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DISTRIBUTION OF 5-HYDROXYTRYPTAMINE (SEROTONIN, ENTERAMINE) IN THE WALL OF THE DIGESTIVE TRACT

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Dalgliesh, Toh & Work (1952) have shown that two substances are responsible for the 'enteramine activity' produced on the rat's atropinized colon by acetone extracts of gastro-intestinal mucosa. The substance responsible for the main part of activity was identified as 5-hydroxytryptamine; the other principle has not been isolated but appeared to be another related indole derivative. This identification of 5-hydroxytryptamine in gastro-intestinal mucosal extracts confirms a suggestion made by Erspamer that the enteramine activity of such extracts and of those of the posterior salivary glands of octopus and of skin of amphibia are due to the same active principle. This was isolated from the latter sources and identified as 5-hydroxytryptamine (Erspamer & Asero, 1951, 1952). 5-Hydroxytryptamine has also been isolated from ox serum under the name of serotonin (Rapport, 1949).

The present paper deals with the assay of extracts containing 5-hydroxytryptamine and substance P and the distribution of 5-hydroxytryptamine in the gastro-intestinal wall.

The atropinized rat's colon provided a sensitive preparation for assaying tissue extracts for 5-hydroxytryptamine in the presence of acetylcholine, histamine and substance P. The guinea-pig's ileum under atropine and mepyramine contracted to 5-hydroxytryptamine and substance P, but could be rendered insensitive to 5-hydroxytryptamine by tryptamine (Gaddum, 1952); it could then be used for the assay of substance P in the presence of 5-hydroxytryptamine.

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METHODS

The rat's colon and the guinea-pig's ileum were suspended in 18 ml. baths. In order to reduce spontaneous activity of the rat's colon, the Ca^{++} content of the bath fluid was reduced and its temperature kept at 22–24° C. The method has been described by Dalgliesh *et al.* (1952). The guinea-pig's ileum was suspended in 18 ml. Tyrode solution at 34° C and, to render the preparation insensitive to acetylcholine and histamine, 0·4 μg atropine sulphate and 1 μg mepyramine maleate were added to the bath fluid each time it was renewed. To render the preparation insensitive to 5-hydroxytryptamine, 200 μg tryptamine hydrochloride were added to the bath and kept in it until the tryptamine contraction had passed off. Sometimes another 200 μg were added after 2–3 min, to see if the preparation had become insensitive to the stimulating action of tryptamine itself or of 5-hydroxytryptamine. When this condition was attained, 200 μg tryptamine were added to the bath fluid each time it was renewed.

A standard powder of substance P was prepared according to the method described by Euler (1942) with slight modification introduced by Dalgliesh *et al.* Substance P activity is expressed in mg of this standard preparation. The 5-hydroxytryptamine activity was assayed against a preparation of serotonin creatinine sulphate which was kindly supplied to us by Dr R. K. Richards of Abbott Laboratories. Only about 50% of the serotonin complex consists of the base. The figures given for 5-hydroxytryptamine in this paper refer to the base and not to the complex.

Acetone and acid-water extracts of tissue. The tissue was cleaned in running tap-water, dried as far as possible over blotting-paper and weighed. It was then finely minced with a pair of curved scissors. Acetone extraction was carried out by adding 4 ml. of acetone to each gram of the minced tissue. The extraction was allowed to continue overnight in the cold at about 2° C. The acetone extract was filtered off and the solid residue washed 2–3 times with small volumes of fresh acetone. The combined filtrates were evaporated to dryness *in vacuo* below 35° C. The dried material was taken up in distilled water, so that 1 ml. solution would correspond to 500 mg fresh tissue, and then assayed.

Acid-water extraction was carried out by adding 1 ml. N-HCl + 4 ml. distilled water to each gram minced tissue and boiling for 2 min. The extract was cooled, neutralized with N-NaOH and centrifuged. The supernatant fluid was decanted off and used for the assays.

RESULTS

Assay of tissue extracts against 5-hydroxytryptamine

When acetone extracts of the gastro-intestinal wall were assayed against 5-hydroxytryptamine on the rat's atropinized colon and on the guinea-pig's ileum which had been rendered insensitive to acetylcholine and histamine by atropine and mepyramine, the values obtained in both assays varied. The greatest differences were obtained with extracts from the gastric mucosa of the rabbit. Table 1 shows the differences for two experiments in which acetone

TABLE 1. Comparison of the activity of acetone extracts of rabbit's gastric mucosa on the rat's atropinized colon (Co) and on the guinea-pig's ileum after atropine and mepyramine (Il) in terms of 5-hydroxytryptamine

	5-Hydroxytryptamine in μg/g mucosa			
	Body		Pyloric region	
	(Co)	(Il)	(Co)	(Il)
Expt. 1	10	22·2	1·25	28·6
Expt. 2	7·5	16·7	1·25	8·3

extracts of gastric mucosa of the body and pyloric region were assayed against 5-hydroxytryptamine. The values obtained on the guinea-pig's ileum preparation were between 2 and 23 times higher than those obtained on the rat's colon. It could be shown that the effect on the guinea-pig's ileum was not entirely due to 5-hydroxytryptamine nor to related indole derivatives for the following reason.

Gaddum (1952) has shown that the 'tryptamine receptors' of the guinea-pig's ileum can be rendered insensitive to 5-hydroxytryptamine by keeping large doses of tryptamine in the bath in which the guinea-pig's ileum is suspended. In this condition, the acetone extracts of the gastric mucosa still show a contractile activity on the ileum. This is illustrated in Fig. 1. In (*a*) it is seen that 1 μg 5-hydroxytryptamine corresponded to about 45 mg mucosa of the body and 35 mg of the pyloric region. After treating the preparation with tryptamine (*b* and *c*) it became insensitive to 2 μg 5-hydroxytryptamine but still contracted to extracts equivalent to 70 mg pyloric and 90 mg fundus mucosa respectively.

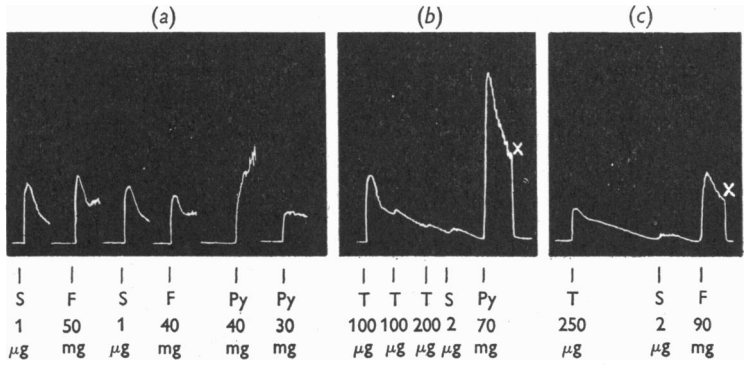

Fig. 1. Guinea-pig's ileum in 18 ml. Tyrode solution containing 0·4 μg atropine sulphate and 1 μg mepyramine maleate. Effects of acetone extracts of body (F) and pyloric region (Py) of rabbit's gastric mucosa; same experiment as no. 1, Table 1. S, 5-hydroxytryptamine (serotonin); T, tryptamine. In (*a*) 5-hydroxytryptamine and extract kept in bath for 1 min; in (*b*) and (*c*) tryptamine, 5-hydroxytryptamine and extract added successively and kept in bath until washed out at *x*.

In corresponding experiments with acetone extracts of the wall of the dog's digestive tract, the differences in the values obtained on the rat's colon and the guinea-pig's ileum were less pronounced and sometimes absent.

The substance in acetone and acidified water extracts which stimulated the guinea-pig's ileum in the presence of tryptamine is probably substance P, the presence of which apparently did not interfere with the assay for 5-hydroxytryptamine on the rat's colon. For this reason, 5-hydroxytryptamine was assayed on the rat's atropinized colon only. Its sensitivity to 5-hydroxytryptamine varied in different experiments. Good contractions could be

obtained with 0·2–0·4 μg; usually the preparations became more sensitive in the course of a prolonged assay, so that they sometimes contracted to 0·02 or 0·04 μg 5-hydroxytryptamine.

It was occasionally observed that the contraction obtained with the crude acetone extracts was stopped short after about 30 sec and that the muscle then relaxed, but when the extract was subsequently washed out, the muscle started to contract again. This was a true after-effect produced by the extract, because in its absence renewal of the bath fluid by itself never produced an effect of this sort. It is best explained by assuming the presence of an inhibitory substance in the crude extracts, the effect of which, however, quickly subsides after washing out, whereas the action of 5-hydroxytryptamine persists for a certain time.

The atropinized rat's colon also contracts to the standard preparation of substance P but is not very sensitive to it. Furthermore, the contraction produced by substance P shows a characteristic difference from the 5-hydroxytryptamine contraction. The latency with substance P is longer; contraction starts only 60–90 sec after its addition to the bath. A similar latency had been observed by Euler (1936) with substance P on the isolated duodenum of the mouse. In addition, the colon, when contracted by substance P, relaxes more slowly after washing out than when contracted by 5-hydroxytryptamine.

The relative insensitivity of the atropinized rat's colon to substance P, and the different time course of its contraction to substance P in comparison to that to 5-hydroxytryptamine, are probably the reasons why the presence of substance P in our extracts did not appear to interfere with the assay for 5-hydroxytryptamine.

Content of 5-hydroxytryptamine in the wall of the digestive tract

Acetone extracts contain, apart from 5-hydroxytryptamine, small amounts of a related substance acting similarly on the rat's colon (Dalgliesh *et al.* 1952). In the following experiments the total activity of the extracts on the rat's colon was expressed in terms of 5-hydroxytryptamine; this has to be kept in mind when assessing the results obtained.

Dog's digestive tract. 5-Hydroxytryptamine is not uniformly distributed in the different layers of the wall and in the various sections of the digestive tract. The mucosa is richest in 5-hydroxytryptamine; the muscularis externa contains only traces. In the mucosa the pyloric region of the stomach usually yielded the highest values and the oesophagus contained only traces. This is shown in Tables 2 and 3.

In Table 2 the distribution of 5-hydroxytryptamine in the various regions of the mucosa is shown. In the first four experiments the samples were assayed against an arbitrary standard acetone extract of dog's intestinal mucosa and the activity expressed as percentage of the most active sample. Expts. 5 and 6

were assayed against 5-hydroxytryptamine, and the absolute values for these two experiments are given in Table 3 which includes values for submucosa and muscularis externa. In one experiment the superficial and deep layer of the duodenal mucosa were extracted and assayed separately. The deep layer containing the base of the glands contained about $2\frac{1}{2}$ times as much 5-hydroxytryptamine per gram fresh tissue as the superficial layer.

In one experiment acid-water extracts from the mucosa of the stomach and duodenum were assayed against 5-hydroxytryptamine. The results are given

TABLE 2. Distribution of 5-hydroxytryptamine activity in the mucosa of the dog's digestive tract. Values expressed as percentage of the most active region

Expt. No.	Oesophagus	Stomach		Duodenum	Jejunum	Ileum	Colon
		Body	Pylorus				
1	—	60	68	68	100	75	36
2	—	25	100	13	16	13	13
3	—	50	100	75	54	50	50
4	—	25	100	40	33	40	40
5	6	50	100	87	40	50	68
6	—	60	96	100	80	61	61

TABLE 3. Content of 5-hydroxytryptamine in acetone extracts of dog's digestive tract

Region	5-Hydroxytryptamine in μg/g fresh weight					
	Mucosa		Submucosa plus muscularis mucosae		Muscularis externa	
	Expt. 5	Expt. 6	Expt. 5	Expt. 6	Expt. 5	Expt. 6
Oesophagus	0·6	—	—	—	—	—
Stomach body	5	5	—•	0·08	—	0·015
Stomach pylorus	10	8	—	0·15	—	Traces
Duodenum	8·7	8·3	—	0·2	—	0·15
Jejunum	4	6	—	—	—	—
Ileum	5·1	5·7	—	—	—	—
Colon	6·8	5·7	—	—	—	—

TABLE 4. Assay of acid-water extracts of mucosa for 5-hydroxytryptamine, histamine and substance P. Substance P assayed on guinea-pig's ileum before and after treatment with tryptamine

Mucosal extract from	5-Hydroxy-tryptamine in μg/g	Histamine in μg/g	Assay against substance P (mg/g) on guinea-pig's ileum	
			Before tryptamine	After tryptamine
Stomach body	5·2	80	About 7	0
Stomach pylorus	9·8	46	20	8
Duodenum	3·2	74	55	45

in Table 4. It will be seen that the values obtained are of the same order as those obtained with acetone extraction and, again, that the pyloric region of the stomach yielded the highest value.

Mucosa of rabbit's stomach. The distribution of 5-hydroxytryptamine in the stomach is different in the rabbit and in the dog. In the dog, pyloric mucosa yielded higher values than the mucosa of the body. In rabbits Erspamer (1940) obtained the opposite result. This we have been able to confirm, as seen from

the experiments given in Table 1. The values for the mucosa of the body of the rabbit's stomach are of the same order (7·5–10 μg/g) as those for the pyloric mucosa of the dog (8–10 μg/g).

In Expt. 1 of Table 1, the mucosa of the body of the stomach after extraction with acetone was re-extracted with distilled water (pH of extract 7); the activity of the aqueous extract was less than 0·4 μg 5-hydroxytryptamine/g tissue, which shows that the acetone extraction had removed almost all 5-hydroxytryptamine.

Assay of tissue extracts against substance P

The fact that both 5-hydroxytryptamine and substance P contract the guinea-pig's ileum which has been rendered insensitive to acetylcholine and histamine has to be kept in mind when this preparation is used for the assay of tissue extracts which may contain substance P as well as 5-hydroxytryptamine. This preparation has, for instance, been used in preliminary experiments for the assay of substance P by Douglas, Feldberg, Paton & Schachter (1951). They obtained extremely high values when assaying their acid-saline extracts of the dog's gastro-intestinal wall in this way. The activity of one extract of duodenal mucosa corresponded to 330 mg substance P per gram tissue. The authors themselves suggested that these high values were probably erroneous and due to activity of substances other than substance P. In fact, it was suggested that enteramine (5-hydroxytryptamine) was present in the extracts and responsible for the high values obtained. That this was probably so is evident from the following experiments in which contractions produced by a given dose of substance P in the presence and absence of small amounts of 5-hydroxytryptamine are compared.

In the experiment of Fig. 2*a*, the addition of 0·2 and 0·4 μg 5-hydroxytryptamine to 1 mg substance P produced contractions slightly larger than those obtained by 1·2 and 1·5 mg substance P alone, although these amounts of 5-hydroxytryptamine in themselves had little stimulating action. In another experiment, 0·2 μg 5-hydroxytryptamine plus 0·5 mg substance P produced an effect given by 1·25 mg substance P alone.

These results suggest that in order to assay tissue extracts for substance P in the presence of 5-hydroxytryptamine, the guinea-pig's ileum preparation must first be rendered insensitive to any 5-hydroxytryptamine that may be present. For this purpose tryptamine was used.

Tryptamine itself has a stimulating action on the guinea-pig's ileum rendered insensitive to acetylcholine and histamine. The contraction is not sustained, and relaxation sets in although the tryptamine remains in the bath. During the relaxation spontaneous contractions often appear or become stronger, as seen in Fig. 4. When the preparation has relaxed, it no longer contracts either to further doses of tryptamine or to 5-hydroxytryptamine. This was first

shown by Gaddum (1952). In addition, the tryptamine produces the following changes: (1) an increase or appearance of spontaneous, small contractions; (2) an increase in the stimulating effect of substance P.

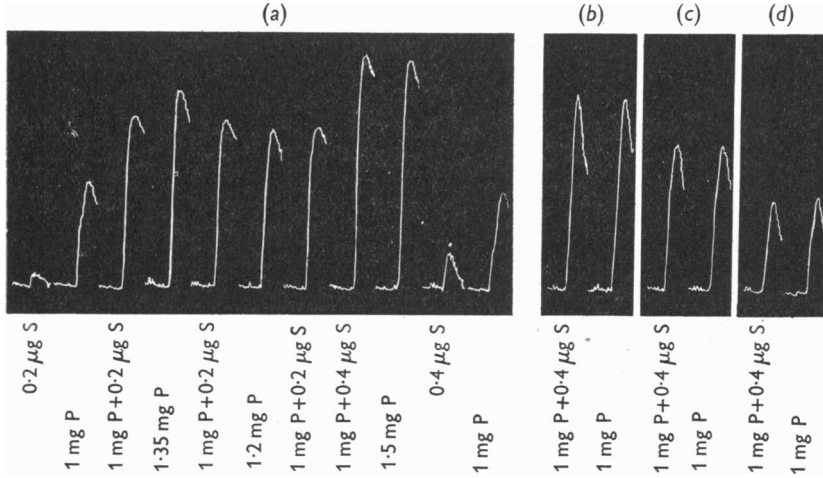

Fig. 2. Contractions of guinea-pig's ileum in 18 ml. Tyrode solution containing 0·4 μg atropine sulphate and 1 mg mepyramine maleate. In (*b*), (*c*) and (*d*) Tyrode solution contained 200 μg tryptamine as well. 5-Hydroxytryptamine (S) and substance P (P) left in the bath for 1 min and given at 5 min intervals. In (*b*) 20 min, in (*c*) 40 min and in (*d*) 95 min had elapsed since tryptamine was first added to the Tyrode solution.

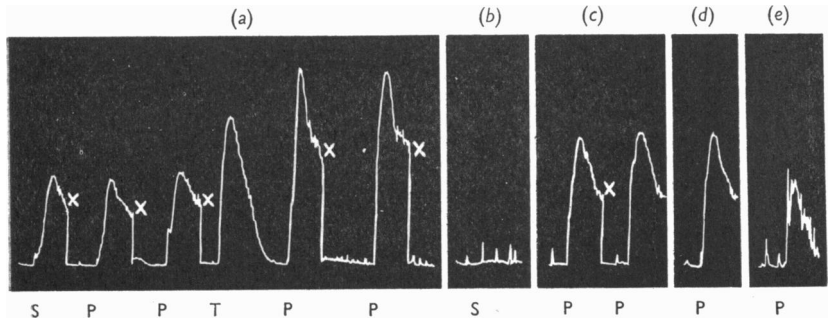

Fig. 3. Guinea-pig's ileum in 18 ml. Tyrode solution containing 0·4 μg atropine sulphate and 1 μg mepyramine maleate. 0·25 μg 5-hydroxytryptamine (S) and 1 mg substance P (P) given at 5 min intervals and left in the bath for 90 sec. 0·2 mg tryptamine (T) added to bath and maintained in the bath until the end of (*d*), when the amount of tryptamine was increased to 2 mg at (*e*). At (*d*) 80 min had elapsed since the beginning of the tryptamine treatment. At (*x*) the bath fluid was renewed whilst the drum was stopped.

The experiment of Fig. 3 illustrates the appearance of spontaneous contractions and the augmentation of the substance P contraction after treatment with tryptamine. In this experiment the effects of 1 mg substance P corresponded to that of 0·25 μg 5-hydroxytryptamine. 200 μg tryptamine

caused a transient contraction and thereafter small, spontaneous contractions occurred and the effect of 1 mg substance P was greatly enhanced, whereas 5-hydroxytryptamine had become ineffective. The increased sensitivity to substance P declines gradually in the course of 1 hr, after which practically identical responses can be obtained with the same dose of substance P on repeated administration. If the dose of tryptamine is now increased to 2 mg, the effect of substance P becomes depressed and remains depressed as long as the high concentration of tryptamine is retained in the bath. Washing out the tryptamine leads to only partial recovery of the sensitivity to substance P and, if a small dose (0·2 mg) of tryptamine is given later, some sensitization to substance P again occurs.

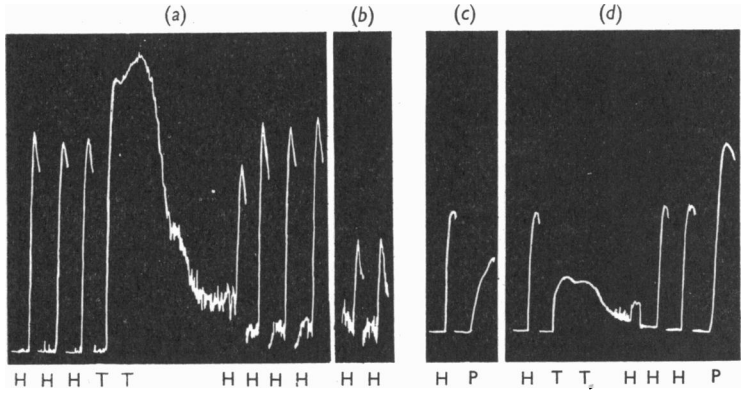

Fig. 4. Two experiments on guinea-pig's ileum in 18 ml. Tyrode solution containing 0·4 μg atropine sulphate. H = 0·075 μg histamine, kept in bath for 20 sec and given at 90 sec intervals. P = 1 mg substance P, kept in bath for 60 sec. T = 200 μg tryptamine which in the second experiment was kept in the bath till the end of (*d*), and in the first experiment till the end of (*a*), when the amount was increased to 600 μg and kept in the bath till the end of (*b*).

The sensitization to substance P by a relatively small dose of tryptamine (200 μg) also occurs in the absence of mepyramine when the preparation is sensitive to histamine. The sensitivity to histamine, however, remains practically unchanged in this condition, as illustrated in the two experiments of Fig. 4. In the first experiment (Fig. 4*a*) tryptamine increases the spontaneous contractions without altering the sensitivity of the preparation to histamine. When the dose of tryptamine is increased, the sensitivity to histamine, however, becomes depressed (Fig. 4*b*). The second experiment (Fig. 4*c*, *d*) shows the increase in sensitivity to substance P but not to histamine.

The importance of rendering the guinea-pig's ileum preparation insensitive to 5-hydroxytryptamine when assaying in its presence for substance P is illustrated by the experiment of Table 4, which gives the results of assays of acid-water extracts prepared from mucosa of the dog's stomach and duodenum.

The content of 5-hydroxytryptamine was first assayed on the rat's atropinized colon. Then the histamine content was assayed on the guinea-pig's atropinized ileum which thereafter was rendered insensitive to histamine by mepyramine. The stimulating action still obtainable with the extracts was then assayed against substance P before and after tryptamine treatment. The duodenal extracts contained relatively little 5-hydroxytryptamine and much substance P. The difference in the assay for substance P before and after tryptamine was accordingly small. The extracts of the pyloric mucosa contained greater amounts of 5-hydroxytryptamine and small amounts of substance P than the duodenal extracts. Accordingly, the difference between the values in the assay for substance P before and after treatment with tryptamine was much greater. In fact, after treatment with tryptamine, the extracts when given alone produced no contraction, because the amounts of substance P present were too small to be detected in this way. The value of 8 mg/g was obtained by the finding that 25 mg extract plus 0·3 mg substance P caused the same small contractions as 37·5 mg extract plus 0·2 mg substance P. Therefore the effect of 0·1 mg substance P corresponded to that of about 12·5 mg extract. It is not certain that the effect of the 12·5 mg extract is actually due to substance P, but the extract certainly contained no more than 8 mg substance P per gram. In the extracts of the mucosa of the body of the stomach no substance P could be detected, even when the assay was performed as with the pyloric extract: i.e. in combination with small amounts of substance P.

DISCUSSION

The results presented in this paper show that it is possible to assay small quantities of 5-hydroxytryptamine and substance P pharmacologically when they are both present in tissue extracts. The presence of substance P does not interfere, or not greatly, with the assay for 5-hydroxytryptamine on the rat's atropinized colon.

With this method it was therefore possible to show that the 5-hydroxytryptamine which is present in the wall of the gastro-intestinal tract is practically all derived from the mucosa and that the muscularis externa contains only traces. In the mucosa the distribution of 5-hydroxytryptamine in different sections of the tract varies in the dog and rabbit. In the dog the highest concentration was found in the pyloric mucosa of the stomach, in the rabbit in the mucosa of the body of the stomach. The amounts present in the mucosa vary between 4 and 10 μg/g. We have to remember, however, that some of the activity which was assayed may have been due not to 5-hydroxytryptamine itself but to a related indole derivative.

It is interesting to note that apart from occurring in platelets 5-hydroxytryptamine has been found only in organs which contain a high proportion of glandular tissue. This may indicate that one of the physiological functions of

5-hydroxytryptamine is concerned with secretory activity. In fact, Bacq, Fischer & Ghiretti (1952) have shown that this substance occurs in the saliva of perfused octopus salivary glands on nerve stimulation and has a secretory effect on these glands. The ability of 5-hydroxytryptamine to stimulate some smooth muscles in low concentrations does not necessarily imply a physiological role of 5-hydroxytryptamine in smooth muscle activity.

In order to assay tissue extracts for substance P on the guinea-pig's ileum rendered insensitive to acetylcholine and histamine, it is first necessary to find out if the extracts contain 5-hydroxytryptamine as well. In that case the extracts can be assayed on the guinea-pig's ileum after treatment with tryptamine. The procedure to be recommended when using an 18 ml. bath would be to add 0·4 μg atropine, 1 μg mepyramine and 200 μg tryptamine to the bath each time the fluid is replaced, to leave the extracts in the bath for 60–90 sec, and to repeat the test every fifth min. Tissue extracts may contain, in addition to substance P, other unknown, similarly acting, smooth muscle stimulating substances. It would be interesting to know if the amount of substance P which is precipitated with ammonium sulphate from crude tissue extracts, according to the method used by Pernow (1951), corresponds to the activity of such crude extracts when assayed on the guinea-pig's ileum rendered insensitive to 5-hydroxytryptamine, histamine and acetylcholine.

Our results show, further, that the very high values for substance P obtained by Douglas *et al.* (1951) in preliminary assays of gastro-intestinal tissue extracts were, at least in part, due to the additional presence of 5-hydroxytryptamine.

The sensitization to substance P which tryptamine induces on the guinea-pig's ileum is peculiar, in that it is not shown by histamine. The fact that the guinea-pig's ileum preparation in this condition also often shows increased spontaneous activity, or the appearance of such activity, is interesting in connexion with Euler's suggestion (1936) that substance P is responsible for spontaneous activity and might suggest that this increased activity is the result of increased sensitivity to the release of endogenous substance P. Against this view there is the fact that, with increasing doses of tryptamine which depress substance P activity, the spontaneous contractions do not always diminish.

SUMMARY

1. The 5-hydroxytryptamine content of extracts of gastro-intestinal tract of dogs and of the gastric mucosa of rabbits was assayed on the rat's atropinized colon.

2. The muscularis externa of the dog's gastro-intestinal tract contains no 5-hydroxytryptamine, or only traces, whereas the mucosa contains between 4 and 10 μg/g. The highest concentration was usually found in the mucosa of

the pyloric region of the stomach; traces were found in the mucosa of the oesophagus.

3. Unlike the mucosa of the dog's stomach, that of the rabbit's stomach contains more 5-hydroxytryptamine in the region of the body than in the pyloric region.

4. The guinea-pig's ileum rendered insensitive to acetylcholine and histamine by atropine and mepyramine can be used for assaying tissue extracts for substance P activity only if the extracts are free from 5-hydroxytryptamine. Otherwise the preparation must first be rendered insensitive to 5-hydroxytryptamine as well, by continuous treatment with tryptamine.

5. Tryptamine has a transient stimulating effect on the guinea-pig's ileum. In doses in which it renders the preparation insensitive to 5-hydroxytryptamine, it renders the preparation more sensitive to the action of substance P but not to that of histamine; in addition, it promotes the appearance of spontaneous activity.

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