

THE INHIBITORY EFFECT OF STILBOESTROL ON GASTRIC SECRETION IN CATS

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It was observed by Nakai (1925), Artz (1926), and Mason (1931), that during pregnancy there is a definite diminution and even an absence of free hydrochloric acid in the gastric juice. According to Artz (1930) 29 out of 50 pregnant women lack free hydrochloric acid during the first trimester. Strauss and Castle (1932) while making observations on anaemia of pregnancy noted that 75 per cent of pregnant women did not secrete normal amounts of free hydrochloric acid or pepsin during more than half of the period of pregnancy. Way (1945) made similar observations and concluded that the secretion is low during pregnancy, rising in the last weeks and rising sharply after delivery. Strauss and Castle (1932) also showed that the gastric response to histamine was greatly diminished during pregnancy.

It is known that oestrogenic activity is increased during pregnancy and blood oestrogen concentration is raised. The possibility that this increase in the concentration of oestrogen in the blood may be directly related to, or even the cause of, the depression of gastric secretion during pregnancy made it worth while to study the action of oestrogens on gastric secretion experimentally. A previous attempt to demonstrate such an action was made by Atkinson and Ivy (1938) on two dogs with Pavlov pouches, using intramuscular injections of 2,000 units theelin daily. They found no effect on the secretion of gastric juice or acidity, but as pointed out by Culmer, Atkinson, and Ivy (1939) the doses of theelin used were low compared with the amounts of oestrogens produced during pregnancy.

METHODS

The cats were anaesthetized with pentobarbitone sodium. Gastric juice in response to histamine was collected by means of a cannula according to the method described by Roth and Ivy (1944). The oesophagus was tied in the neck and the duodenum was also ligated about three quarters of an inch beyond the pylorus proximal to the entry of the bile duct. The cannula was passed into the stomach through an incision in the duodenum. A solution of histamine acid phosphate was infused intravenously so that the animal received 15 μ g. of histamine base in 0.5 ml. saline per minute.

Observations were made on normal male and female cats and on cats treated with stilboestrol dipropionate (later referred to as stilboestrol) in oil, 0.1 to 0.25 mg. being given intramuscularly to each cat every day for a period of 6–15 days.

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Secretion was collected at ten minute intervals for periods up to three hours, by which time a steady secretion rate had been reached. Free and total acidity were determined by titration of juice with 0.05N-NaOH, thymol blue being used as indicator. The secretory responses of the normal and treated groups of cats were compared in terms of the steady rates of secretion of juice and of the concentration of free hydrochloric acid during that period.

With the doses of stilboestrol used, the animals' appetites usually were good until the last few days on the larger doses. Except in males given 0.1 mg. daily, there was evidence of oestrogenic effects such as enlargement of the mammary glands in both sexes and uterine hypertrophy in the females.

RESULTS

Sex difference in response of normal cats.—The average responses of groups of male and female cats to the same rate of histamine infusion are shown in Table I

TABLE I

SEX DIFFERENCE IN MEAN GASTRIC SECRETORY RESPONSE TO 15 μ G. HISTAMINE PER MIN. IN CATS
Number of animals in parentheses

	Males	Females	P
Volume of juice secreted in 10 min. (ml.) ..	3.24 ± 0.31 (13)	1.74 ± 0.22 (13)	<0.001
Average concentration of free HCl (mm.) ..	125 ± 6.5 (10)	105 ± 5.5 (11)	$0.05 > P > 0.02$
Average rate of secretion of free HCl (mm./10 min.)	377 ± 38 (10)	196 ± 26.5 (11)	<0.01

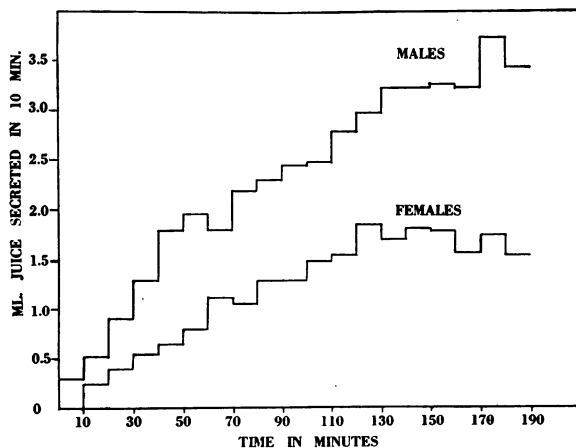


FIG. 1.—Graph showing sex difference in the average secretion of gastric juice in normal cats in response to continuous histamine infusion given intravenously at the rate of 15 μ g. per min. starting at 0. Abscissa, time in min.; ordinate, ml. juice secreted in 10 min.

and Fig. 1. It can be seen that there is a significant difference between the responses of the two sexes. The average secretion rate of juice in female cats was about 55 per cent of that in males and the difference is statistically highly significant ($P < 0.001$).

TABLE II

EFFECT OF STILBOESTROL TREATMENT ON GASTRIC SECRETORY RESPONSE OF CATS TO 15 μ G.
HISTAMINE PER MIN. I.V.I.

Number of animals in parentheses

	Average volume of juice (ml.) secreted in 10 min.		P Between normals and treated
	Males	Females	
Normal	3.24 \pm 0.31 (13)	1.74 \pm 0.22 (13)	
Stilboestrol 0.1 mg. daily 10-14 days.. ..	2.46 \pm 0.09 (4)	0.95 \pm 0.16 (6)	0.2 0.02 <P < 0.05
Stilboestrol 0.2 mg. daily 9-14 days	(0.43) (1)	0.77 \pm 0.16 (5)	0.02 <P < 0.05
Stilboestrol 0.25 mg. daily 6-14 days.. ..	0.45 \pm 0.10 (4)	0.49 \pm 0.07 (5)	<0.001 <0.01

Stilboestrol and the secretion of juice.—Cats treated with stilboestrol were given either 0.1, 0.2, or 0.25 mg. daily for periods of from 6-15 days. The results are presented in Table II and Figs. 2 and 3. In male cats there was no significant reduction in the rate of secretion of juice with the smallest dose of 0.1 mg. In one male

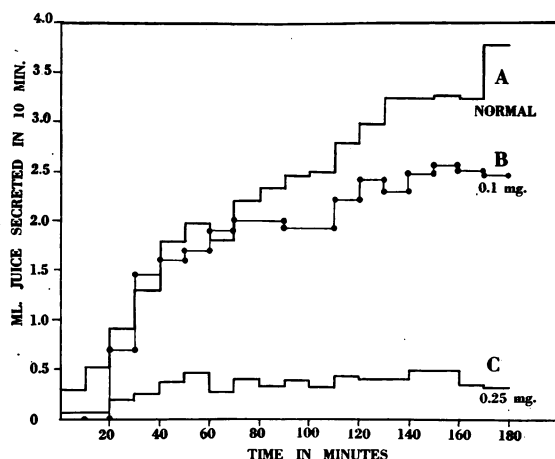
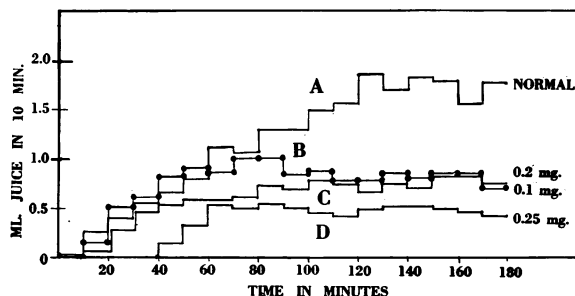


FIG. 2.—Effect of stilboestrol treatment. Average secretion of gastric juice in *male* cats, given 15 μ G. histamine per min. intravenously from 0. (A) 13 normal cats. (B) 4 cats given 0.1 mg. stilboestrol daily for 6-15 days. (C) 4 cats given 0.25 mg. stilboestrol daily for 6-15 days.

FIG. 3.—Effect of stilboestrol treatment. Average secretion of gastric juice in *female* cats. (A) 13 normal cats. (B) 6 cats given 0.1 mg. stilboestrol daily for 10-14 days. (C) 5 cats given 0.2 mg. stilboestrol daily for 9-14 days. (D) 5 cats given 0.25 mg. stilboestrol daily for 6-10 days.



given 0.2 mg. daily, the secretory response was lower than normal, while the dose of 0.25 mg. caused a very significant fall. In female cats, even the smallest dose of 0.1 mg. caused a very significant reduction in the secretion of juice and the effect was greater with the bigger doses.

Stilboestrol and secretion of acid.—In cats treated with stilboestrol, in addition to a reduction in the rate of secretion of gastric juice, there was a fall in the concentration of free hydrochloric acid in the juice and thus an even more pronounced fall in the rate of secretion of the acid (Table III). In females receiving 0.1 mg.

TABLE III
EFFECT OF STILBOESTROL ON AVERAGE CONCENTRATION AND AVERAGE RATE OF SECRETION OF FREE HYDROCHLORIC ACID IN GASTRIC JUICE OF CATS GIVEN 15 μ G. HISTAMINE PER MINUTE INTRAVENOUSLY
Number of animals in parentheses

		Males				Females			
		Average concn. of free HCl mm.	P	Average rate of secretion of free HCl mm. per 10 min.	P	Average concn. of free HCl mm.	P	Average rate of secretion of free HCl mm. per 10 min.	P
Normal	125 \pm 6.5 (10)	—	377 \pm 38 (10)	—	105 \pm 5.5 (11)	—	196 \pm 26.5 (18)	—
Stilboestrol	0.1 mg. daily	92.5 \pm 22 (4)	<0.10	227 \pm 53 (4)	<0.05	47 \pm 15.5 (6)	<0.001	53 \pm 19 (6)	<0.01
Stilboestrol	0.2 mg. daily	—	—	—	—	54 \pm 27.5 (5)	<0.05	67 \pm 39 (5)	<0.02
Stilboestrol	0.25 mg. daily	14.5 \pm 16.5 (4)	<0.001	10 \pm 10 (4)	<0.001	15.5 \pm 9.5 (5)	<0.001	9 \pm 6.5 (5)	<0.001

stilboestrol daily, the average concentration of free hydrochloric acid was 47 ± 15.5 mm., compared with 105 ± 5.5 mm. in normal females. The rate of acid secretion in the treated females was only 53 millimol. in 10 minutes compared with 227 in normal females. The apparent fall in acid concentration with this small dose in males is not significant ($P < 0.10$), but the average rate of secretion of hydrochloric acid, 227 millimol. in 10 minutes, is significantly less than in normals (377 millimol. in 10 minutes, $P < 0.05$).

Duration of treatment.—The average figures in Table II were obtained from cats receiving stilboestrol for 6–15 days. The detailed figures in our protocols, which do not appear in the Table, show that there is a tendency for the effect of stilboestrol to be more pronounced as the duration of treatment increases.

Single intravenous injections of oestrone (Menformon or Theelin) or of stilboestrol in anaesthetized untreated cats do not alter the rate of secretion of gastric juice in response to histamine infusion.

Similarly, two cats treated with the large dose of 4 mg. stilboestrol daily for 2 and 4 days respectively did not show any significant fall in secretory rate compared with normal cats.

DISCUSSION

The striking sex difference in the gastric secretory response towards histamine may well be due to the different gonadal secretions in the two sexes. The average 10 minute secretion being less in the females under identical conditions suggests

that female hormones depress the secretory functions of the gastric glands. Probably for the same reason, a small dose of stilboestrol is not effective in males, but causes depression of the secretion of juice in females. The fact that the 0.1 mg. dose of stilboestrol had no effect on gastric secretion in males and did not cause any obvious mammary enlargement, while doses effective in reducing gastric secretion also had obvious oestrogenic effects, may indicate that the secretory depressant action parallels oestrogenic activity. Some preliminary experiments show that female cats have an exaggerated gastric secretory response to histamine when tested about four weeks after bilateral ovariectomy. The drug is not effective in acute experiments, and the magnitude of the response in the chronic experiments appears to depend not only on the dose but also on the duration of treatment.

Some possible explanations of the reduced secretory response after stilboestrol may be considered. The effects may result from changes in the gastric mucosa. Nasio (1946) observed that there was macroscopic hypertrophy of the gastric mucosa in dogs which he protected against experimental cinchophen ulcers with stilboestrol dipropionate administered for long periods. It was noticed that the stomachs of our treated cats, especially those receiving 0.2 or 0.25 mg. stilboestrol daily for 10 days or more and whose rate of secretion of gastric juice was much reduced, showed mucosal thickening and loss of rugae. Histological examination of these stomachs did not show any consistent changes compared with stomachs from untreated cats whose response to histamine infusion had been tested. In particular, there were no differences in the appearance or distribution of parietal cells. There was considerable variation in the amounts of epithelial and glandular tissue of the mucosa in both treated and untreated cats.

In view of the recent work of Case and Dickens (1948) and of Quastel and Hochster (1949) which indicates an action of oestrogenic hormones, including stilboestrol, on cell enzyme systems, a direct action on the parietal cells seems a very probable explanation for the observed effects of stilboestrol on gastric secretion.

It is possible that the effect may be exerted indirectly through other endocrine glands, most probably the pituitary and the adrenal glands; we have as yet no experimental evidence about this.

While studying the influence of endocrines on the synthesis of serum cholinesterase in rats, Sawyer and Everett (1946) observed that serum enzyme levels are increased in conditions in which sustained high oestrogen levels are known, such as during the last half of pregnancy or in a state of pseudo-pregnancy induced by injecting oestrogen. Everett and Sawyer (1946) observed that castration of female rats resulted in an immediate rapid decline of the serum cholinesterase and that oestrogen administration to castrated rats of both sexes was followed by elevation of serum cholinesterase. As acetylcholine may play a part in the production of gastric juice (Babkin, 1944) a rise in cholinesterase activity was thought to be another possible factor in the inhibition of gastric secretion in stilboestrol treated cats. Estimation of specific and non-specific cholinesterase during these experiments has shown that with the dosages we have used in cats, there is no significant change in the plasma cholinesterase activity (Davies and Ojha, 1950). This finding does not exclude the further possibility that there may be a change in cholinesterase activity in the gastric mucosal cells.

SUMMARY

1. There is a striking difference between the average gastric secretory responses of groups of male and female cats to continuous infusion of histamine during pentobarbitone anaesthesia.

2. Daily intramuscular injection of stilboestrol for 6–15 days reduces significantly the average secretory response to histamine; males are less sensitive than females to the action of small doses.

3. Larger daily doses of stilboestrol may inhibit almost completely the secretion of hydrochloric acid by the cat stomach, in response to histamine.

4. Neither stilboestrol nor aqueous preparations of oestrone had any inhibitory effect on the gastric secretory response to histamine when large single doses were injected intravenously in anaesthetized cats.

5. Possible explanations of the effect are discussed. It is probably due to a direct action of stilboestrol on the parietal cell.

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