Effect of 4-Bromo-3-hydroxybenzyloxyamine (Brocresine) on Gastric Secretion in Pouch Dogs[1] (34181)

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The title compound is a potent inhibitor of both the specific histidine decarboxylase and the nonspecific aromatic L-amino acid decarboxylase (1). Levine *et al.* (2) demonstrated that administration of this drug to rats caused rapid depletion of the histamine pool in the tissues. This resulted (3) in inhibition of gastric secretion in this animal, an inhibition which did not counteract the effects of exogenous (administered) histamine, but did inhibit response to exogenous gastrin. This, it was concluded, provided strong support for the hypothesis that histamine is the common mediator for stimuli of gastric secretion in the rat. Levine (4) concluded from work with Brocresine in a patient with Zollinger-Ellison Syndrome (5), who showed no significant change in the response to administered histamine, that histamine may mediate the effects of gastrin on gastric secretion in man.

The validity of this hypothesis, as generally true for all species, seems to be more believed than proved (6). That there are substantial doubts regarding its truth has also been noted, among others, by Davenport (7). Even for the rat, results have been presented (8) which seem to indicate that vagal stimulation uses, at least in part, a mechanism other than histamine release. We felt that an examination of the effect of Brocresine on gastric secretion in other species might shed light on this problem which has "formidable obstacles" (6) in the way of its solution. Accordingly we have investigated the gastric secretory response, during treatment with the drug, of two kinds of pouch dog, to administered histamine, to porcine gastrin, and to synthetic pentagastrin. We have also measured the secretory response to feeding in dogs with innervated pouches before, during, and after cessation of treatment of these animals with Brocresine.

Materials and Methods. I. Experimental preparations. Eleven mongrel dogs weighing from 14 to 18 kg were used. Seven had denervated (Heidenhain) pouches and 4 had innervated (Pavlov) pouches. The latter were constructed by the modified technic of Gregory *et al.* (9), and the integrity of vagal innervation was confirmed by a positive secretory response to insulin from all 4 pouches. Dogs used on more than one of the experiments, described below, were rested at least 2 wks between experiments.

The dogs were fed a daily meal at 10:30 a.m. consisting of 10 oz of "Friskies," 6 oz of freshly cooked horse meat, and 0.5 cup of evaporated milk. Electrolyte balance was maintained by supplemental salt (1 tsp) in each feeding.

II. Secretory stimulants. Histamine, as the phosphate; synthetic gastrin pentapeptide (ICI-50,123) which is the *N*-*t*-butyloxycarbonyl-β-alanyl derivative of the naturally occurring terminal tetrapepide of gastrin; and natural porcine gastrin were used to stimulate secretory responses. Dosages were as indicated in the Tables. The gastrin was prepared by a modification of the Gregory and Tracy method (10) which has consistently given us good yields of highly active material as follows:

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Porcine antral mucosal strips (2–3 cm wide × 15 cm long), stripped at the abattoir, are placed in boiling water (25 strips/liter) within 2 hr of slaughter, boiled for 10 min, removed and placed in fresh boiling water and boiled for 45 min. The strips are then removed and discarded. To the solution enough normal NaOH is added to bring the pH to 11.5. The mixture is stirred for 15 min and then refrigerated (4°) overnight. Fat is skimmed off and the pH is lowered from 11.5 to 7.0 with HCl, with continuous stirring until the pH is constant. After centrifugation the supernatant is filtered through glass wool and the pH further lowered from 7.0 to 4.0. This is centrifuged, and the precipitate is washed with acetone (3 times) and ether, and air dried. From 150 strips, 5–6 g is obtained at this state. The material is active in our Heidenhain pouch dogs in single subcutaneous doses of 2 to 5 mg and is histamine-free.

The foregoing is suspended in isopropanol (3 g/500 ml), stirred for 2 hr, centrifuged and the precipitate is washed with ether (90% recovery). This (1 g) is dissolved in 20 ml of 0.04 *M* NH_4HCO_3 solution and the pH is brought to 10.7 with NH_4OH with stirring. Hyflo-supercel (5 g) is stirred in and the suspension is centrifuged and the supernatant decanted off. The Hyflo-supercel is washed with water, centrifuged, and the supernatant is added to the previous supernatant. This is repeated 5 times. All supernatants are combined and filtered through Whatman no. 41 and then no. 42 filter papers, with gentle suction. The filtrate is lyophilized to give 0.8 g.

The latter (400 mg) is dissolved in 0.04 *M* NH_4HCO_3 and the pH is raised to 10.7. This solution is passed through Sephadex, G-50. The active fraction (9–10 mg), which elicits a good response in our Heidenhain pouch dogs at 0.2–0.3 mg per single subcutaneous dose, is again dissolved in NH_4HCO_3 (pH 10.7) and passed through Sephadex G-100. The active fraction from this step, amounting to 4 mg causes a good response in the dogs when 50–100 μg is injected as a single subcutaneous dose. The total yield from 150 strips is ~ 16 mg.

III. Experiments. The dogs were fasted for 24 hr prior to each test. In all tests the dogs were placed in Pavlov stands and basal secretions were collected for 0.5–1 hr. In the dogs with innervated pouches the tests were not started until the Pavlov response had subsided and the basal secretion had stabilized, usually at 11:00 a.m. During all tests the pouch secretions were collected at 15-min intervals. Each test lasted 4–5 hr, following which the dogs were given 500 ml of lactated Ringer's solution intravenously to replace electrolytes.

Before any treatment with Brocresine had been started, histamine and gastrin "staircase" responses were run on each dog in order to determine a (standard) submaximal dose of each at approximately the same level for each dog. These doses were used as the control injections for each experiment.

Titrations. The total volume of each collection was recorded and 1-ml aliquots were titrated with 0.01 *N* NaOH to pH 7.0, using a Beckman expanded scale pH meter. Total milliequivalents (as HCl) were calculated for each collection period.

Acute tests. At 2-week intervals a group of 4 Heidenhain and 1 Pavlov pouch dogs were tested for any change in their response to a standard (as defined above) dose of histamine or gastrin when given Brocresine as follows: Four capsules (40 mg/kg) were fed orally at intervals beginning with 1 in the morning and 1 in the late afternoon, on the day before the test, followed by 1 just before the start of the test and the fourth when the secretory response had reached a plateau. The total dose was thus 160 mg/kg (Tables IA, B).

Chronic tests. A. At approximately monthly intervals, 3 Heidenhain pouch dogs were tested for any change in their response to histamine or gastrin while under chronic treatment with Brocresine. The drug was given orally at a level of 50 mg/kg/day for 3 weeks, then at 75 mg/kg/day for 4 weeks, followed by 100 mg/kg/day for 3 days at which time all of the dogs showed definite signs of sickness. The dosage was immediately stopped and resumed 4 days later at 75 mg/kg/day and held at this level for 15

TABLE IA. Acute Tests on Four Denervated Pouch Dogs: Stimulants Alone Compared with Stimulants in the Drug-Treated Dog.

Dog no.	Stimulant or stimulant plus Brocresine[a]	Vol of gastric secretion in 4-hr period (ml) Total	Range	H^+ (meq) Total	Range	Percentage increase (+) or decrease (—) with use of drug; ( ), no. of expts.
BS-2	Histamine[b] (H)	90.7	± 13.0	11.73	± 1.0	(3)
	H + Brocresine (B)	100.4		13.03		(1) +11
BS-6	H	167.9	± 9.0	23.37	± 1.9	(4)
	H + B	190.0	± 7.0	26.16	± 3.0	(2) +11
BS-7	H	107.7	± 10.0	13.79	± 2.1	(3)
	H + B	112.1	± 4.0	13.99	± 0.5	(2) +1
BS-8	H	69.0	± 3.0	7.85		(1)
	H + B	70.3	± 1.7	8.18	± 0.05	(2) +10.4
BS-2	Gastrin[b] (G)	54.2	± 13.0	6.10	± 1.6	(3)
	G + B	65.0	± 7.0	6.62	± 2.4	(2) +10.8
BS-6	G	21.3	± 0.6	1.61	± 0.13	(2)
	G + B	17.7	± 4.0	1.53	± 0.5	(2) —4
BS-7	G	53.1	± 7.4	5.80	± 0.8	(3)
	G + B	65.8	± 0.4	6.76	± 0.9	(2) +11.5
BS-8	G	21.9		1.73		(1)
	G + B	21.8		1.66		(1) —4

[a] Brocresine, 160 mg/kg/24 hr (see text).

[b] Continuous intravenous standard submaximal dose (see text).

weeks. The total time on the drug was 23 weeks (Table II). It is apparent (e.g., dog No. BS-3) from this data that some pouch dogs have maximal responses to gastrin which are very much lower than such responses in other dogs relative to their maximal response to histamine. There may be subtle mechanical differences in the making of the pouch which affects the response to gastrin more than to histamine, thus indirectly supporting the hypothesis that gastric stimulation by gastrin is mediated differently, in the dog, than stimulation by histamine.

B. Two innervated pouch dogs were tested similarly, but at a constant dose level of 75 mg/kg/day over a 10-week period (Table III).

Feeding tests. Two innervated pouch dogs were given a 10-week course of Brocresine daily (75 mg/kg) with weekly feeding tests

TABLE IB. Acute Tests on an Innervated Pouch Dog: Stimulants Alone Compared with Stimulants in the Drug-Treated Dog.

Dog no.	Stimulant or stimulant plus Brocresine[a]	Vol of gastric secretion in 4-hr period (ml) Total	Range	H^+ (meq) Total	Range	Percentage increase (+) or decrease (—) with use of drug; ( ), no. of expts.
P-5	Histamine[b] (H)	256.8	± 6.1	39.15	± 1.1	(2)
	H + Brocresine (B)	232.6	± 28.0	33.50	± 2.7	(2) —14
P-5	Gastrin[b] (G)	123.4	± 18.0	16.93	± 1.7	(6)
	G + B	152.4	± 20.0	21.99	± 3.9	(4) +16

[a] Brocresine, 160 mg/kg/24 hr (see text).

[b] Continuous intravenous standard submaximal dose (see text).

TABLE II. Effect of Long-Term Use of Brocresine on Stimulated Gastric Secretion in Three Denervated Pouch Dogs.

Dog no.	Total H^+ (meq/4 hr) Before Brocresine	3 Weeks	12 Weeks	16 Weeks	20 Weeks	23 Weeks	After 4-week post-Brocresine
Response to Histamine,[a] at stated no. of weeks after start of Brocresine administration[b]							
S-14	20.05	26.30	23.87	22.96	21.09		22.50
BS-3	27.15	22.00	23.88	21.00	19.49		25.02
BS-4	25.61	30.73	29.12	30.99	19.14	26.15	—[c]
Response to Gastrin,[a] at stated no. of weeks after start of Brocresine administration[b]		2 Weeks	11 Weeks	15 Weeks	19 Weeks		
S-14	14.19	14.95	11.00	8.14	8.91		7.29
BS-3	1.56	0.71	0.85	1.57	0.90		2.95
BS-4	9.84	14.36	11.70	8.12	10.90		—[c]

[a] Continuous intravenous standard submaximal dose (see text).

[b] See text for dosage schedule. The second elapsed period included a brief overdosage of the drug, and a 4-day rest period followed by 4 weeks at the 75-mg/kg/day level which was kept constant for the rest of the experiment.

[c] Dog died.

for several weeks before the start of Brocresine dosage, and for the duration of the experiment, and with similar periodic tests for 2 months after cessation of the drug regimen. Each individual feeding test was begun at the same time by collecting basal secretion for 1 hr. Then the dogs were given a standard meal consisting of 0.5 can (8 oz) of dog food mixed with 1 can (3.5 oz) of beef liver. The dogs invariably ate all of the food (in ~ 1 min). Secretory output was collected over the next 4 to 5 hr (Fig. 1).

Intravenous and subcutaneous administration of Brocresine. (Fig. 2, 3) Experiments were run on 2 Heidenhain pouch dogs using these 2 additional methods of dosing with the drug during stimulation by gastrin.

Use of ICI-50,123 as a gastric secretory

TABLE III. Effect of Chronic Dosage with Brocresine (B) on Stimulated (H, Histamine; G, Gastrin; P, Gastrin Pentapeptide) Gastric Secretion in Two Innervated Pouch Dogs.

Dog no.	Treatment	No. of expts.	Vol (ml) in 4 hr Av total	Range	H^+ (meq) Av total	Range	Percentage change in acid output
BS-11	H[a] alone	1	142.8		20.20		
	H with B	3[b]	142.1	± 12.0	20.07	± 2.2	
P-5	H alone	3	187.4	± 16.0	26.73	± 4.0	+13
	H with B	2[b]	247.5	± 22.0	34.71	± 3.0	
BS-11	G[a] alone	2	16.2	± 1.8	1.40	± 0.3	+14.3
	G with B	3[b]	26.2	± 3.4	2.63	± 0.5	
P-5	P[a] alone	3	139.4	± 4.0	20.48	± 1.8	—22.3[c]
	P with B	2[b]	129.0	± 28.0	15.92	± 4.0	

[a] Continuous intravenous standard submaximal dose (see text).

[b] These experiments were spaced evenly through the 10-week period at least a week apart.

[c] This dog developed bloody secretions during the latter part of the test which may account for the decrease (with gastrin, but not with histamine).

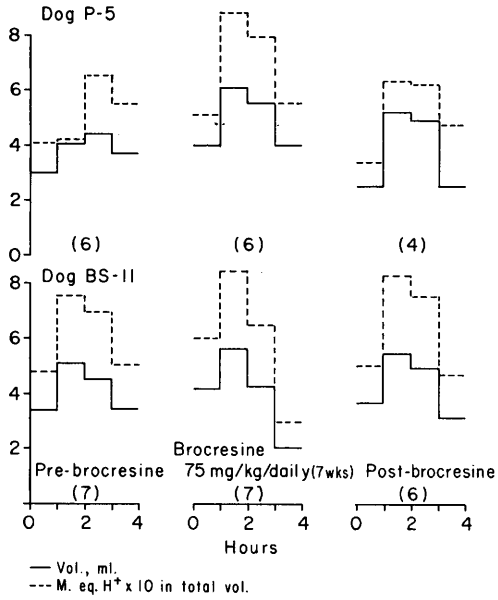

FIG. 1. Average of secretory responses to feeding in two innervated pouch dogs; no. of weekly tests shown in ( ).

stimulant. (Table IV). To compare the effect of Brocresine on response to the gastrin-like synthetic pentapeptide, ICI-50,123, with our previous results using porcine gastrin as a stimulant, we used 3 innervated pouch dogs each of which had been standardized on histamine and gastrin before and after experiments using Brocresine, and which had not been given Brocresine for several weeks. In this test response to a standard dose of the pentapeptide was measured before dosage with the drug, on the sixth day of 7 days of drug use (100 mg/kg/day) and again 7 days after use of the drug had stopped. Histamine

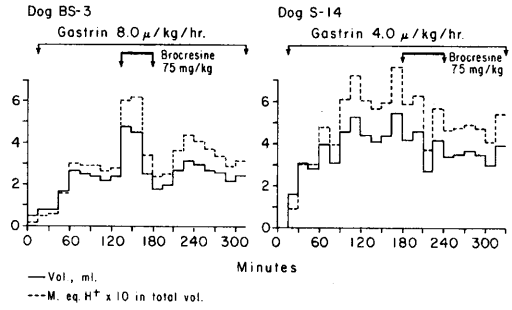

FIG. 2. Gastric secretory response to intravenous Brocresine in two denervated pouch dogs stimulated by gastrin (see [b] Table I).

was also tested in some of these dogs before drug dosage and the day after cessation of drug use.

Results. In the acute experiments with denervated pouch dogs (Table I) there was ~ 10% increase in the total amount of acid secreted following Brocresine dosage and histamine stimulation, as compared with histamine stimulation alone; with gastrin stimulation there was a comparable increase after Brocresine (3 dogs) but a slight decrease (4%) with 1 dog. The 1 innervated pouch dog on this experiment (Table IB) showed some decrease in responses to histamine after Brocresine, but an increase with

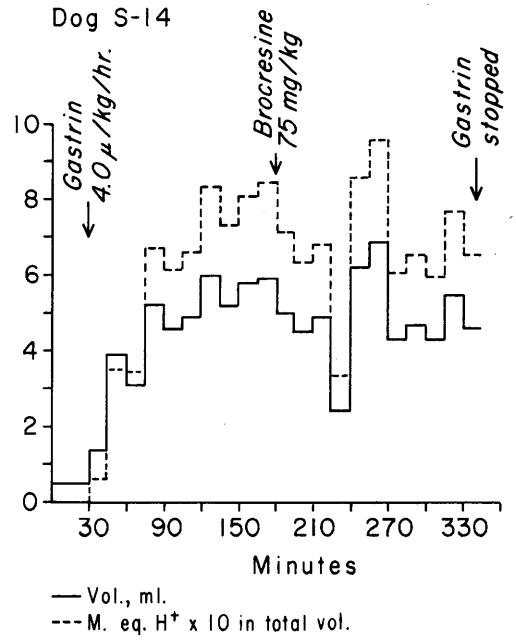

FIG. 3. Gastric secretory response to a subcutaneous injection of Brocresine in a denervated pouch dog stimulated by intravenous gastrin (see [b] Table I).

gastrin, following the drug, in 1 set of experiments, and a small decrease in a later set.

In the chronic experiments, as shown in Table II, over a period of several months of administration of the drug, response to histamine stimulation (3 dogs with denervated pouches) was variable, but with very small variations. Parallel experiments with gastrin showed a decrease in response with 2 dogs and a slight increase with a third. In the

TABLE IV. Effect of Short-Term Use of Brocresine[a] on Gastric Secretion, in Three Innervated Pouch Dogs, Stimulated by Histamine[b] and by Synthetic Gastrin-Like Pentapeptide[b] (ICI-50,123).

	Brocresine							
	Before		Sixth day of adm		1 day after		7 days after	
Dog no.	Vol (ml/4 hr)	H^+ (meq/4 hr)	Vol (ml/4 hr)	H^+ (meq/4 hr)	Vol (ml/4 hr)	H^+ (meq/4 hr)	Vol (ml/4 hr)	H^+ (meq/4 hr)
				Histamine test				
P-5	254.1	35.15			199.2	27.59		
BS-11	142.8	20.20			157.3	22.55		
BS-12	202.5	29.47			227.8	33.34		
				Gastrin pentapeptide tests				
P-5	69.0	8.12	60.0	6.39			68.8	8.27
BS-11	55.6	7.00	66.8	8.51			53.7	6.67
BS-12	163.5	23.22	158.7	22.43			162.5	22.93

[a] Daily for 7 days at 100 mg/kg.
[b] Continuous intravenous standard submaximal dose (see text).

Pavlov (innervated) pouch dogs (Table III), response to histamine was constant in 1 dog and response to gastrin increased (14%) over the course of the experiment. In a second dog, response to histamine increased (13%) and response to the pentapeptide decreased. However, this decrease became apparent at about the time the dog's general condition deteriorated.

Figure 1 shows the results of the feeding tests. There appears to have been no significant change in gastric secretion from either (innervated pouch) dog during the course of the experiment.

Figure 2 shows the response to exogenous gastrin of 2 Heidenhain pouch dogs with administration of Brocresine intravenously during the experiment. Except for a sharp increase (0.5 hr duration) in response by 1 of the dogs, there was no significant change in the response of either pouch. Following subcutaneous administration (Fig. 3) of Brocresine, there was a transient, moderate decrease in response to continuous infusion of gastrin solution, followed by recovery.

Results in the brief chronic experiment shown in Table IV compared with results in Tables I–III, reveal no significant differences between the effect of the drug on stimulation by ICI-50,123 and by porcine gastrin. The response of these innervated pouch dogs was approximately level through the course of the experiment.

Discussion. Under the conditions of this study, Brocresine did not inhibit gastric secretory response of either the Heidenhain or the Pavlov pouches to administered histamine, to gastrin, to synthetic gastrin pentapeptide, or to a standard feeding test. The variations in response of the dogs appear, for the most part, to be no more than would be observed without the use of the drug.

It is apparent that in these experiments the effect of Brocresine in dogs is markedly different from the effect in rats (3). If the results in rats support the concept that histamine is the final mediator of gastric acid stimulation in that species, then our data indicate a species difference in this regard, as supported in preliminary results by Jam-

ieson (11) and Thompson *et al.* (12).

Summary. We studied the effect of Brocresine on the gastric secretory response of denervated and innervated pouch dogs. Brocresine is a potent inhibitor of histidine decarboxylase. It has been shown that in the rat (2, 3) and perhaps in the human (4), this drug inhibits gastric secretory response to administration of exogenous gastrin, but not of exogenous histamine. In the present work we found that responses to exogenous gastrin, or gastrin-like synthetic pentapeptide, or histamine, are not significantly altered by administration of Brocresine in either acute experiments, or over a period of several months of chronic dosage. The responses to feeding in a group of Brocresine-treated innervated pouch dogs were also not significantly altered over a period of several weeks. The results suggest that in the dog histamine may not be the final mediator of all gastric stimulation. This hypothesis is also supported by the fact shown (but not newly-discovered here) that the ratio of maximal response with gastrin to maximal response to histamine varies widely from 1 pouch dog to another. Response of the pouch to gastrin stimulation appears to be much more subject to differences (perhaps subtle) in surgical technique than response to histamine stimulation.

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