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Biochemical and Histochemical Studies on the Distribution of Histamine in the Digestive Tract of Man, Dog and Other Mammals*

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Summary. 1. The distribution of histamine was determined in tissues of the digestive tract of man, dog, pig, cow, and sheep, especially in the oral mucosa, stomach, gallbladder, and pancreas.

2. After treatment with compound 48/80, histamine was released from the frenulum linguae, soft palate, tongue, and thyroid gland of dogs, but not from the vestibulum oris, hard palate, pharynx, oesophagus, stomach, and pancreas (experiments in the dog). The release of histamine from the tongue showed regional differences and was lowest in the root and highest in the tip.

3. A parallelism could be shown between the histamine content and the mast cell density in different parts of the tongue, stomach and in the soft palate of untreated dogs and dogs treated with 48/80. The mast cells in the gastric mucosa could be characterized by their staining properties as "atypical" mast cells, whereas those in the musculature of the tongue were "typical" mast cells.

4. The histamine content of the single mast cell was similar in all tissues (3.2 pg/cell in the tongue, 3.3 in the stomach, 4.8 in the soft palate and 3.4 in the submaxillary gland). Only the mast cells in the fundic mucosa showed a significantly lower histamine content (1.9 pg/cell). The mast cells of the fundus and body of the stomach of the dog seemed to store histamine which was released by 48/80.

5. A new classification of histamine stores is proposed: "unspecific mast cell stores" and "tissue specific stores".

Key-Words: Histamine — Alimentary Canal — Mammals — Mast Cells.

In the digestive tract of mammals, the histamine content of the tissues has been examined more frequently than of other organ systems (for a survey see Lorenz and Werle, 1969). But in some of them, like the pancreas, gallbladder, and oral mucosa, nothing or very little is known about its concentration and distribution.

In the dog, cellular localization of the amine had been studied intensely in many tissues of the alimentary canal (Feldberg and Harris, 1953; Arvy and Quivy, 1955, 1955a; Mota *et al.*, 1956; Lorenz *et al.*, 1968c; Aures *et al.*, 1968). Nevertheless, the results obtained by bio-

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chemical and histochemical methods are not sufficient to characterize various types of histamine stores, which may occur in one single tissue. Especially since Enerbäck (1966a—d) had differentiated two kinds of mast cells by their biochemical and histochemical properties, which he had called "typical" and "atypical" mast cells, it seemed necessary to investigate the problem whether histamine is actually localized in two types of stores only: the "mast cell store" and the "nonmast cell store" (Brodie *et al.*, 1966).

In this study the concentration and distribution of histamine were determined in many tissues of the digestive tract, especially in some parts of the oral mucosa, in the pancreas, gallbladder, and stomach. Furthermore, by the aid of biochemical and histochemical methods we tried to characterize the stores of histamine in different organs of the alimentary canal of the dog.

Methods

Materials. The human tissues were obtained from four men who died of accidents. The tissues of the animals were obtained from a local slaughter house or from our laboratory. Mongrel dogs weighing 8—12 kg were narcotized by 10—15 mg/kg Nembutal® and bled. The organs were removed immediately after the death of the animals, frozen by CO_2 snow and held at $-$ 20° C, until the assay of histamine was performed.

Reagents: o-phthaldialdehyde (recrystallized from ligroin B. P. 50—70° C), histamine dihydrochloride (Fluka, Basle), n-butanol (for chromatography, Riedel de Haën, Seelze), n-heptane, perchloric acid (Uvasol®, Merck, Darmstadt); toluidine blue 0 for microscopy, lead acetate and Entellan (Merck, Darmstadt). We thank very much the Imperial Chemistry Industries, Manchester, for the gift of compound 48/80.

Determination of Histamine. Histamine was measured spectrofluorometrically according to the method of Shore *et al.* (1959). Blanks were obtained by omission of the condensation step and by carrying 4 ml of 0.4 N $HClO_4$ through the whole procedure. The recoveries of 0.5—20.0 μg histamine hydrochloride added to the tissue homogenates were 70—80%.

The specifity of the method was proved by comparing the fluorescence spectra of the tissue extracts (in 0,1 N HCl) with that of standard histamine, and by comparing the histamine values obtained by the method of Shore *et al.* (1959) with those obtained by the biological assay of histamine on the isolated guinea-pig ileum using the highly specific antihistaminic drug antazoline (Lorenz and Werle, 1969a) as an antagonist. The results of the two methods corresponded upto a difference of only $\pm$ 5%, the fluorescence spectra of the extracts were identical with those of standard histamine. All histamine values are expressed as μg histamine dihydrochloride/g wet weight.

Treatment of the Dogs with Compound 48/80. Adult mongrel dogs (pairs of the same litter) were treated on three successive days with i.m. injections of increased doses of 48/80 (2.5 mg/kg, 3.5 mg/kg and 4.0 mg/kg daily). Then the animals were sacrificed and the tissues removed, as described under materials.

Staining of the Mast Cells with Toluidine Blue and Estimation of the Mast Cell Density. Specimens of the tissue adjacent to the ones used for the determination of histamine were fixed for 24 hours with 4% basic lead acetate. Then they were embedded in paraffin; 10 μ sections were stained for 30 min with a 0.5% toluidine blue solution at pH 4.0 and mounted in Entellan. For the characterization of the "typical" or, "atypical" mast cells, the same sections or those following immediately on the paraffin block (5 μ thickness) were stained for 30—45 sec or 10 min with 0.1% toluidine blue solution at pH 0.3, 0.5, 1.5 and 4.0, according to the method of Enerbäck (1966b). The gastric mucosa was cut vertically to its surface. In each section the number of mast cells was counted in 25 fields of nearly 1 mm² each. 2—3 sections in layers of different depth were examined in each specimen of tissue. Also, cells degranulated to a large extent by the treatment of 48/80 were counted. The density of mast cells is expressed as the number of cells/25 fields (averages of 2—3 sections).

Results

1. Histamine Content of Different Tissues in the Alimentary Tract of Man, Dog and other Mammals

In the digestive tract of the *dog*, the highest histamine concentrations were found in the stomach, duodenum, and palatine tonsils, the lowest in the pancreas, thyroid gland, gallbladder, and mucosa of the hard palate and vestibulum oris (Table 1). In the oral mucosa the histamine

Table 1. *Histamine content of different organs in the digestive tract of the dog*

Organ	Histamine content (μg/g)	
	Mean value $\pm s_{\bar{x}}$	Extreme values
Head		
Vestibulum oris	6.2 ± 1.8	3.8— 8.2
Frenulum linguae	16.9 ± 1.6	14.6—18.1
Tongue	14.9 ± 3.8	10.8—21.4
Hard palate	4.1 ± 1.1	3.2— 5.9
Soft palate	26.5 ± 6.5	20.6—90.3
Palatine tonsil	61.5 ± 16.5	7.6—42.4
Pharynx	22.7 ± 14.8	
Neck and thorax		
Oesophagus	14.4 ± 3.0	10.1—17.7
Thyroid gland	6.4 ± 3.1	3.2— 9.9
Thymus	24.9 ± 26.0	6.7—66.4
Lymph node	14.5 ± 12.0	6.2—34.7
Abdomen		
Stomach	66.5 ± 12.3	36.4—101.9
Duodenum	72.3 ± 9.7	62.1— 77.7
Ileum	40.5 ± 10.2	27.0— 50.4
Pancreas	5.4 ± 0.7	3.9— 6.1
Gallbladder	4.8 ± 0.7	3.1— 5.7
Spleen	13.8 ± 6.7	6.2— 24.3

N = 5—9 animals. The mucosa of the vestibulum oris was removed from the cheeks.

content increased from oral to aboral. The highest histamine concentrations were found in the soft palate and pharynx (Table 1). Since we obtained the same results in *man* (Table 2), it seems possible that histamine plays a role in the genesis of the inflammatory or allergic oedema of this region (*glottic oedema*).

Table 2. *Histamine content of different tissues of the human mouth and pharynx*

Organ	Histamine content (μg/g)	
	Mean value $\pm s_{\bar{x}}$	Extreme values[a]
Tongue, tip	7.6 ± 3.6	4.8–12.9
body	5.9 ± 0.9	4.6– 6.8
root	7.4 ± 1.0	6.2– 8.5
Mucosa in the region of the isthmus faucium	19.4 ± 9.5	13.3–30.4
Palatine tonsil	15.4 ± 2.7	12.7–19.0
Pharynx	16.5 ± 2.1	13.2–20.3

[a] $N = 4$ (number of human beings tested).

Table 3. *Histamine content of the pancreas and the gallbladder of different mammals*

Organ, species	N	Histamine content (μg/g)	
		Mean value $\pm s_{\bar{x}}$	Extreme values
Pancreas pig	5	5.1 ± 0.6	4.6– 5.4
cow	6	13.8 ± 1.5	11.7– 15.8
calf	6	6.7 ± 2.3	3.8– 10.0
sheep	5	22.6 ± 6.2	16.8– 33.0
rat	6	8.4 ± 6.5	3.0– 21.1
Gallbladder pig	4	81.6 ± 37.0	38.5–114.0
cow	4	30.3 ± 10.0	22.4– 49.8
sheep	5	8.1 ± 4.9	2.1– 13.4

N = number of animals tested.

The histamine concentration of the *pancreas* and *gallbladder* varied from one species to another (Table 3). The highest histamine content of the pancreas was found in that of sheep, the lowest in dogs and pigs. The very low density of mast cells in the pancreas of the dog (ARVY and QUIVY, 1955a) can explain this finding. A very high histamine concentration was discovered in the gallbladder of the pig. It could be possible that histamine plays a role in the etiology of *biliary dyskinesia*.

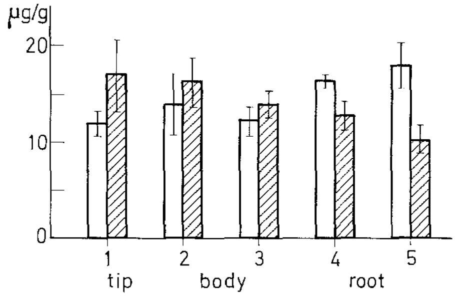


Fig. 1. *Distribution of histamine in the tongue of the dog.* Histamine in µg/g, $\bar{x} \pm s_{\bar{x}}$, 5 animals tested. Strips of the mucosa and musculature were obtained by dissecting the tongue into 5 equal, 2—3 cm large pieces. Strip 1 corresponds to the tip, 2—4 to the body and 5 to the root of the tongue. Differences of the histamine content in the *mucosa:* 4/1 and 5/1 $p < 0.05$, in the *musculature:* 1/5 and 2/5 $p < 0.05$. ▨ mucosa; □ musculature

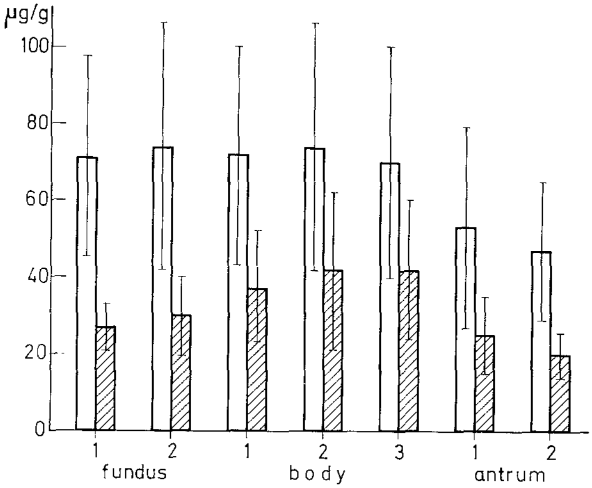


Fig. 2. *Distribution of histamine in the stomach of the dog.* Histamine in µg/g. $\bar{x} \pm s_{\bar{x}}$, 5 animals tested. Strips of the mucosa and musculature were obtained by dissecting the stomach in 7 equal, 3—4 cm large pieces along the circular musculature. The mucosa was separated from the musculature (controlled by microscopy). Correlation between the histamine content of mucosa and musculature: $r = 0{,}77$; $p < 0.05$. ▨ musculature; □ mucosa

2. Distribution of Histamine in the Tongue, Stomach and Pancreas

Histamine is not uniformly distributed in tongue of dogs. In the mucosa the concentration increased from the tip to the root, in the muscular tissue from the root to the tip (Fig. 1), corresponding to the change of the mast cell density (Table 8).

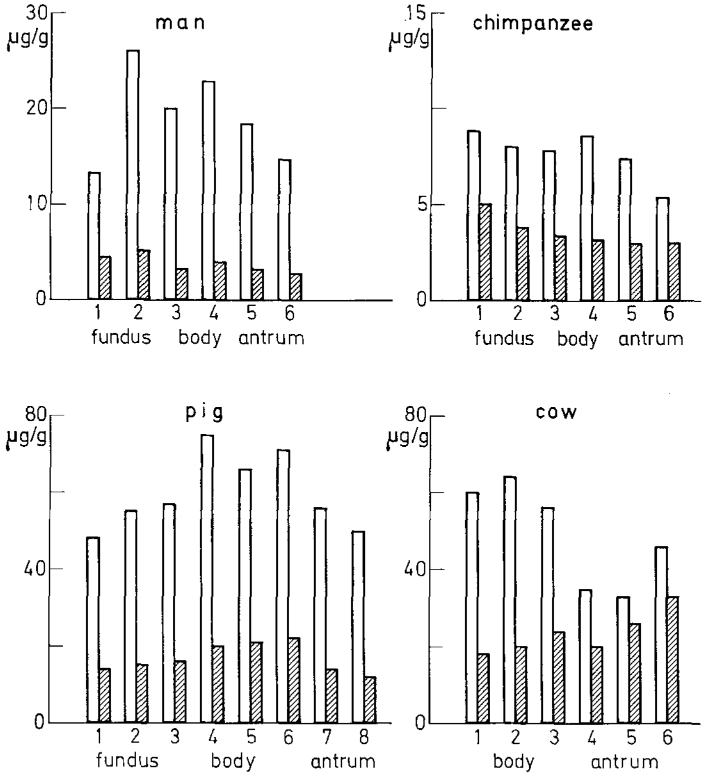


Fig. 3. *Distribution of histamine in the stomach of man, chimpanzee, pig and cow.* Histamine in µg/g, mean values $\pm s_{\bar{x}}$., 2 animals tested. Preparation of the mucosa and musculature according to Fig. 2. Correlation between the histamine content of mucosa and musculature: man: $r = 0.97$, $p < 0.001$; chimpanzee: $r = 0.17$; cow: $r = 0.35$; pig: $r = 0.91$, $p < 0.001$. □ mucosa, ▨ musculature

In the *gastric mucosa* of the dog the highest histamine concentrations were found in the fundus and body, the lowest in the antrum (Fig. 2). Also in man, monkey, pig and cow (Fig. 3), the highest histamine content was measured in those regions of the gastric mucosa, showing the highest density of the acid forming parietal cells (Ellenberger and Baum, 1912). Only in the rennet-bag of the cow, the histamine concentration strongly increased immediately at the pylorus, where parietal cells were very seldom (Lorenz and Pfleger, 1968d). The meaning of this interesting finding is not known, but it may be possible, that histamine in some species plays a role in the sphincter mechanism of the pylorus.

The muscularis propria and serosa of the dog's stomach showed only $48 \pm 8\%$ of the histamine concentration of the mucosa, in the body

58% (Fig. 2). Whether this higher histamine content of the body muscularis has any influence on the blood flow in this region, is not known. But a parallelism could be demonstrated between the histamine content of the mucosa and that of the muscularis propria, not only in dogs (Fig. 2), but also in men and pigs (Fig. 3), whereas this correlation could not be shown in monkeys and cows. The histamine concentration of the muscularis propria was 18 ± 1% of that of the mucosa in men, 28 ± 3 in pigs, 52 ± 21 in cows and 55 ± 8 in monkeys (Fig. 3). Therefore, the histamine content of the gastric muscularis propria was relatively high in monkeys, cows and dogs and low in men and pigs.

In the pancreas of dogs, cows and calves, histamine was uniformly distributed. If the two limbs of the dog's pancreas (cauda and caput pancreatis) were divided in 3 equal parts, the following histamine concentrations were found as mean values of 6 animals (beginning at the end of the cauda = left limb): 4.1 ± 1.1; 4.1 ± 0.8; 4.3 ± 0.5; 4.4 ± 1.2; 4.3 ± 1.0; 4.8 ± 1.4 (μg/g).

3. Histamine Concentrations in Tissues of the Digestive Tract of Dogs after Treatment with 48/80

In the *tongue* of dogs, histamine was released by compound 48/80 to about 50—60% (Table 4). No significant difference existed between the mucosa and the musculature. However, similar to the histamine concentration, the histamine release by 48/80 was not equal in all parts of the

Table 4. *Histamine release in the tongue of the dog after treatment with compound 48/80*

Tissue	N	Histamine content (μg/g)		Release (%)	Significance (p)
		Untreated	Treated		
Whole tongue	9	14.9 ± 3.8	7.1 ± 4.3	52	< 0.001
Mucosa	5	15.9 ± 1.9	7.0 ± 0.3	56	< 0.001
Musculature	5	14.7 ± 3.8	5.8 ± 0.3	61	< 0.001
Mucosa					
1. strip	9	14.9 ± 5.0	4.0 ± 1.5	73	< 0.001
2. strip	5	15.3 ± 6.1	5.2 ± 2.4	66	< 0.001
3. strip	9	14.1 ± 3.9	5.6 ± 2.2	60	< 0.001
4. strip	5	16.4 ± 1.7	10.1 ± 0.9	35	< 0.025
5. strip	9	18.6 ± 5.0	10.0 ± 3.9	46	< 0.001
Musculature					
1. strip	9	18.4 ± 6.7	6.1 ± 2.5	67	< 0.001
2. strip	5	17.7 ± 9.1	6.6 ± 2.4	63	< 0.001
3. strip	9	14.5 ± 3.2	6.5 ± 2.4	55	< 0.001
4. strip	5	13.0 ± 3.3	6.3 ± 2.0	52	< 0.001
5. strip	9	9.4 ± 3.0	4.3 ± 1.1	64	< 0.001

$\bar{x} \pm s_{\bar{x}}$. N = number of animals tested. Preparation of the tissue strips according to Fig. 1.

mucosa, whereas it showed only insignificant variations in the musculature (Table 4). Contrary to the histamine content, the histamine release by 48/80 decreased in the mucosa from the tip to the root of the tongue ($73 \pm 14\%$ in the first strip, $35 \pm 24\%$ in the fourth strip, $p < 0.02$).

Table 5. *Histamine release in the oral mucosa, pharynx and different organs of neck and thorax in the dog after treatment with compound 48/80*

Organ or tissue	Histamine content (μg/g)		Release (%)	Significance (p)
	Untreated	Treated		
Vestibulum oris	6.2 ± 1.8	7.8 ± 5.7	0	—
Frenulum linguae	16.9 ± 1.6	11.0 ± 4.7	35	< 0.05
Hard palate	4.1 ± 1.1	7.6 ± 3.5	0	—
Soft palate	26.5 ± 6.5	17.0 ± 5.5	36	< 0.02
Pharynx	22.7 ± 14.8	20.5 ± 2.4	10	> 0.05
Thyroid gland	6.4 ± 3.0	2.7 ± 1.4	58	< 0.025
Thymus	24.9 ± 26.0	13.7 ± 6.5	45	> 0.05
Lymph node	14.5 ± 12.0	5.1 ± 1.3	65	> 0.05
Upper oesophagus	12.5 ± 3.8	13.6 ± 4.0	—	—
Lower oesophagus	16.3 ± 2.2	17.2 ± 2.9	—	—

$\bar{x} \pm s_{\bar{x}}$. 5 pairs of animals were tested. The upper oesophagus reaches from the pharynx up to the division of the trachea, the lower part from the division of the trachea up to the cardia.

In the mucosa and the submucosa of the *frenulum linguae* and the *soft palate*, about 35% of histamine was released (Table 5), which was less than in the tongue. No histamine release could be shown in the mucosa and the submucosa of the *vestibulum oris* and the *hard palate*, which contained relatively much solid connective tissue, and in the walls of the *pharynx* (Table 5). In the regions of collum and thorax a significant histamine release could be demonstrated only in the *thyroid gland* (Table 5), which was similar to that of the tongue. No significant change of the histamine content could be observed in the *thymus* and *lymph node*, since the histamine values showed considerable variations in treated and untreated dogs.

Furthermore, no histamine release by 48/80 could be shown in the upper and lower parts of the *oesophagus* and in different parts of the mucosa and musculature of the *stomach* (Table 6). On the contrary, the histamine concentration increased in the mucosa and musculature, but the difference between treated and untreated animals was of no statistical significance. Furthermore, no histamine release could be observed in the pancreas after i.m. injection of 48/80 in dogs.

Table 6

Histamine content of different parts of the dog's stomach after treatment with 48/80

Tissue	Histamine content (µg/g)		Change (%)	Significance (p)
	Untreated	Treated		
Mucosa				
Fundus 1	71.2 ± 26.2	88.4 ± 16.6	+ 25	> 0.05
2	74.3 ± 32.2	83.1 ± 12.7	+ 12	> 0.05
Body 1	71.6 ± 28.1	84.8 ± 12.3	+ 18	> 0.05
2	74.1 ± 31.8	89.2 ± 8.7	+ 20	> 0.05
3	74.8 ± 35.4	89.5 ± 10.8	+ 19	> 0.05
Antrum 1	52.5 ± 25.8	67.7 ± 14.9	+ 30	> 0.05
2	46.7 ± 17.6	46.6 ± 13.7	—	> 0.05
Musculature				
Fundus 1	27.2 ± 6.1	39.9 ± 11.6	+ 48	$0.05 < p < 0.1$
2	29.7 ± 10.2	37.5 ± 11.0	+ 26	$p < 0.1$
Body 1	36.6 ± 15.0	41.5 ± 10.4	+ 13	—
2	43,6 ± 22.6	38.4 ± 6.7	− 12	—
3	41.5 ± 18.0	42.9 ± 6.1	—	—
Antrum 1	26.4 ± 10.2	28.8 ± 11.6	—	—
2	19.4 ± 6.4	17.8 ± 4.8	—	—

$\bar{x} \pm s_{\bar{x}}$. Preparation of the stomach according to Fig. 2. 5 pairs of animals tested.

4. Correlation between the Histamine Content and the Mast Cell Density in Tissues of the Dog

The highest mast cell density of all dog tissues was found in the gastric mucosa, the lowest in the submaxillary gland (Table 7). A highly significant correlation between the mast cell density and the histamine concentration of the tissues could be demonstrated (Fig. 4). Since the regression line passed through the origin, nearly all histamine in these tissues seemed to be localized in mast cells. Only in the submaxillary gland, about 40% of the amine was non-mast cell histamine, as previously shown (Lorenz *et al.*, 1968c). Therefore, the histamine value of the submaxillary gland in Fig. 4 was corrected for mast cell histamine.

After treatment with 48/80, a parallel decrease of the histamine concentration and the mast cell density could be demonstrated in different parts of the mucosa and musculature of the tongue and in the *soft palate* (Table 8). The remaining histamine concentrations and mast cell densities in the tongue showed a correlation, the regression line of which passed through the origin (Fig. 5a). These findings support the statement that nearly all histamine of those tissues was localized in *mast cells*.

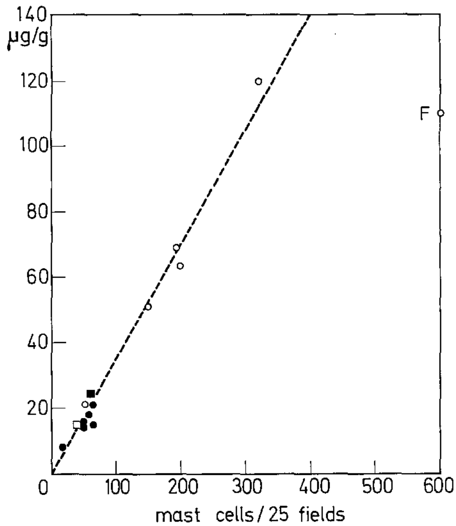


Fig. 4. *Correlation between the histamine content and the mast cell density of different organs in the digestive tract of dogs.* Histamine content in μg/g, mast cell density in number of mast cells/25 fields. The strips 1, 3, 5 according to Fig. 1 were studied in the mucosa and musculature of the tongue, fundus 1, body 2 and antrum 2 in the gastric mucosa and musculature according to Fig. 2. *F* fundic mucosa. Each point in the graph corresponds to a mean value obtained from determinations in 5 animals. Coefficient of correlation $r = 0.91$, $p < 0.001$. • tongue, ○ stomach, ▪ soft palate, □ submaxillary gland. The histamine value of the submaxillary gland was corrected for mast cell histamine (see text)

Table 7. *Mast cell density in different organs or tissues of the dog*

Organ or tissue	N	Mast cell density	
		Mean value $\pm s_{\bar{x}}$	Extreme values
Tongue, mucosa	15	60 ± 20	31–103
musculature	15	44 ± 26	13–112
Soft palate	5	51 ± 13	29– 61
Submaxillary gland	5	44 ± 23	17– 68
Stomach, mucosa	15	341 ± 197	55–705
musculature	15	131 ± 89	14–335

Mast cell density given in number of cells/25 fields ≃ number of cells/mm². N = number of tests. In the gastric mucosa and musculature one piece of tissue was removed from the fundus, body and antrum, in the tongue from strip 1, 3 and 5 according to Fig. 1.

In the *gastric* mucosa and musculature of the fundus and body, we found a decrease of the mast cell density after treatment with 48/80, which was significant only in the mucosa of the fundus (Table 8), and

Table 8. *Comparison of the histamine content and the mast cell density in different tissues of the dog after treatment with 48/80*

Tissue	Histamine content (µg/g)		Change (%)	Number of mast cells/ 25 fields		Change (%)
	Untreated	Treated		Untreated	Treated	
Tongue						
Mucosa:						
1. strip	15.7 ± 6.2	3.7 ± 1.7	– 76	50 ± 13	6 ± 6	– 88
3. strip	14.6 ± 4.9	4.1 ± 1.3	– 72	69 ± 29	17 ± 7	– 86
5. strip	20.3 ± 5.4	9.4 ± 4.9	– 56	71 ± 12	36 ± 26[a]	– 50
Musculature:						
1. strip	17.7 ± 6.3	5.4 ± 2.6	– 70	57 ± 33	16 ± 12	– 72
3. strip	14.6 ± 3.7	5.6 ± 2.5	– 62	49 ± 29	17 ± 13	– 65
5. strip	7.8 ± 2.2	2.9 ± 1.0	– 63	16 ± 11	16 ± 11	– 63
Soft palate	26.5 ± 6.5	17.0 ± 5.5	– 36	51 ± 13	32 ± 13	– 37
Stomach						
Mucosa:						
Fundus 1	137 ± 40	117 ± 47	– 15	545 ± 169	313 ± 62	– 43
Body 2	141 ± 54	143 ± 72	–	323 ± 78	261 ± 88	– 19
Antrum 2	68 ± 31	71 ± 39	–	157 ± 85	172 ± 75	+ 10
Musculature:						
Fundus 1	51 ± 20	56 ± 23	+ 10	148 ± 74	118 ± 45	– 20
Body 2	69 ± 25	66 ± 21	–	196 ± 87	125 ± 46	– 36
Antrum 2	21 ± 7	27 ± 15	+ 29	56 ± 24	88 ± 50	+ 57

$\bar{x} \pm s_{\bar{x}}$. 5 pairs of animals tested. The changes of the histamine content and mast cell density are given in ± per cent, the tissue pieces were named according to Fig. 1 and 2.

Significance: tongue: In all cases $p < 0.001$, in [a] $p < 0.01$. Soft palate: $p < 0.02$. Stomach: With the exception of the number of mast cells in the mucosa of fundus 1 ($p < 0.025$) no significant changes.

an insignificant increase in the antrum. Contrary to these changes of the mast cell density, the histamine concentration of the gastric mucosa and musculature remained by far constant (Table 8, see above). Since the change of the mast cell density was relatively small, a significant correlation between the histamine content and the mast cell density before and after treatment with 48/80 could be shown in different parts of the stomach as well as in the tongue (Fig. 5b). These findings indicated that nearly all histamine in the stomach was localized in *mast cells*. But the decrease of the number of mast cell needs further explanation (see below).

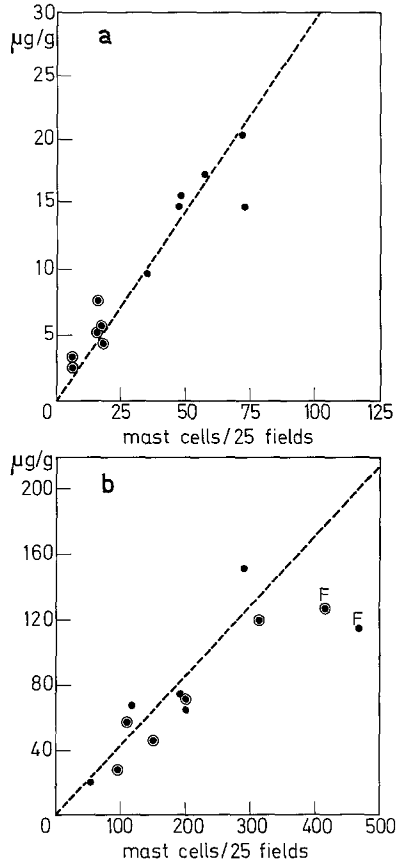
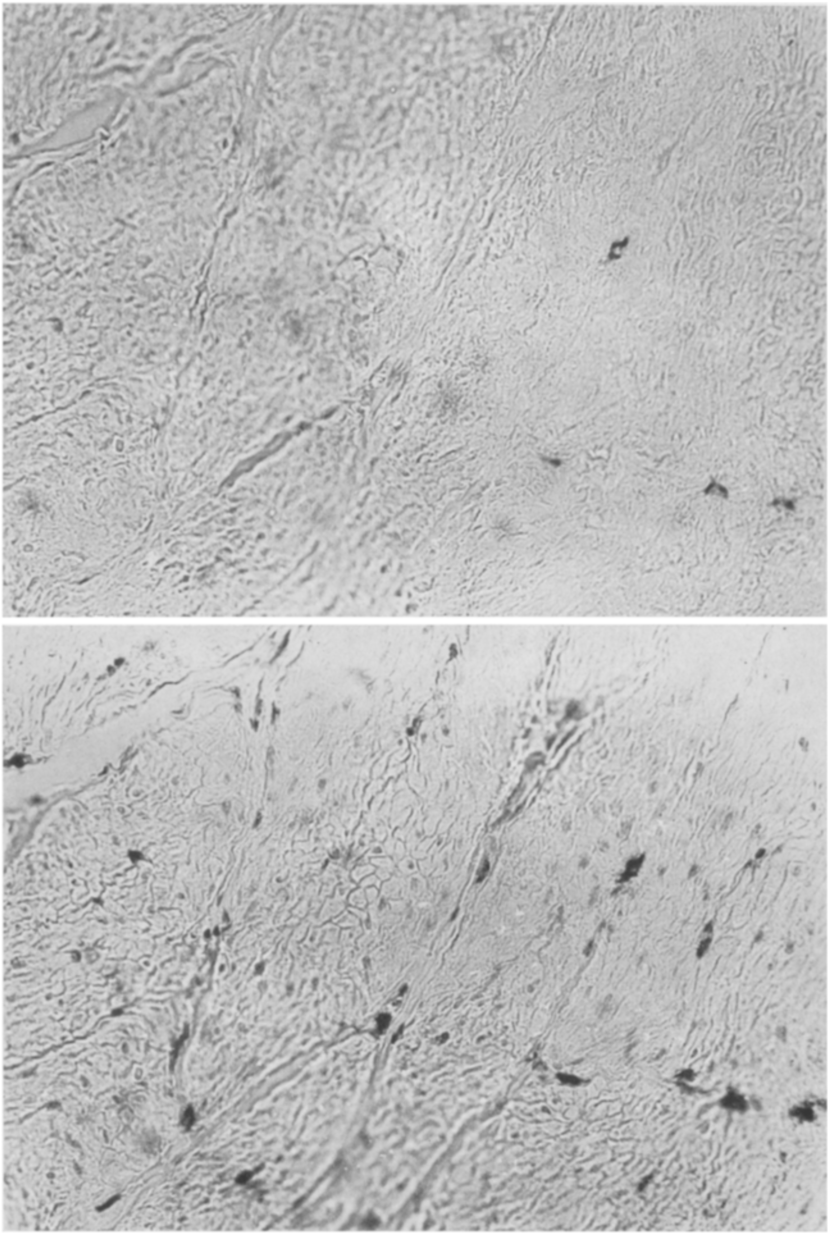


Fig. 5 a and b. *Correlation between the histamine content and the mast cell density of the tongue and the stomach in normal dogs and those treated with 48/80.* Histamine content in µg/g, mast cell density in number of mast cells/25 fields. Each point in the graph corrseponds to a mean value obtained from determinations in 5 normal dogs (•) and those treated with 48/80 (⊙). Pieces of tissue in the tongue and stomach according to Fig. 4. *F* fundic mucosa. a) *tongue:* $r = 0.97$, $p < 0.001$; b) *stomach:* $r = 0.86$, $p < 0.001$

5. Characterization of the Mast Cells in the Tongue and the Stomach by Staining with Toluidine blue at Different pH Values

The mast cells of the gastric mucosa, duodenum and ileum of the rat could be differentiated from those of other tissues (Riley, 1959; Schauer, 1964): They were strongly resistant to 48/80 and stained by 0.1 % toluidine blue at pH 4.0, but not at pH 0.3. Enerbäck (1966a—d) called these cells "atypical mast cells".



a

b

Fig. 6 a and b. *Staining of a section of the gastric musculature of the dog with toluidine blue (0.1%) at pH 4.0 and 0.3.* The same section was stained for 45 sec at pH 4.0 and for 10 min at pH 0.3 according to ENERBÄCK (1966b). It was obtained from body 2 according to Fig. 2. a = pH 0.3, b = pH 4.0. The mast cells appear as deeply black spots, note the great difference between the number of these cells at pH 0.3 and 4.0. Magnification 300 ×

Since the mast cells of some tissues of the dog were resistant to 48/80, we examined the staining behaviour of these cells with 0.1 % toluidine blue at pH 4.0; 1.5; 0.5 and 0.3. In all parts of the musculature of the tongue (according to Fig. 1) no difference could be fond in the mast cell density at different pH-values before and after treatment with 48/80. In the mucosa, this was only the case in the tip and body of the tongue, but not in the root, where about 40% of the mast cells disappeared at pH 0.3. Therefore, the mast cells of the tongue seemed to be typical mast cells, with the exception of 40% in the mucosa of the radix linguae, which were atypical mast cells. In the *gastric mucosa* and musculature of fundus, body and antrum of untreated dogs, only 39% of the mast cells could be stained with 0.1% toluidine blue at pH 1,5, and only 18% at pH 0.3 (for example see Fig. 6a, b). In dogs treated with 48/80, practically no mast cells could be shown in the gastric mucosa at pH 0.3. Thus about 20% of the mast cells of the gastric mucosa seemed to be typical mast cells by their staining properties and their sensitivity towards 48/80 (see above) and *80*% seemed to be „atypical" mast cells. Indeed, the mast cells of the gastric mucosa were much smaller than those in the musculature of the tongue. The increase of the histamine content and the mast cell density in the antrum mucosa after 48/80 was somewhat surprising, but ENERBÄCK (1966c) obtained the same results in the duodenum of rats after the treatment with 48/80.

In the stomach of the rat, the typical mast cells had been found only in the submucosa, while all mucosal mast cells were "atypical" (AURES *et al.*, 1968). In the dog's stomach too, the "typical" mast cells occurred only in the basal layers of the mucosa and in the submucosa. This could be shown by staining of the sections with 0.1% toluidine blue at pH 0.3, and by a reduction of the mast cell density after treatment of the animal with 48/80 (Table 9). In the upper two thirds of the corpus mucosa the number of mast cells/mm^2 was the same as in the basal third and in the submucosa. Contrary to that, in the fundic mucosa it was in the upper layers about 25% higher than in the basal layers and in the submucosa. In dogs treated with 48/80, this difference was statistically significant ($p < 0.05$).

6. Histamine Content of the Single Mast Cell in Normal Dogs and Those Treated with 48/80

The histamine content of the single mast cell has been calculated from the histamine concentration per g tissue and the number of mast cells per cm^3. It was 3.2 ± 0.9 pg (1.4—7.1) in the tongue, 3.3 ± 0.7 pg (1.6—14.1) in the stomach, 4.8 ± 1.4 pg (2.0—8.4) in the soft palate and 3.4 ± 0.9 (1.5—6.7) in the submaxillary gland.

Table. 9. *Mast cell density in different layers of the gastric mucosa and the muscularis mucosae in the fundus and body of the dog after treatment with 48/80*

Layer	Mast cell density (number of cells/ 25 fields)		Decrease (%)
	Untreated	Treated	
Fundic mucosa			
Upper two thirds	583 ± 215	575 ± 74	—
Basal third and muscularis mucosae	474 ± 66	445 ± 99	6
Body			
Upper two thirds	303 ± 69	299 ± 152	—
Basal third and muscularis mucosae	316 ± 124	261 ± 129	18

$\bar{x} \pm s_{\bar{x}}$. 5 pairs of animals tested, the fundic mucosa corresponded to fundus 1, the body mucosa to body 2 in Fig. 2.

Table 10. *Histamine content of the single mast cell in different tissues of the dog after treatment with 48/80*

Tissue	N	Histamine content (pg/cell)		Increase (%)	Significance (p)
		Untreated	Treated		
Stomach					
Mucosa					
Fundus 1	9	1.9 ± 0.8	3.5 ± 1.0	85	< 0.005
Body 2	9	4.0 ± 1.8	6.2 ± 2.0	55	< 0.05
Antrum 2	9	3.8 ± 2.2	4.0 ± 1.3	—	—
Musculature					
Fundus 1	5	4.5 ± 2.7	5.3 ± 2.5	18	< 0.2
Body 2	5	4.1 ± 2.1	5.4 ± 0.9	32	< 0.1
Antrum 2	5	4.2 ± 2.3	3.8 ± 2.1	—	—
Tongue					
Mucosa (strip 3)	5	2.5 ± 0.8	2.5 ± 1.6	—	—
Musculature (strip 3)	5	3.6 ± 2.3	3.6 ± 1.4	—	—

$\bar{x} \pm s_{\bar{x}}$. Tissue pieces named according to Fig. 1 and 2. Calculation of the histamine content of the single mast cell from the histamine content/g and the number of mast cells/cm^3 = number of mast cells/25 fields × 100000 (thickness of the section 0.01 mm, area of 1 mm^2).

The mean content of at all investigated was 3.7 ± 0.8 pg. For comparison, the values found by other investigators are given: 1.14 pg in the cat skin (RILEY, 1959), 10—15 pg in the mesentery of the rat (UVNÄS and THON, 1966), 1 pg in the basophile granulocyte (VAN ARSDEL and BRAY, 1961), 6.7—15.6 pg in different tissues of the dog (GRAHAM *et al.*, 1955), 18 and 24 pg in the liver capsule of calves and oxes (RILEY, 1959), 25—34 pg in different tissues of the guinea-pig (BORÉUS and CHAKRAVARTY, 1960).

The histamine content of the single mast cell was nearly the same in all tissues studied in the dog. Only the mast cells of the fundic mucosa showed a histamine concentration which was about 50% lower than that of the body mucosa ($p < 0.01$) and that of the other tissues examined (Table 10, cf. Fig. 5b). Since the animals were starved 12 hours before death, an influence of feeding could not explain these findings. But after treatment of the dogs with 48/80, the histamine content of the mast cells of the fundic mucosa increased to about 85%, and was now similar to that of the other tissues. Also, the histamine concentration of the mast cells of the body mucosa increased to about 55% and reached values which were the highest of all mast cells in the dog as far as investigated (Table 10). Furthermore, a small, not significant increase in the histamine content of the single mast cell could be observed in the musculature of the fundus and body after application of 48/80 (Table 10). In the other tissues the histamine content of the mast cells remained unchanged. Since it is known, that the "atypical" mast cells can store monoamines (ENERBÄCK, 1966d), it seems probable that the increase of the histamine content of the single mast cell in the fundus and body is due to an uptake and storage of histamine released from typical mast cells in the whole body by 48/80. An uptake of exogenous histamine by the gastric mucosa of dogs has been described (CODE, 1965). Contrary to the "atypical" mast cells, the typical mast cells cannot take up and store exogenous histamine (SCHAYER, 1956).

Discussion

The studies on a possible physiological rôle of histamine in the digestive tract were focussed on the stimulation of gastric secretion (CODE, 1966; LORENZ and PFLEGER, 1968d). But histamine has probably some other physiological functions in the alimentary tract, too.

For instance, a mediator rôle of this amine was discussed in the *detoxication* mechanisms of the Waldeyer's tonsillar ring by the enhancement of the permeability for different toxins (GASTPAR and LORENZ, 1968). Specific histidine decarboxylase and diamine oxidase were demonstrated in the tonsils (GASTPAR and LORENZ, 1968). The induction of the histidine decarboxylase by the toxins could play a rôle in the focal infection, since the "induced" histamine (SCHAYER, 1966) would enhance the permeability not only in the direction from the blood into the lympoid tissue, but also from this tissue into the blood-stream (GASTPAR and LORENZ, 1968).

Distribution and metabolism of histamine in the *pancreas* have been studied only in the rat (EHINGER *et al.*, 1968; LORENZ *et al.*, 1968a). A release of histamine into the pancreatic juice of dogs during secretion could be shown by LORENZ *et al.* (1968b). But, contrary to the submaxillary and parotid glands (LORENZ and PFLEGER, 1968d; LORENZ and WERLE, 1969a), the pancreatic secretion induced by histamine and secretin could not be inhibited by several antihistaminic drugs (LORENZ *et al.*, in preparation). Since even high doses of antihistiminics with a strong cholinolytic action (promethazine and piprinhydrinate) were ineffective after injection into the A. pancreaticoduodenalis, the conclusion was that these drugs could penetrate only in small amounts into the pancreatic tissue (LORENZ, 1969). Further studies are necessary to elucidate the function of histamine in this gland.

In all studies on the possible physiological function of histamine, the cellular localization of this amine plays an important rôle. Different methods of solving this problem should be combined, since no biochemical or histochemical assay by its own is able to characterize the histamine stores qualitatively and quantitatively with a complete reliability. The fluorescence microscopical techniques seem to have two sources of error: These methods suffer from a limited sensitivity, and tissue components, like guanidines and thioimidazoles, could inhibit the formation of the complex between o-phthalaldehyde and histamine (LORENZ *et al.*, 1968e). The following procedures were chiefly used: Determination of the correlation between the histamine content and the mast cell density (RILEY and WEST, 1953); estimation of the histamine release by typical liberators, like 48/80 (mast cell histamine) or reserpine (non-mast cell histamine) (MACINTOSH and PATON, 1949; BRODIE *et al.*, 1966); histochemical (SCHAUER and WERLE, 1959) and fluorescence microscopical demonstration of histamine (JUHLIN and SHELLEY, 1966; THUNBERG, 1967; HÅKANSON *et al.*, 1967, 1967a, b); histochemical characterization of the mast cell type (ENERBÄCK, 1966a to d). Other methods, which are not so often used, should be mentioned: Autoradiography after application of ^{14}C-histamine (WEINSHELBAUM and FERGUSON, 1966), the fractionating of cells of the blood (CODE, 1937; MINARD, 1940), and of cell particles of the tissues (MOTA *et al.*, 1954; SNYNDER *et al.*, 1966; KATAOKA and DE ROBERTIS, 1967).

Since the cellular localization of histamine in the gastric mucosa and the musculature of the tongue has been studied with the aid of the four methods chiefly used (FELDBERG and TALESNIK, 1953; LORENZ and PFLEGER, 1968d; AURES *et al.*, 1968; and this paper) complete evidence is presented, that histamine in gastric mucosa (fundus and body) is localized to about 80% in "atypical" and to about 20% in "typical" mast cells and in the musculature of the tongue to about 100% in typical mast cells. In all of the other tissues of the dog further studies seem to be necessary, especially by the fluorescence microscopy.

BRODIE *et al.* (1966) differentiated two types of histamine stores, mast cell and non-mast cell store. Histamine can be released from the mast cell store by the compound 48/80 and from the non-mast cell store by reserpine and by parasympathetic stimuli. Since the "atypical" mast cells with properties of the non-mast cell stores (ENERBÄCK, 1966d) have been discovered, this classification seems to be questionable. According to the morphology and the functional significance we would prefer the terms "unspecific mast cell" stores and "tissue specific" stores. The former are found in all tissues, the latter only in one or few tissues. The former are localized only in mast cells, the latter in "atypical" mast cells, enterochromaffin and enterochromaffinlike cells (HÅKANSON *et al.*, 1967, 1967a, b), nerve endings (SNYDER *et al.*, 1966; KATAOKA and DE ROBERTIS, 1967) or in a new cell system in the pancreas and hypophysis (EHINGER *et al.*, 1968). The former may have a similar function in all tissues, like regulation of the blood flow, the latter seem to have a specific function, like stimulation of the gastric or salivary secretion (LORENZ *et al.*, 1968b).

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