol. Neilson and Simpson[10] administered a single dose of diethylstilbesterol to male noncastrated turkeys and found an increase in plasma cholesterol. Our results conflict with those of Fewster et al. who found that estradiol decreased plasma cholesterol[12]; however their dosage was 1.7 mg estradiol benzoate daily, and the decrease they found with this high dose would be consistent with the biphasic effect described by Uchida et al.[13].

The finding that estrogen was associated with a rise in plasma cholesterol in the presence as well as the absence of naturally occurring testosterone indicates that the estrogen effect was overriding in comparison to the effects of testosterone on plasma cholesterol. These effects of estrogen on plasma cholesterol in male rats would appear to be contrary to the proposed protective role of estrogen in the development of atherosclerosis. In this connection a recent study by Phillips[17] revealed a significantly increased plasma estrogen level in a sample of 15 men who had had a myocardial infarction between the ages of 32 and 42 as compared to control subjects. The findings of our study as well as those of others indicate that the role of sex hormones in cardiovascular disease is a very complex one and that probably there are many factors other than lipid metabolism which play a role in the protective effect of estrogen. Thus the mechanism by which estrogen purportedly protects the female remains obscure.

The study reported herein was done in conjunction with a long-term study in our laboratory on the effect of sex hormones on vascular connective tissue[18]. This long-term study, as well as an earlier one[19], indicates that estrogen decreases collagen and elastin accumulation in rat aorta. Since collagen and elastin are increased in atherosclerotic lesions, the effect of estrogen to decrease their accumulation could be hypothesized to be 1 factor contributing to the protective role of estrogen in the female.

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Gastrin stimulated H^+ secretion in amphibian gastric mucosa: Effect of tetrodotoxin[1]

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Summary. The role of neural mechanisms in gastrin stimulated H^+ secretion was studied using amphibian gastric fundic mucosa. Spontaneously secreting mucosae were converted to resting state (zero H^+ secretory rate) using Burimamide. Following removal of burimamide, 3×10^{-6} M tetrodotoxin did not block gastrin stimulation of H^+ secretion indicating that neural mechanisms are not required.

A strong interrelationship between neural and hormonal control of gastrointestinal secretory and motor function has been suggested by in vivo and in vitro experiments. In intestinal muscle tetrodotoxin blocks the effect of gastrin on acetylcholine release and motor activity[2] suggesting a similar mechanism may exist for secretory stimulation by gastrin. This paper reports studies into the requirement of neural mediation for gastrin stimulation of gastric H^+ secretion. Tetrodotoxin, a compound which blocks neural Na^+ channels, and the non-secreting bullfrog mucosa form the model system for this investigation.

Materials and methods. All chemicals used in preparation of solutions were reagent grade, Mallinkrodt Chemical Co.; pentagastrin was a gift from Ayerst Pharmaceutical Co., and tetrodotoxin was purchased from Sigma Chemical Co. Potency of the tetrodotoxin was confirmed by lethality in rat following i.p. injection.

Adult bullfrogs were killed by severing the spinal cord in the neck and pithing. The abdomen was quickly opened and the gastric fundus removed. It was placed in amphibian Ringer's solution bubbled with 95% O_2/5% CO_2, and the outer muscle layer was stripped from the underlying mucosa. The mucosal tube was opened, stretched, and mounted between the halves of a lucite chamber. The composition of solutions bathing the mucosal and serosal surfaces have been reported previously[3]. The solutions were

circulated from external reservoirs by 95% O_2/5% CO_2 gas lift system. Transmucosal pd and resistance were quantitated as previously reported[3,4]. H^+ secretion was measured by the pH stat technique at a mucosal pH of 4.5. Tetrodotoxin, lyophilized in sodium citrate, was dissolved in H_2O. Pentagastrin was prepared according to the recommendation of the manufacturer. All additions were made in the serosal solution; concentrations given are those in the serosal solution.

Results. Frog mucosae, when initially mounted, are almost invariably secreting. In these experiments the H^+ rate after initial mounting and equilibration in the chamber was 3.83 μmoles/cm²/h. 5-butyl-methylthiourea (burimamide) 10^{-4}-10^{-3} M routinely produced a zero H^+ rate as is shown in the figure.

The protocol followed was a) burimamide inhibition of H^+ secretion, b) removal of burimamide by several changes of the serosal solution, c) addition of 3.0×10^{-6} M tetrodotoxin (serosal) and d) addition of 3.3×10^{-6} M pentagastrin to the serosal solution. The figure shows the results of a typical experiment and the table gives the results of 5 consecutive experiments.

Burimamide produced a rise of transmucosal pd and resistance. These changes coincided with reduction of H^+ rate. If a 2nd addition of burimamide was used, no additional change of pd or resistance was seen. Tetrodotoxin added to either the spontaneously secreting or resting mucosa had no effect on H^+ secretion. Tetrodotoxin produced no consistent or significant change of potential difference or resistance.

Pentagastrin added in the continued presence of 3.0×10^{-6} M tetrodotoxin was effective as a stimulant of gastric H^+ secretion. The pd decreased and resistance declined. In 5 experiments (table) the H^+ secretion rose from 0 to 5.22 μmoles/cm²/h. Thus in the presence of a substance which blocks neural Na^+ channels, pentagastrin was effective in stimulating H^+ secretion.

Discussion. Gastric H^+ secretion demonstrates strong interdependence among the secretory stimuli. Vagotomy in dog and man causes inhibition of gastrin[5,6] stimulated secretion which can be reversed by cholinergic drugs[7,8]. We previously demonstrated in the necturus mucosae[9] and others have confirmed in dog[10] and man[11] that the new H_2 antagonists blocks not only histamine-stimulated secretion but also gastrin and urecholine or 2-deoxy-D-glucose stimulated secretion.

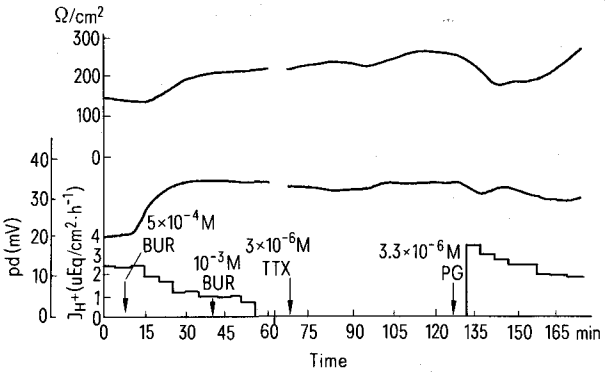
Interrelation of cholinergic mechanisms and gastrin have been studied in lower esophageal sphincter[12], gastric[13] and intestinal smooth muscle[2]. Vizi et al.[14] studied preparations of guinea-pig intestinal muscle containing Auerbach's plexus; gastrin dose responsively released acetylcholine from neurones of Auerbach's plexus. Contraction of esophageal[12], gastric[12] and intestinal smooth muscle[2] in response to gastrin, cholecystokinin and caerulein was blocked by tetrodotoxin (3×10^{-6} M); acetylcholine remained effective. Inhibitors of nicotinic receptors were ineffective in preventing gastrin stimulated contraction.

Urushibara et al.[15] previously found no inhibition of the gastrin stimulated bullfrog fundus by cocaine, morphine, tetrodotoxin, succinyl choline, and dimethyl tubocurarine but the high rate of basal secretion precludes a firm conclusion regarding the role of neural mechanisms.

Our studies are in agreement with those of Davidson et al.[16] who concluded that failure of lidocaine benzyl chloride to block gastrin stimulation of H^+ secretion indicated the neural plexus was not involved. Thus our studies indicate that intramucosal neural mechanisms are not required for gastrin stimulation of H^+ secretion in contrast to the role of neural mechanisms in gastrin stimulated motor activity.

Experiment	H^+ Rates Basal J_{H^+} (μmoles/cm² · h¹)	Pentagastrin-stimulated J_{H^+} (μmoles/cm² · h¹)
1	5.00	7.49
2	4.70	5.09
3	2.69	4.32
4	3.94	3.65
5	2.83	5.57
$\bar{X}$	3.83	5.22
SE	0.47	0.65

Basal J_{H^+} is the H^+ secretory rate after initial equilibration of the mucosa in the chamber before addition of burimamide. Pentagastrin stimulated J_{H^+} is the H^+ secretory rate elicited by pentagastrin in the presence of 3×10^{-6} M tetrodotoxin.



The bullfrog stomach when mounted is secreting H^+. Following burimamide (BUR) inhibition and removal, tetrodotoxin (TTX) is ineffective in preventing pentagastrin (PG) stimulation of H^+ secretion.

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