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DISTRIBUTION OF HISTAMINE AND SUBSTANCE P IN THE WALL OF THE DOG'S DIGESTIVE TRACT

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The experiments described deal with the histamine content of the different regions of the digestive tract, from the oesophagus to the rectum, and of the different layers of the wall at each region studied. Although various authors have described the presence of histamine in the wall of the digestive tract, a systematic survey of its distribution has so far not been attempted. The paper also includes preliminary experiments on the distribution of another smooth muscle stimulating substance which resembles the substance *P* of Gaddum & Schild, and may be identical with it.

The results of previous work on histamine in the wall of the digestive tract may be summarized as follows: Histamine has been isolated chemically from gastric and intestinal mucosa of various species (Barger & Dale, 1911; Abel & Kubota, 1919), and under conditions which exclude the possibility of bacterial origin or the formation from putrefactive changes during the extraction (Gerard, 1922; Sacks, Ivy, Burgess & Vandolah, 1932). Schild's experiments (1939) on the guinea-pig's digestive tract indicate that histamine is present along its whole length. He found that histamine was released from the oesophagus, stomach, small and large intestine during the antigen-antibody reaction of anaphylaxis. Quantitative estimates of the histamine present in the wall of the intestine and stomach have been obtained by biological assay in various species. In the small intestine the values per gram of fresh tissue were for the horse 7–8 μg. (Gaddum & Schild, 1934), for the dog 35 μg. (Gaddum, 1936), and for the guinea-pig 8·5–20 μg. (Schild, 1939). The values for the horse and dog were obtained after removal of the mucosa. The histamine present in the gastric mucosa has been determined by Gavin, McHenry & Wilson (1933), by Emmelin & Kahlson (1944), and by Trach, Code & Wangenteen (1944). The values for dogs were much higher than those for cats and

human beings and in all three species the histamine concentration in the mucosa of the fundus was about twice that of the pyloric and antral region. The values in μg. histamine per gram mucosa were as follows: dog, fundus 48–180 (average 85), pyloric region 24–80 (average 47); cat, fundus 5–34 (average 16), pyloric region 4–16 (average 9); human beings, fundus 4–24 (average 10), antral region 3–12 (average 6). Gavin *et al.* showed further that the muscularis propria contained less histamine than the mucosa, which contains 80% of the histamine of the stomach wall. Histamine appears also in the gastric juice and is thought to be derived from the mucosa histamine (for references see Babkin, 1950).

The name *substance P* was given by Gaddum & Schild (1934) to an unknown substance found first by Euler & Gaddum (1931) in extracts of the muscle coat from the small intestine and of brain and believed to be a polypeptide. The extracts caused a fall of arterial blood pressure in the atropinized rabbit and a slow contraction of the isolated atropinized intestinal preparation of the rabbit. Euler (1934, 1936*a*) found large amounts of what appeared to be the same substance in human semen and in extracts of the prostrate gland of various animals. Substance *P* has a stimulating action on a number of smooth muscle preparations and on smooth muscles *in vivo*, but it has apparently no effect on the bronchi (Euler, 1936*b*; Bjurstedt, Euler & Gernandt, 1940; Gernandt, 1942; Vogt, 1949, 1950; Kuck & Vogt, 1950). The fact that substance *P* can be precipitated with half-saturated ammonium sulphate has been made use of to prepare highly active powders (Euler, 1936*c*, 1942).

Euler (1936 *a*,*b*) suggests that the release of substance *P* in the intestinal wall may be responsible for its spontaneous movements. It diffuses out from the wall of a piece of intestine suspended in physiological saline solution. According to Vogt (1949) substance *P*, or a related substance, is released in increased amounts from the wall of the frog's stomach during vagus stimulation, and is therefore thought to be responsible for the atropine-resistant motor effects of vagus stimulation on the digestive tract. Recent findings by Vogt (1950), and particularly an analysis by Fischer & Vogt (1950) with paper chromatography, have shown that substance *P* may consist of two related substances, the one with a greater action on the blood pressure of the rabbit the other on the atropinized rabbit's intestine. The term substance *P* may refer to more than two polypeptides, each with slightly different actions.

Erspamer (1940) has described the presence of yet another smooth muscle stimulating substance of unknown constitution in extracts of gastric and intestinal mucosa, but not of muscularis propria. This substance is thought to be a di- or polyphenol derivative and has been called *enteramin*; it is also found in spleen extracts. Like substance *P* it lowers the arterial blood pressure in the atropinized rabbit, but differs from substance *P* in many of its physical chemical properties as well as in the fact that it has a very strong stimulating

action on the atropinized rat's intestine, but practically none on the guinea-pig's and rabbit's intestine.

Recently a substance with properties similar to those of substance *P* has been obtained by Rocha e Silva, Beraldo & Rosenfeld (1949) by the action of proteolytic venoms or trypsin on globulin. This substance, which is thought to be a polypeptide, was given the name *bradykinin*. Its relation to substance *P* is unknown. Its action on the intestine resembles the 'slow reacting substance' obtained by the action of venoms on lecithin (Feldberg & Kellaway, 1938; Feldberg, Holden & Kellaway, 1938), but the two substances are not identical, since the one is a split product of lecithin, the other of globulin.

METHODS

Dogs were bled under chloralose anaesthesia, and the whole digestive tract removed from the still living animal. Oesophagus, stomach, small intestine and colon with rectum were separated, their contents washed out with tap water, and were then kept in saline solution in the refrigerator until required for preparing the extracts.

In each experiment pieces about 2 cm. long were cut out from the different sections and separated into their layers. Six to nine areas were taken from the small intestine after its approximate length had been measured. The position of these areas along the intestine is shown in Fig. 1. However, the different pieces were not removed and extracted in the order given in the figure. For Expt. 1, the order was 6, 14, 10, 13, 8, 7, for Expt. 2 it was 6, 14, 10, 8, 7, 13, and for Expt. 3 it was 6, 14, 10, 7, 12, 13, 9, 8, 11. Adjacent areas gave practically the same values, when one was prepared first and the other last. Similarly, it made no difference whether the extracts were first made from the oesophagus, stomach or intestine. The time, 2½–3 hr., required for extraction of all areas in a single experiment did not apparently affect the result.

Of each area of the small intestine, colon and rectum, four layers were usually separated, and extracted separately. Starting from the lumen, the layers will be referred to as: (1) glandularis mucosae or gl. mucosae, (2) muscularis mucosae or m. mucosae, (3) submucosa, and (4) muscularis externa or m. externa with serosa attached to it. The first two layers represent the mucosa; its m. mucosae consists of a thick layer of smooth muscle fibres. The term gl. mucosae refers to that part which is easily scraped off from the m. mucosae. The submucosa consists of a tough membranous sheath of connective tissue which is easily removed intact.

The wall of the oesophagus was readily separable into two layers only: the m. externa and the submucosa plus mucosa. Nor was it possible to divide the mucosa of the stomach into two clearly defined layers, and the mucosa was therefore extracted as a whole.

The procedure of extraction was as follows: the fresh tissue was weighed and then ground in a mortar with sand and with about 2 ml. N-HCl/g. tissue. When the tissue was partly macerated, about 10 ml. H_2O/g. tissue, as well as a few ml. of saline solution, were added to the mortar and grinding continued. The macerated tissue, with the fluid, was boiled for about a minute and filtered. The filtrate was either kept in the refrigerator overnight, or tested at once for histamine, after neutralization with N-NaOH, on the guinea-pig's ileum preparation suspended in a 15 ml. bath, to which 0·2–0·4 μg. atropine was added. All histamine values are given as base.

No systematic identification of the active principle with histamine was attempted, because previous work had clearly shown that histamine itself is responsible for the histamine-like effects of such extracts. It was shown, however, (1) that the extracts lowered the arterial blood pressure in the atropinized cat, and (2) that the effect on the intestine was abolished by small doses of mepyramine (0·2 μg. added to the 15 ml. bath) and on washing out this anti-histamine compound, the sensitivity of the preparation to histamine returned together with that to the active principle in the extracts. It is of course possible that the contractions produced by the extracts, although

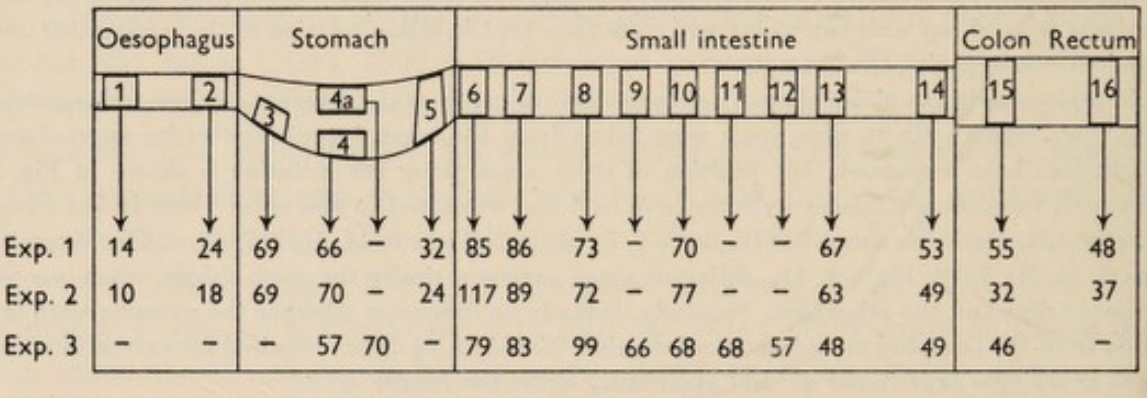
due to histamine, are augmented by the effect of sensitizing substances in the extracts. If so, the error introduced by this factor can only be slight, because the amounts of tissue extract required for testing were small.

The assay for substance *P* was made on the atropinized guinea-pig's ileum preparation after it had been made insensitive to histamine by the addition of 0·2 μg. of mepyramine to the bath. In some experiments the effect was compared with that of an old sample of substance *P* given to us by Professor von Euler, to whom we should like to express our thanks. In one experiment extracts were also assayed on the arterial blood-pressure of the atropinized rabbit under urethane anaesthesia.

RESULTS

Distribution of histamine in the wall of the digestive tract

Preliminary experiments showed that histamine-like activity was obtained in the extracts from all sections of the digestive tract and from all layers of the wall when tested on the atropinized guinea-pig's ileum, but the activity varied



Exp. 1	14	24	69	66	–	32	85	86	73	–	70	–	–	67	53	55	48
Exp. 2	10	18	69	70	–	24	117	89	72	–	77	–	–	63	49	32	37
Exp. 3	–	–	–	57	70	–	79	83	99	66	68	68	57	48	49	46	–

Fig. 1. Diagram of digestive tract to illustrate the position of the pieces taken for extraction and histamine assay. The figures below the diagram are μg. histamine/g. entire wall of the respective areas.

greatly according to the source of tissue extracted. A systematic examination of the various layers and the different sections of the digestive tract was therefore carried out in three dogs; in each between 46 and 50 pieces of tissue were extracted and assayed separately. Fig. 1 gives the regions of the digestive tract from which the different pieces were obtained. The histamine values shown in this figure for the whole wall were not obtained from assaying pieces of the whole wall, but by calculations from the values obtained from the different layers in each piece, and their weights.

Oesophagus. Of all sections of the digestive tract the wall of the oesophagus yielded the lowest histamine equivalents. In the two experiments of Fig. the values varied between 10 and 24 μg./g. fresh tissue and the values were higher in the lower than in the upper part of the oesophagus; both m. externa and mucosa showed this increase. The histamine is mainly in the mucosa plus submucosa, although they together form less than 50% of the wall; the m. externa only contributed between 15 and 39% of the histamine of the wall (Table 1).

Stomach. Fig. 1 shows that, whereas the wall of the fundus and corpus contained nearly 70 μg. histamine per gram wet tissue, the wall of the pyloric region contained less than half this amount. Again the main contribution was from the mucosa. The histamine values found here corresponded to those given by Gavin *et al.* (1933) and by Emmelin & Kahlson (1944). The mucosa of the fundus and corpus contained 65–133 μg., that of the pyloric region 40 and 63 μg. histamine per gram fresh weight, which corresponded to between 47 and 70% of the whole histamine of the wall (Table 2). The highest values were found in the mucosa of the fundus (120 and 133 μg./g.). The relatively lower histamine content of the wall of the pyloric region is mainly accounted for by this lower histamine content in the mucosa.

TABLE 1. Histamine equivalent in wall of oesophagus

Area in Fig. 1	Expt. No.	μg. histamine/g. in: Whole wall	Individual layers: M. + SM.	Individual layers: ME.	Contribution of each layer to wall: % weight of tissue: M. + SM.	% weight of tissue: ME.	% of histamine: M. + SM.	% of histamine: ME.
1	1	14	25	5·5	42	58	77	23
	2	10	20	2·8	39	61	82	18
2	1	24	32	9·2	46	54	61	39
	2	18	35	5·0	44	56	85	15

M. = mucosa; SM. = submucosa; ME. = muscularis externa.

TABLE 2. Histamine equivalent in wall of stomach

Area in Fig. 1	Expt. No.	μg. histamine/g. in: Whole wall	Individual layers: M.	Individual layers: SM.	Individual layers: ME.	Contribution of each layer to wall: % weight of tissue: M.	% weight of tissue: SM.	% weight of tissue: ME.	% of histamine: M.	% of histamine: SM.	% of histamine: ME.
Fundus	1	69	133	56	23	27	18	55	67	15	18
3	2	69	120	60	41	30	22	48	52	20	28
Corpus	1	66	93	109	25	44	16	40	60	25	15
4	2	70	100	90	25	43	16	41	62	23	15
	3	57	65	86	30	58	13	29	66	15	19
4*a*	3	70	90	104	42	38·5	14·5	47	50	22	28
Pyloric region	1	32	63	46	12·5	35	7	58	70	7	23
5	2	24	40	50	15	28	5	67	47	11	42

M. = mucosa; SM. = submucosa; ME. = muscularis externa.

In Expt. 2 the superficial layer of the mucosa of the corpus was scraped off; it amounted to 24% of the mucosa, and contained 35 μg./g. histamine as compared with 108 μg./g. of the remaining deeper part of the mucosa. The same procedure was performed with the mucosa of the pyloric part; the tissue scraped off amounted to 34% of the mucosa, and contained 18 μg. histamine as compared with 86 μg./g. in the rest of the mucosa.

Surprisingly high values of histamine (46–109 μg./g.) were found in the membranous sheath of the submucosa; they were sometimes as high as, or even higher than, those of the mucosa. In all but one piece, between 72 and

85% of the histamine of the stomach wall was accounted for by that present in mucosa and submucosa.

In all parts of the stomach the histamine equivalent of the m. externa was low, the lowest values being obtained in the pyloric region, although here the m. externa may form about two-thirds of the wall. These results are in agreement with those of Gavin *et al.* (1933).

In Expt. 3, two pieces were taken from the corpus of the stomach, one along the greater curvature, the other, opposite to it, along the lesser curvature. The piece from the lesser curvature contained more histamine than that from the greater curvature; the difference was shared by all layers but it was not great (see Table 2).

TABLE 3. Histamine equivalent of wall of intestine

Area in Fig. 1	Expt. no.	μg. histamine/g. in Whole wall	Individual layers GM.	MM.	SM.	ME.	Contribution of each layer to wall: % weight of tissue GM.	MM.	SM.	ME.	% weight of histamine GM.	MM.	SM.	ME.
	1	85	100	115	87	42	20	36	12	32	24	48	12	16
6	2	117	85	155	80	41	24	35	13	28	17	64	9	10
	3	79	68	117	70	37	24	39	11	26	20	58	10	12
	1	86	105	115	85	34	23	37	10	30	28	50	10	12
7	2	89	90	150	50	46	24	32	10	30	24	54	6	16
	3	83	77	117	73	37	25	40	10	25	23	57	9	11
	1	73	95	100	55	27	29	31	9	31	38	43	7	12
8	2	72	90	140	36	24	30	23	10	37	38	45	5	12
	3	99	97	190	70	26	29	29	10	32	29	56	7	8
9	3	66	77	93	55	22	25	38	9	28	29	54	8	9
	1	70	100	90	55	27	22	38	10	30	31	49	8	12
10	2	77	70	160	34	30	25	28	10	37	23	60	3	14
	3	68	80	100	60	24	23·5	36	9·5	31	28	53	8	11
11	3	68	65	125	43	20	17·5	35	12·5	35	17	65	8	11
12	3	57	38	103	28	18	19	36	11	34	13	71	5	11
	1	67	60	130	48	20	30	29	9	32	27	57	7	9
13	2	63	50	180	22	39	33	16	10	41	26	45	4	25
	3	48	16	80	23	13	12	48	12	28	5	79	6	10
	1	53	50	100	40	18	9	35	14	42	9	67	10	14
14	2	49	23	145	25	25	25	20	13	42	12	60	7	21
	3	49	29	87	30	19	10	40	15	35	6	71	9	14
	1	55	11	180	34	7	5	25	16	54	1	82	10	7
15	2	32	11	115	22	6	10	20	20	50	3	73	14	10
	3	46	5	135	32	7	6	27	18	49	1	79	7	13
	1	48	9	160	38	10	5	22	16	57	1	74	13	12
16	2	37	5	100	24	18	8	22	26	44	1	60	17	22

GM=glandularis mucosae; MM.=muscularis mucosae; SM.=submucosa; ME.=muscularis externa.

Small intestine. As seen from Fig. 1, the wall of the small intestine has a high histamine equivalent which gradually decreases from duodenum to ileum and, in the duodenum, the histamine equivalent is even higher than that of the stomach wall of the fundus or corpus. Again, as can be seen from Table 3, the main contribution of histamine is made from the mucosa, and again relatively high values are obtained from the submucosa, whereas the m. externa usually contains less histamine. This layer contributes only between 8 and 25% (average 12%) to the histamine of the wall, although it represents 25–42% (average 33%) of its weight. The highest values (up to 190 μg./g.) were obtained from the m. mucosae, which in all but one piece yielded higher

histamine equivalents than those of the gl. mucosae. The reduction in the histamine content of the wall from duodenum to ileum is shared by all layers except the m. mucosae, so that, in the lower part of the ileum, the m. mucosae is many times richer in histamine than any other layer. The gradient in the histamine content from duodenum to ileum is in part also accounted for by the fact that the m. externa presents a higher percentage of the wall in the ileum than in the duodenum.

The relatively high histamine content of the submucosa is not derived from the adjacent muscularis mucosae by diffusion during the delay in extraction, as shown by the following experiment. A loop of duodenum was opened under chloralose anaesthesia. The mucosa and the m. mucosae were scraped away from the submucosa, allowing the blood to flow freely during this time, and leaving the submucosa in contact only with the histamine-poor m. externa. The portion of duodenum was then excised and the submucosa dissected off and extracted. Its histamine content was 60 μg./g., as compared with 65 μg./g., the histamine content of an adjacent piece of submucosa prepared with the usual technique.

In Expt. 1, the m. externa from the piece lying between nos. 6 and 7 was separated into its two layers, which were extracted separately. The tissue of the circular muscle layer made up two-thirds of the wall and contained 41 μg./g. histamine, whereas the longitudinal muscle layer with serosa contained 23 μg./g. The value for the m. externa calculated from these figures would be 35 μg./g., which has to be compared with 42 and 34 μg./g., the corresponding values found for the m. externa in the adjacent pieces.

Colon and rectum. The histamine values of colon and rectum are lower than those of the lower ileum. The histamine gradient in the small intestines thus continues in the colon and rectum. Only a thin layer of gl. mucosae could be scraped off, and this layer contained very little histamine. No histological examination was made to see if the remainder of the mucosa was free from glandular tissue and consisted solely of m. mucosae; it certainly had a histamine content as high as that of the m. mucosae of the small intestine, whereas the thick m. externa, with a histamine content of 7–18 μg./g. contributed 7–22% of all histamine of the wall.

Presence of an unidentified smooth muscle stimulating substance (substance P) in extracts of the wall of the digestive tract.

When the atropinized ileum preparation was made insensitive to histamine by mepyramine, amounts of extract which had previously caused contraction became inactive, but if the dose of extract was increased five to tenfold, some of the extracts again caused contractions which developed more slowly than those previously produced. These slow contractions were not due to histamine

since the preparation was found to have become insensitive to far greater amounts of histamine than those present in the increased amounts of extract used for testing (Fig. 2). The experiment of Fig. 2 shows further that the substance acting on the preparation insensitive to histamine was destroyed by boiling the extract for a few minutes in strong acid solution. In this experiment *A* corresponds to unboiled extract, *B* to the same extract after having been boiled with N-HCl solution for 10 min. Before mepyramine was given, 1 mg. treated and untreated extract produced the same contraction which was stronger than that produced by 0·1 μg. histamine (not shown in the figure). After treatment with mepyramine, even 2 μg. histamine was ineffective, but progressively larger slow contractions resulted from 4, 6 and 10 mg. of untreated extract, whereas 10 mg. of the treated extract caused no contraction at all.

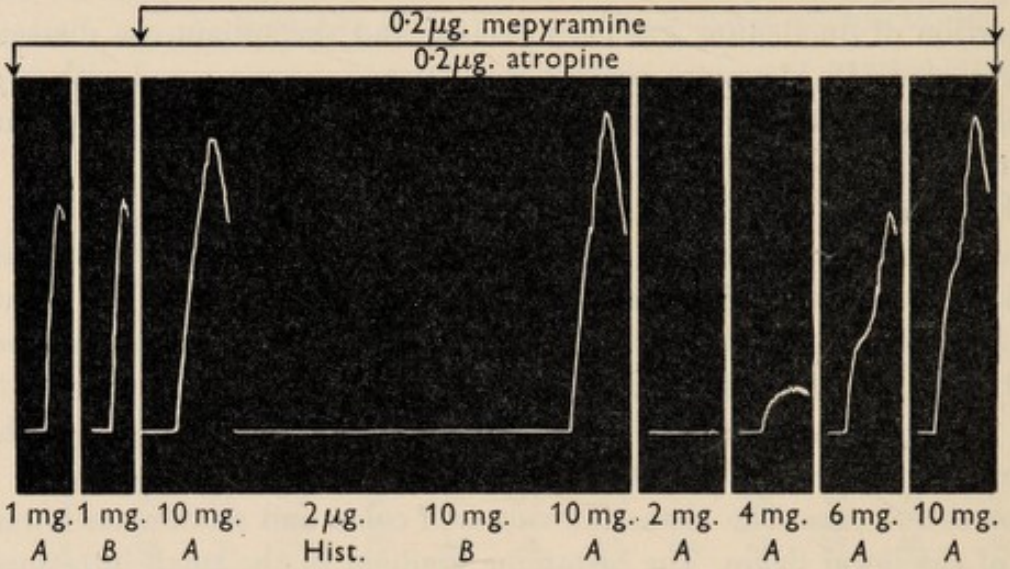


Fig. 2. Guinea-pig's ileum in 15 ml. Tyrode solution containing atropine 1 in 75,000,000. Effects of histamine (Hist.) and extracts from m. mucosae of dog's colon. *A*, untreated extract; *B*, extract after destruction of substance *P* by boiling in strong acid. The mg. refer to fresh weight of m. mucosae. After the second contraction, mepyramine 1 in 75,000,000. (For details see text.)

The activity of the extracts on the histamine-insensitive preparation was probably due to the presence of the substance which Euler & Gaddum (1931) and Gaddum & Schild (1934) found in extracts of intestinal wall, and named substance *P*. The slowness of the contraction, its resistance to atropine and mepyramine resemble the contraction produced by substance *P*. In addition, like substance *P*, the contraction in response to the extracts became successively smaller when the same doses were given at intervals of less than 5 min. Further, the active principle of our extract was destroyed, like substance *P*, by boiling in strong acid or in alkaline solution. In addition, the active extracts cause a slowly developing contraction of the atropinized rabbit's ileum, and a fall of arterial blood-pressure in the atropinized rabbit. In one experiment,

a potent extract was compared with the effects of a sample of substance *P* obtained from von Euler. 1 mg. of this sample had approximately the same activity as the extract from 9 mg. of tissue, both on the intestine and on the blood pressure. Although these preliminary tests do not entitle us to state definitely that the substance of our extract is identical with substance *P*, we shall refer to it as such in the following paragraphs.

As seen from the graded responses obtained with different amounts of extract on the histamine-insensitive ileum preparation of the guinea-pig (Fig. 2), it is possible roughly to compare and assay the activity of different extracts. The comparison is sometimes rendered difficult on account of the presence of an inhibitory substance in the extracts. Similar inhibitory effects have been observed by Gaddum & Schild and could be attributed to the presence of adenosine compounds.

Distribution. There was little or no substance *P* in the extracts from the layers of the wall of the oesophagus, but there was such activity in extracts of the mucosa of the stomach; none was detectable in the extracts of its submucosa or m. externa.

TABLE 4. Substance *P* activity of extracts of the wall of the intestine

Area in Fig. 1	Substance *P* activity of individual layers expressed as percentage of most active extract							
	Expt. 1				Expt. 3			
	GM.	MM.	SM.	ME.	GM.	MM.	SM.	ME.
6	61	61	24	8	85	100	56	23
7	61	55	30	8	100	67	59	20
8	42	52	28	8	55	67	63	23
9	—	—	—	—	20	20	18	5
10	29	45	30	8	30	48	55	18
11	—	—	—	—	45	50	10	11
12	—	—	—	—	20	44	30	10
13	36	33	21	6	20	67	40	17
14	30	39	30	9	40	50	32	20
15	n.d.	100	24	6	25	—	—	—
16	n.d.	76	14	n.d.	—	—	—	—

GM. = glandularis mucosae; MM. = muscularis mucosae; SM. = submucosa; ME. = muscularis externa; n.d. = not detectable.

In the intestine the substance *P* activity, in many extracts, runs parallel to their histamine content. In Expts. 1 and 3 of Fig. 1, the activity of the different samples was assayed against each other, and the highest value obtained referred to in Table 4 as 100. In Expt. 1, a potent sample was also assayed against the sample of substance *P* prepared by von Euler which was at our disposal, and the value 100 corresponded to an activity of 330 mg. substance *P*/g. tissue. It will be seen from Table 4 that the lowest values were obtained with extracts of m. externa, the highest with those of mucosa. More frequently, extracts of m. mucosae were more active than those of

gl. mucosae. The substance *P* activity of extracts of the submucosa was relatively high. The stimulating effect of extracts diminished from duodenum to ileum. This may not have been due entirely to changes in substance *P* content but to an increase of an inhibitory substance present in the extracts.

DISCUSSION

The question of the function of histamine in the wall of the digestive tract is not satisfactorily answered by a study of its distribution alone, but any attempt to ascribe a function to that histamine must take into account (1) that the mucosa is much richer in histamine than the m. externa, (2) that in the mucosa the histamine is not confined to the glandular tissue, but that as far as differentiation between the layers of the mucosa was possible, the m. mucosae contained more than the gl. mucosae, and (3) that the submucosa, a tough membranous sheath which contains neither glandular nor muscular tissue, is relatively rich in histamine.

The presence of histamine in the submucosa raises the question of the structural elements as the source of the histamine. This layer contains nerve fibres and the nerve cells of the submucous plexus, and is highly vascularized. In the dog the mesenteric nerves to the intestine contain large amounts of histamine, but the values found by Euler (1949) were on the whole lower, and certainly not higher than those we found for the submucosa, which consists to a small extent only of nervous tissues. So only part of the histamine in the submucosa would be accounted for by these structures, or we would have to assume that the fine terminal nerve fibres and the cells of the submucous plexus have a much higher histamine content than the mesenteric nerves themselves. If the nervous elements of the submucous plexus were the source of the histamine in the submucosa, it would be expected that the site of the myenteric plexus would also be characterized by a high histamine concentration. This is not so. The plexus is situated between the two layers of the m. externa, and the nerve cells of the plexus adhere to the longitudinal muscle layer when stripped off. This layer, however, was found to contain not more, but slightly less, histamine than the circular muscle layer. There is also insufficient evidence to conclude that the histamine in the submucosa is derived from its vessels. We have knowledge about the histamine content of the large vessels only, and in their walls it occurs in amounts which are at most a few μg./g. tissue (Schild, 1939; Schmitterlöw, 1948).

Previous speculations as to the functions of histamine in the mucosa have been focussed on that of the stomach, but without taking into account the presence of an equally high histamine concentration in the intestinal mucosa. It has been suggested (Babkin, 1938; MacIntosh, 1938; Emmelin & Kahlson, 1944) that the liberation of histamine is an integral link in the excitation of

the oxyntic cells, and that the released histamine appears then in the gastric juice. A strong case can be made out for such an assumption, but there are a few facts which suggest that this function would not account for all the histamine present in the gastric mucosa. The pyloric region contains no oxyntic cells, yet its mucosa is not free of histamine. True, the histamine is reduced to about half that of the fundic mucosa, but even this reduced value represents a high histamine content. It is interesting to note that enteramin has a distribution similar to that of histamine in the mucosa of the stomach (Erspamer, 1940). Furthermore, we find that in the intestine the high histamine content of the mucosa is not confined to gland cells; it is even greater in the m. mucosae. A similar situation therefore cannot be excluded for the mucosa of the stomach.

In the intestinal mucosa we encounter the same difficulty in assessing the function of its histamine as in the gastric mucosa. Histamine stimulates secretion of succus entericus (Koskowski, 1926) just as it does secretion of gastric juice, and it may therefore be a physiological stimulus for intestinal secretion. On the other hand, its role may concern motility of the m. mucosae. Or again, it may play a part in local vascular reactions in response to stimuli comparable in some respects to the role of histamine in the human skin. Perhaps it functions in all three ways.

The histamine of the wall of the digestive tract is not confined to the mucosa, but its concentration in the m. externa is 3–25 times lower than that in the muscle layer of the mucosa, the m. mucosae. It is remarkable that the two layers of smooth muscle in the intestinal wall should differ so greatly in this respect. If motility of smooth muscle in the wall of the digestive tract depends even in part on the histamine content, than a relatively greater state of muscular activity should be readily demonstrable in the m. mucosae than in the m. externa. There have been several attempts in the past to find, in the intestinal wall, gradients either in sensitivity to smooth muscle stimulating substances or in the concentration of such substances in the wall, in order to explain the unidirectional action of peristalsis. Our results show that such a gradient clearly exists for the histamine content of the wall which diminishes from duodenum to ileum. But the gradient is not peculiar to the m. externa but is shared by all layers except the m. mucosae. It is therefore not possible without additional evidence to associate this gradient with the unidirectional nature of peristalsis.

We do not know for certain whether the stimulating effect of our extracts on the atropinized guinea-pig's ileum, rendered insensitive to histamine by mepyramine, was due to the presence of the same principle which Euler & Gaddum (1931), and Gaddum & Schild (1934) found in extracts of the horse intestine, and named substance *P*. This is very likely, but the possibility must be kept in mind that we were dealing with more than one smooth muscle

stimulating substance. In the intestinal wall of the horse most of the substance *P* was found in the muscle layer and not, as in the dog's intestine, in the mucosa. Here the activity was maximal in extracts from m. mucosae. The active substance of our extracts cannot have been enteramin because of its strong stimulating effects on the atropinized guinea-pig's and rabbit's gut. Nevertheless, our extracts probably contained enteramin, which may have augmented the action of substance *P* and so influenced the quantitative aspect of our results. If, as suggested by Euler, substance *P* is responsible for the spontaneous rhythmic movements of the intestine which are not abolished by either atropine or mepyramine, its distribution may be related to the degree of this activity in different species and in the different layers of the intestinal wall. In that case the m. mucosa of the dog should exhibit a high degree of spontaneous activity. Apart from the movements of the villi such activity has been described for the large masses of the m. mucosae of the dog's small intestine by King & Church (1923), and by King & Robinson (1944). In man the muscle is thought to be responsible for a great deal of the intestinal motility. The fact that this layer contains two very active smooth muscle-stimulating substances in high concentration, shows that from the auto-pharmacological point of view this layer, at least in dogs, is endowed with the requirements for such high activity.

Histamine and substance *P* are not the only smooth muscle-stimulating substances found in the wall of the digestive tract. We have already referred to enteramin, but the wall of the digestive tract contains, in addition, choline and acetylcholine which have been extracted from the m. externa and from the mucosa. According to Abderhalden & Paffrath (1925) the mucosa is the main source for the choline of the intestinal wall. No systematic survey has been made of the distribution of acetylcholine in the different layers of the intestinal wall. But the distribution of the enzyme for synthesis of acetylcholine, the choline acetylase, is known (Feldberg & Lin, 1950); it probably runs parallel to the acetylcholine content. In some respects the distribution of the choline acetylase resembles that of histamine and substance *P*. There is more in the mucosa than in the m. externa; in the duodenum it accounts for 70%, in the ileum, 60%. On the other hand, there is this difference, the concentration of choline acetylase is higher in the gl. mucosae than in the m. mucosae, and there is none in the submucosa.

A great deal is known about the specific secretory, absorptive and motor activities of the different hormones found in the gastric and intestinal mucosa; but this is not so with these other more generally distributed and highly active substances, histamine, substance *P*, enteramin, choline and acetylcholine. Yet until their activities are more clearly established, we cannot identify any of them for certain as the physiological stimuli for any of the diverse functions of the wall of the digestive tract.

SUMMARY

1. The histamine equivalents of the different sections and layers of the wall of the dog's digestive tract were determined in extracts prepared by grinding the tissue in acid and boiling the mixture. The extract was assayed after neutralization on the atropinized guinea-pig's ileum with the following results.

(*a*) The wall of the oesophagus contains relatively little histamine.

(*b*) The fundus and the corpus of the stomach wall contain considerable amounts of histamine, and about twice as much as the pyloric region.

(*c*) The wall of the intestine contains large amounts of histamine; the values are highest in duodenum and decrease gradually and continuously down to the rectum.

(*d*) In all regions the greater part of the histamine comes from the mucosa. The submucosa contains relatively high histamine values; those of the muscularis externa are lower than that of any other layer.

(*e*) In those parts of the digestive tract where it is possible to separate the mucosa into two layers, glandularis mucosae and muscularis mucosae, most of the histamine is usually found in the latter.

(*f*) In the intestinal wall the m. mucosae, alone of all layers, retains a consistently high content of histamine from duodenum to rectum. All other layers contain progressively less as the caudal end of the intestine is approached.

2. In addition to histamine a smooth muscle-stimulating substance, resembling substance *P* of Euler & Gaddum, was found in the extracts of the wall of the digestive tract. They caused a contraction of the atropinized guinea-pig's intestine, made insensitive to histamine by mepyramine, and a fall of arterial blood-pressure in the atropinized rabbit. There was little substance *P* activity in extracts of oesophagus, some in extracts of stomach, and much in extracts of small and large intestine. In the intestine it occurred mainly in the mucosa, particularly in the m. mucosae.

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