Relationship of Mast Cell Population and Endogenous Histamine Concentration in the Canine Stomach.* (32016)

ERNESTO MOLINA, WALLACE P. RITCHIE, JR., DAVID GRIGG, AND OWEN H. WANGENSTEEN
Department of Surgery, University of Minnesota Medical School, Minneapolis

Histamine has been isolated from virtually all mammalian tissues(1,2) and has been reported to exist in particularly high concentrations in the gastrointestinal tract of the dog and man(3,4,5). The intriguing possibility that endogenous histamine may be the final common mediator of gastric acid secretion prompted us to investigate the relationship between histamine concentration and the mast cell content of the fundic, corporal and antral mucost of the canine stomach.

Methods and materials. Under sodium pentobarbital anesthesia (2.6 mg/kg) the complete stomachs from 4 adult mongrel dogs were rapidly excised through a midline abdominal incision. The animals were then sacrificed. Each stomach was opened along the greater curvature and twelve 3 × 3 cm specimens of full thickness gastric wall were obtained from the areas indicated in Fig. 1. Each sample was divided into 2 equal parts for independent histologic examination and histamine assay. Specimens for histological examination were fixed for 12 hours in 4% aqueous lead subacetate and then for an additional 12 hours in 10% formalin. The tissue was then dehydrated in the usual manner and imbedded in paraffin. Sections were cut to 10 μ thickness, stained with 1% toluidine blue and mounted in "Permount".† The advantage of this mounting technique is that the specimens retain their staining characteristics for several months. Quantitation of mast cell populations was carried out by a modification of the technique originally described by Ritchie(6). A square grid measuring .223 mm on a side and divided into 49 squares ordered in 7 rows was placed in the eyepiece of the light microscope. Under 430 magnifications, a "swath" of mucosa, 0.223 mm in width, was quantitated for mast cells in 6 areas on each slide. Care was taken to utilize only those areas which were cut perpendicular to the mucosal surface as evidenced by the fact that the lumina of the glands were apparent throughout their entire course. In addition, the mast cell profile of the mucosa was determined by quantitating the number of cells which fell under each row of squares when the specimen was examined with the grid. The random counting error of this technique was determined by blindly recounting 20 slides after 4-6 weeks and was found to be 11.3%.

The glandular mucosa from the specimen for histamine assay was carefully dissected free from the submucosal and muscular layers and was homogenized in 9 volumes of 0.4 N perchloric acid. The amount of endogenous histamine present in the specimens was then assayed by the fluorometric method of Shore, Burkhalter, and Cohn(7). Results are reported as micrograms per gram of wet tissue. In this laboratory, the standard deviation of the technique, determined by assaying a large number of replicate samples, is ± 3.09 μg.

Results. The histamine content and the average number of mast cells per swath of full thickness mucosa .223 mm in width from the areas of mucosa sampled is indicated in Table I. It is evident that no significant difference exists between the number of cells present in the specimens of fundic, corporal, or antral mucosa. This circumstance is in marked contrast to the concentration of endogenous histamine in these same areas. The histamine content of the fundic and corporal glandular mucosa averaged 133.6 μg/g(± 1.4) while that of the antral glandular mucosa averaged only 73.3 μg/g(± 4.9). This difference was statistically significant to the .05 level of biological confidence.

The mast cell profile of the fundic, corporal

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† Fisher Scientific Co., Fair Lawn, N. J.

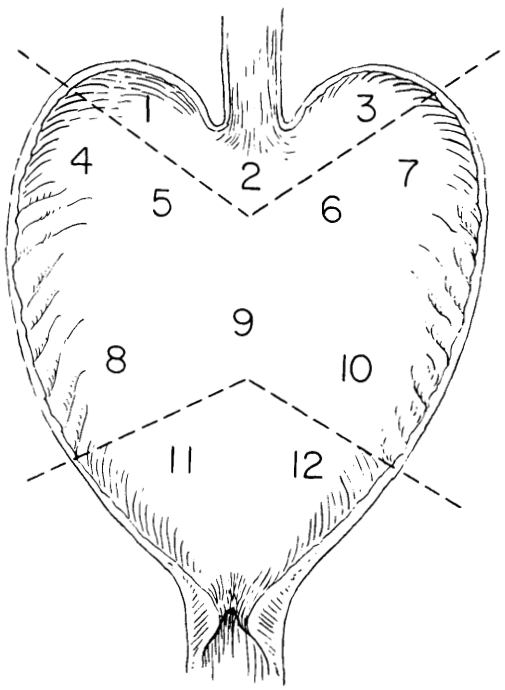

FIG. 1. Geographic distribution of biopsy sites—stomach opened along greater curvature.

and antral mucosa is indicated in Fig. 2. In the fundus and corpus, the greatest concentration of cells occurs principally in the region of the surface epithelium and gastric pits. The greatest number of cells, however, are located in the glandular neck region coincident with the greatest number of parietal cells and with the histamine profile described by Feldberg and Harris(9). While the total number of mast cells per .223 mm swath of full thickness mucosa is essentially the same in antral and corporal mucosa, they are nevertheless most abundant .030 mm to .045 mm deeper in the antral glands than are those of the corpus.

TABLE I. Number of Mast Cells per .223 mm "Swath" of Full Thickness Mucosa and Tissue Histamine Concentration ($\mu g/g$) in the Fundic, Corporal and Antral Mucosa of the Canine Stomach (Average of 4 Dogs).

	Biopsy position	No. mast cells	Tissue histamine
Fundus	1	31.9	130
	2	30.6	134
	3	25.0	139
Corpus	4	26.8	132.5
	5	25.6	129.5
	6	29.1	135
	7	29.0	142.5
	8	23.5	135.5
	9	31.2	125
	10	31.2	133
Antrum	11	30.0	71.5
	12	30.6	75

Discussion. The observation that the fundus and corpus of the canine stomach contain significantly higher levels of endogenous histamine than the antrum is in substantial agreement with the work of Gavin *et al*(8), Feldberg and Harris(9), and Hill and Code (4). Since degranulated mast cells cannot be identified by the technique employed, it is possible that fixation of histamine by mucin following its release from the cell could account for the considerable differences in histamine content noted in these areas. However, assuming that the turnover rate of mast cells is similar throughout the canine stomach, an alternative possibility is that the amount of

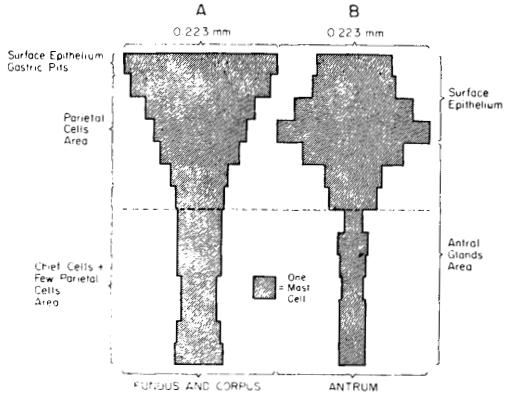

FIG. 2. Mast cell profile of fundic, corporal and antral mucosa.

histamine present in the gastric mucosa of the dog is not critically related to the mast cell population since the mast cell populations of both body and antrum are substantially the same, even though the former contains twice as much histamine as the latter. Support for this view is found in the work of Mongar and Schild(10) who noted that the tissues of the gastrointestinal tract loose only small amounts of histamine when treated with 48/80, a compound which liberates histamine principally from mast cells. It is interesting to hypothesize that there may exist a dual pattern of histamine storage in the gastric mucosa: viz,

the histamine present in the antrum may be bound to mast cells while the major portion of corporal and fundic histamine is more closely associated with the secretory cells themselves, possibly bound by mucins(1).

Summary. The distribution of histamine and mast cells in the fundic, corporal and antral mucosa of the canine stomach was assayed. Twelve areas from each of 4 stomachs were studied. Mast cell profiles of these areas showed the cell population to be similar in all mucosal areas, although the greatest number of cells occurred deeper in the glandular mucosa of the antrum than in the glandular mucosa of the fundus and corpus. In contrast, the amount of histamine in the antral mucosa (73.3 $\pm$ 4.9 μg/g) was roughly one half of that noted in fundic and corporal mucosa (133.6 $\pm$ 1.4 μg/g), a difference which is statistically significant, ($p<.05$). One possible explanation for these observations is that the major portion of corporal and fundic histamine is not associated with mast cells.

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An Excitatory Adrenergic Alpha Receptor Mechanism of Terminal Guinea-Pig Ileum. (32017)

DAVID G. REYNOLDS, GALE E. DEMAREE*, AND MELVIN H. HEIFFER*
Department of Gastroenterology, Division of Medicine, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, D.C.

The action of catecholamines on intestinal smooth muscle is generally taken to be that of inhibition of contractile activity. An exception to this expected and usual action exists in the observation of Munro(1) in which epinephrine is reported to have a definite excitatory action on terminal guinea-pig ileum. This observation was verified in a report by McDougal and West(2). Munro characterized the response to the following extent: it occurs optimally in smaller animals(3); it does not occur in fasted animals(1); it is not antagonized by atropine(1) and may even be potentiated by that agent(3); and is due to direct action on the smooth muscle that possibly involves a receptor surface(1,4).

The general inhibitory action of the catecholamines on intestinal smooth muscle has been shown to be mediated through both alpha and beta adrenergic receptor mechanisms(5). This study was undertaken to test the possibility that the excitatory action of catecholamines on terminal guinea-pig ileum involves either an alpha or beta receptor mechanism. The possibility of catecholamines acting as cholinergic, histaminic or serotonin releasing agents was tested as was the possibility that they act directly on serotonin receptors.

Methods. Ten guinea-pigs (300-400 g) were used in these studies. The animals were killed by cervical dislocation and 2 to 3 cm segments of terminal ileum were removed immediately. They were flushed with electrolyte solution and mounted on their long axis in a chamber

* Dept. of Pharmacology, Division of Medicinal Chemistry, Walter Reed Army Inst. of Research.