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FRACTIONATION OF THE SMOOTH MUSCLE STIMULANTS PRESENT IN EXTRACTS OF GASTRO-INTESTINAL TRACT. IDENTIFICATION OF 5-HYDROXYTRYPTAMINE AND ITS DISTINCTION FROM SUBSTANCE P

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Extracts of the gastro-intestinal tract contain a number of smooth-muscle stimulating substances, three of these being choline, acetylcholine and histamine. Smooth-muscle preparations rendered insensitive to all three substances by suitable inhibitors are still stimulated by crude extracts, and at various times efforts have been made to characterize the active substances present in such extracts. The situation has been confused by the invention of a series of names for these unidentified smooth-muscle stimulants without the provision of satisfactory evidence that each name represents a chemical entity. Ivy & Oldberg (1928) attributed the activity to cholecystokinin; Euler & Gaddum (1931) obtained an active extract to which they gave the name 'substance P'; Kokas & Ludány (1933) called their active preparation villikinin, and finally Erspamer (1940) added the name enteramine. Each of these names has been regarded as representing a separate substance, but evidence for this view has not always been convincing.

In the present investigation we have been able to show that the enteramine activity of extracts of the gastro-intestinal tract is due to 5-hydroxytryptamine and that these extracts contain another smooth-muscle stimulating substance which is probably an indole derivative of related structure. Evidence is also presented that the substance P activity of crude extracts is not due to 5-hydroxytryptamine.

During the later course of our investigation, Erspamer & Asero (1951, 1952) announced the isolation of 5-hydroxytryptamine from extracts of the posterior salivary glands of octopus and from the skin of amphibia. Their identification of 5-hydroxytryptamine with enteramine has been confirmed by our characterization of the substance from mammalian intestine. 5-Hydroxytryptamine

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has also been isolated from beef serum under the name of serotonin (Rapport, 1949).

Substance P and 5-hydroxytryptamine have been shown to have different pharmacological activities.

MATERIALS AND METHODS

In the absence of any knowledge of the nature of substance P and of enteramine, it was necessary in the first instance to prepare a crude concentrate of each and to assign to this a standard potency. This had to be done despite our belief that in measuring our standard by a suitable physiological response we might well be measuring a summation of responses due to several different materials.

Standard preparation of substance P. Small intestine from the horse (10 kg) was cleaned, minced and extracted with water (20 l.) containing a trace of HCl (pH about 3·0). The method of Euler (1942) for the preparation of substance P was then followed as far as the precipitation with saturated $(NH_4)_2SO_4$. The dried precipitate weighed 12 g. In order to remove ammonia, the solid extract (12 g) was suspended in water (50 ml.) and NaOH added to pH 8·0. Ethanol (250 ml.) was added and the alcoholic solution was heated at 35–40° *in vacuo* until no more ammonia was evolved. The ethanol which had evaporated was replaced and the solution stored at 0° for 20 hr. The precipitate was discarded and the filtrate taken to dryness *in vacuo*. The residual powder (5 g) was the standard preparation of substance P.

Assay of the powder on a guinea-pig ileum preparation before and after treatment with mepyramine showed the presence of a small histamine-like reaction equivalent to less than 0·04 μg histamine/mg powder. The preparation was free from detectable amounts of acetylcholine and choline.

Standard preparation of enteramine. A dog was bled under pentobarbitone and the small intestine removed. The tissue was irrigated with tap water and freed, as far as possible, from mesenteric fat. The intestine was slit longitudinally and the surface of the mucosa swabbed with dry cotton-wool. The mucosa was scraped from the submucosa and muscularis propria with a large scalpel and transferred to a blender. The homogenate from the blender was poured into cold acetone (3·5 vol.) and the suspension stored at 0° for 48 hr with occasional stirring. The suspension was filtered, acetone was removed *in vacuo* without raising the temperature above 35° and the residual aqueous solution was extracted twice with half its volume of light petroleum (b.p. 60–80°) and twice with half its volume of ethyl acetate. Dissolved ethyl acetate was extracted from the water layer with peroxide-free ether and the dissolved ether removed *in vacuo*. From 219 g mucosa treated in this way we obtained 2·266 g solids.

Assay of substance P and enteramine

Substance P activity was assayed on the guinea-pig ileum suspended in 18 ml. Tyrode solution, and rendered insensitive to acetylcholine and choline by atropine and to histamine by mepyramine (Douglas, Feldberg, Paton & Schachter, 1951). The extract was kept in the bath for 60 sec before being washed out by overflow. Successive tests were made at intervals of 4 min. A contractile response was obtained with 200 μg of standard preparation of substance P. Discrimination between increasing doses of substance P was not sharp; a dosage difference of 20% could not be detected with certainty, but a difference of 30% was always detectable. It will be shown elsewhere that this kind of assay of substance P activity becomes inaccurate when extracts contain a high proportion of enteramine activity as well, and that the preparation has then to be rendered insensitive to enteramine (Feldberg & Toh, 1953).

Enteramine. Erspamer (1940, 1948) used for the routine assay of enteramine a uterus preparation obtained from ovariectomized rats brought into oestrus by injection of ovarian hormone. This procedure was regarded as cumbersome and satisfactory results were obtained with the rat's colon.

Use was made of the observation of Garcia de Jalon, Bayo Bayo & Garcia de Jalon (1945) that the spontaneous activity of rat smooth muscle (uterus) could be reduced by lowering the temperature and Ca^{++} content of the bathing solution. A rat's colon, the part nearer the caecum, was suspended in 18 ml. of solution of the following composition: NaCl 9·0, KCl 0·4, $CaCl_2$ 0·03, $NaHCO_3$ 0·15, glucose 1·0 g/l. (Gaddum, Peart & Vogt, 1949). The temperature of the bath was 22–24°. With this preparation there was no response with histamine up to 100 μg. Response to acetylcholine was abolished by atropine. A strong contraction was produced in the presence of atropine by 0·4 mg of our standard preparation of enteramine. This quantity was then designated as one unit. The test sample was left in the bath for 1·25 min and successive tests were made at intervals of 4·5 min (see Fig. 1).

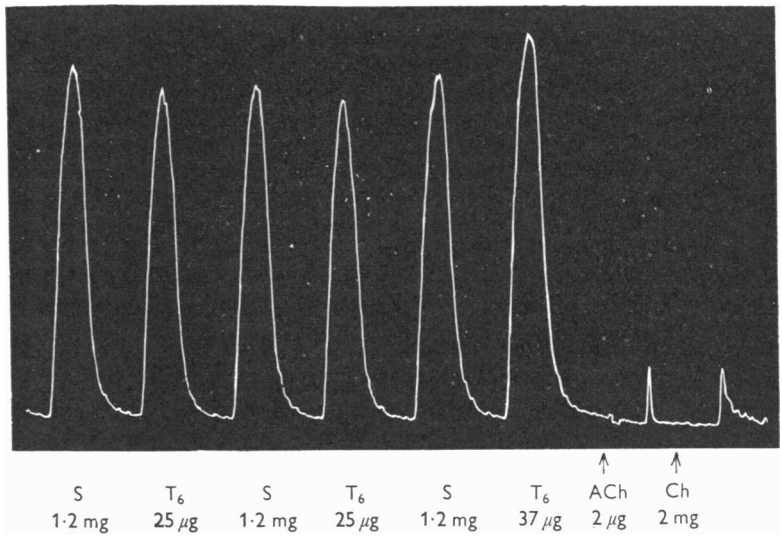

Fig. 1. Rat's colon suspended in 18 ml. saline solution (Gaddum *et al.* 1949) containing 0·5 μg atropine sulphate. Test substances were added to the bath at 4·5 min intervals and kept in the bath for 1·25 min. S = standard preparation of enteramine (see text). T_6 = fraction from a counter-current distribution experiment. ACh = acetylcholine. Ch = choline.

RESULTS

Fractionation of enteramine concentrates

An aqueous solution of our standard preparation was stable at 100° over the range pH 3–7, but in more acidic or alkaline solutions it was destroyed. The activity was not extractable from aqueous solutions by benzene, ether, chloroform, ethyl acetate or light petroleum, but was extractable by *n*-butanol, *iso*butanol, *sec.*-butanol or ethyl methyl ketone. Various solid absorbents such as charcoal, kieselguhr and alumina were tried in the hope that one or another might be useful in purifying the active principle, but in every case the solid either failed to adsorb the active component, or if it did so we failed to find a suitable elution procedure.

Counter-current distribution. The observation that enteramine activity could be extracted from aqueous solutions by some organic solvents suggested the possibility of applying the counter-current methods of Craig (1949) or Bush & Densen (1948). Preliminary experiments showed that the distribution of enteramine between water and *n*-butanol was not sensitive to small changes in pH and accordingly, in large-scale experiments, water and *n*-butanol were used as the two phases without any buffer.

Experiments using the method of Bush & Densen (1948) and a three-funnel distribution between water (1 vol.) and butanol (2 vol.) suggested a partition coefficient of the active component in favour of butanol. A mucosal extract prepared by the method used for the standard enteramine preparation (976 mg) was then partitioned in a 12-tube all-glass counter-current apparatus of the type described by Craig & Post (1949). The heavier phase was water-saturated with butanol (92 ml.) and the lighter moving phase, butanol saturated with water (25 ml.). The sample was dissolved in the lower phase of tube 1 and all the other tubes filled with lower phase only. After equilibration of the two phases in tube 1 the upper phase in this tube was transferred to tube 2 and replaced by fresh water-saturated butanol. The process was repeated until all twelve tubes contained a fully equilibrated two-phase system and the total contents of each tube were then evaporated to dryness *in vacuo* below 35° C. The dry material in each tube was weighed, dissolved in water and assayed on the atropinized rat colon. The results are shown in Table 1.

TABLE 1. Counter-current fractionation of acetone extract of dog intestinal mucosa partitioned between butanol and water. Twelve-tube fractionation; upper layers 25 ml., lower 92 ml.

Tube no.	Total solids (mg)	Activity (units/mg)	Total activity in tube (units)
1	726	0·33	242
2	94·4	—	—
3	27·2	1·3	35·6
4	12·3	1·9	23·4
5	6·6	4·0	26·4
6	4·1	6·7	27·5
7	1·1	53·3	58·6
8	1·2	53·3	63·9
9	2·0	26·3	52·6
10	3·1	10·0	31·0
11	9·1	1·2	10·8
12	6·8	1·1	7·5
Total recovery	894	—	599·3
Total before fractionation	976	—	1180

(For definition of the unit see text.)

From the data given in Table 1 it seemed probable that two active substances were present in the standard enteramine preparation, and that one of these was readily extractable from water into butanol.

Fractionation of extracts of horse's small intestine

To confirm the suggestion of the existence of two active components in the standard enteramine preparation it was necessary to fractionate a larger quantity of material. It was not possible to obtain sufficient material for this purpose from a single dog, and further experiments were therefore made using extracts of horse intestine.

The small intestine from a horse (4 kg) was minced and extracted with cold acetone in the usual way. The acetone extract was concentrated to about 1 l. and extracted three times with half its volume of light petroleum (b.p. 60–80° C) and thrice with similar volumes of ethyl acetate. The residual aqueous phase contained 38 g solids. It was concentrated *in vacuo* to 400 ml. and extracted eight times with 200 ml. lots of butanol. The butanol extracts were combined and evaporated *in vacuo* below 45° C. The residue was dissolved in cold methanol (20 ml.) and filtered after 45 min at 0° C. The solid (1 g) was discarded and the filtrate taken to dryness *in vacuo*. The residue (2·98 g) was dissolved in water (100 ml.), and a portion assayed on the atropinized rat colon for enteramine activity and compared with the standard enteramine preparation from dog mucosa. The total activity in 2·98 g was equivalent to about 20,000 units of the standard preparation.

Counter-current distribution. Fractionation was carried out in the 12-tube all-glass apparatus already described. Instead, however, of a 12-stage fractionation, the method of Bush & Densen (1948) was followed so that twelve butanol (each 41 ml.) and twelve water (each 92 ml.) fractions were obtained. The fractionation was begun with all the material (2742 mg) in the water phase of tube 1 and the distribution of material and of activity in the twenty-four fractions is shown in Table 2.

The distribution of activity again suggested the presence of at least two active components in the enteramine concentrate. The considerable loss in total activity may be due to partial decomposition of the active principles, or possibly also to the action of one of the active principles being potentiated by the presence of the other.

Distinction between enteramine concentrates and substance P

It seemed possible that one or other of the activity peaks in the counter-current fractionation (Table 2) might be due to substance P, and a comparison was therefore made between the pooled aqueous fractions 2–6, hereafter designated FA, the pooled butanol fractions 16–22, designated FC, and the standard preparation of substance P (p. 299). The following tests were used: (*a*) Guinea-pig ileum preparation in a bath of magnesium-free Tyrode solution (18 ml.) containing atropine (0·4 μg) and mepyramine (0·4 μg). The substance under test was left in contact with the preparation for 90 sec and then washed

out. (*b*) Rabbit small intestine preparation in magnesium-free Tyrode solution (18 ml.) containing atropine (0·5 μg). Each test sample was washed out after 1·5–2 min. (*c*) Atropinized rat colon preparation; the conditions for use of this preparation with enteramine concentrates have already been described (p. 299). Owing to the considerable latent period with substance P, samples were kept in the bath for up to 2·5 min. (*d*) Blood pressure test on rabbit.

TABLE 2. Counter-current fractionation of acetone extract of horse small intestine partitioned between butanol and water. 24-stage fractionation by method of Bush & Densen (1948), upper layers 41 ml., lower 92 ml.

Fraction no.		Total solids (mg)	Activity (units/mg)	Total activity in fraction (units)
Water	1	1373·5	0·3	412
	2	380·8	3·3	1257
	3	186·5	7·0	1305
	4	107·8	8·0	860
	5	72·8	8·5	604
	6	53·5	10·0	535
	7	27·8	10·0	278
	8	25·0	16·0	460
	9	19·3	20·0	386
	10	16·0	29·2	467
	11	11·3	26·7	302
	12	11·2	30·0	336
Butanol	13	6·0	35·0	210
	14	5·9	44·4	262
	15	6·2	28·5	177
	16	9·6	20·0	192
	17	7·1	40·0	284
	18	8·2	40·0	328
	19	7·6	57·0	433
	20	4·3	62·5	269
	21	7·5	55·0	413
	22	11·5	12·5	144
	23	18·2	4·4	80
	24	14·8	3·1	46
Total recovery		2392·4	—	10040
Total before fractionation		2742	—	18370

(For definition of the unit see text.)

The depressor effect of each sample on the arterial blood pressure of the rabbit was measured under pentobarbitone anaesthesia after intravenous injection of atropine (1 mg/kg) and mepyramine (1 mg/kg). The effects of FA, FC and our standard preparation of substance P are compared in Table 3.

Neither FA nor FC showed the latency period on the rat colon preparation characteristic of substance P and, although on this preparation both FA and FC were very much more active than substance P, they were not significantly more active than substance P in any of the other tests. It was concluded, therefore, that fractions FA and FC contained smooth-muscle stimulating substances different from that present in substance P and most effectively assayed on the rat colon preparation. Small qualitative differences between the responses to the different samples were also observed, but since all three

samples were relatively crude concentrates, all possibly containing more than one smooth-muscle stimulating substance, it is inadvisable to attach too much significance to these differences. A trial partition of the standard preparation of substance P between butanol and water suggested that the preparation contained at least two smooth-muscle stimulating substances. This point has not been pursued.

TABLE 3. Comparison of the pharmacological activity of substance P, enteramine fraction FA and enteramine fraction FC. (The figures refer to the weight of each substance required to produce comparable responses in each test.)

	Amounts giving equal response		
	FA (mg)	P (mg)	FA : P
Rabbit's blood pressure	1·0	1·0	1 : 1
Guinea-pig's ileum	1·0	1·0	1 : 1
Rabbit's intestine	1·7	0·5	1 : 0·3
Rat's colon	0·1	2·0	1 : 20
	FC (mg)	P (mg)	FC : P
Rabbit's blood pressure	0·5	1·0	1 : 2
Guinea-pig's ileum	0·06	0·6	1 : 10
Rabbit's intestine	0·05	0·5	1 : 10
Rat's colon	0·015	1·0	1 : 66

Identification of 5-hydroxytryptamine in extracts of small intestine

While experiments were in progress on the further purification of fractions FA and FC, Erspamer & Asero (1951, 1952) reported the isolation of 5-hydroxytryptamine from octopus salivary gland and suggested that it was identical with enteramine. Since our enteramine preparation appeared to contain two smooth-muscle contracting substances, it seemed possible that one of these might be 5-hydroxytryptamine; further experiments confirmed this suspicion.

Preparation of concentrate. The first 10 ft. of pig small intestine were cleaned, freed from fat, minced (6·3 kg) and extracted with cold acetone in the usual way. The acetone extract was concentrated to about 1 l. and washed thrice with its own volume of light petroleum (b.p. 60–80°). The pH of the aqueous solution was adjusted to 4·7.

Electrodialysis. The apparatus used was a four-chamber cell of the type described by Synge (1951). The enteramine concentrate (400 ml.) was placed in chamber 2, no. 1 being the cathode. The other chambers were filled with 0·01 N-HCl and a potential difference of 200 V applied across the electrodes (platinum). The pH of the cathode compartment was kept below 8 by frequent addition of small lots of 2 N-HCl. After 2 hr the cathode solution was replaced by fresh 0·01 N-HCl and electrodialysis continued for another 2 hr. During electrodialysis samples were withdrawn and tested on the atropinized rat colon. Chambers 3 and 4 never contained any active material. The initial activity of chamber 2 declined and activity appeared in the cathode compartment. The two cathode solutions were combined, adjusted to pH 4·5 and

concentrated *in vacuo*. The whole of the enteramine extract processed in this way gave 170 ml. of concentrated catholyte.

Counter-current fractionation. The concentrated catholyte was fractionated by the method already described for the extract from horse intestine. Twelve butanol fractions (46 ml. each) and twelve water fractions (92 ml. each) were collected, and the information given in Table 2 was used as a guide in grouping the various fractions. Water fractions F_1, F_2 and F_3 were kept separate and concentrated *in vacuo* below 35° C. Fractions F_{4-6}, F_{7-9} and F_{10-12} and butanol fractions F_{13-15}, F_{16-18}, F_{19-21} and F_{22-24} were grouped and similarly concentrated.

Paper chromatography. No quantitative estimation was made of the biological activity of the various fractions from the counter-current fractionation, nor were the total solids estimated. A small sample from each concentrate was transferred to Whatman no. 4 or no. 1 paper, and the correct quantity for chromatography found by trial. Synthetic 5-hydroxytryptamine and tryptamine were used as reference substances and one-dimensional chromatograms (descending) were developed with butanol–acetic acid (4:1:5 mixture of Partridge, 1946). No. 4 paper was found most suitable for fractions 1–12 and no. 1 paper for fractions 13–24. After drying, the papers were sprayed with one or other of the following reagents: Ehrlich's *p*-dimethylaminobenzaldehyde reagent (Dalgliesh, 1952), Pauly's diazotized sulphanilic acid reagent (Dalgliesh, 1952), the Folin-Denis reagent for phenols (Folin & Denis, 1912), ninhydrin or silver nitrate.

Aqueous fractions: identification of 5-hydroxytryptamine

The aqueous fractions F_2, F_3, F_{4-6}, F_{7-9} and F_{10-12} all contained a substance which had the same R_F value (0·40) in butanol–acetic acid on Whatman no. 4 paper as 5-hydroxytryptamine. As judged by colour intensity in reaction with Ehrlich's reagent, the main bulk of this substance was concentrated in F_3 and F_{4-6}. Reference to Table 2 showed that the main peak of biological activity was to be expected in these fractions from a counter-current distribution, and further experiments were therefore made to show, first, that these fractions did in fact contain 5-hydroxytryptamine and, secondly, that their biological activity on the rat colon preparation was due to this substance. Tables 4 and 5 give the results of paper chromatography of samples of F_{4-6} developed with different solvents, sprayed with all five colour reagents and compared with 5-hydroxytryptamine.

F_{4-6} was also mixed with 5-hydroxytryptamine, and attempts were made to separate the two substances by paper chromatography in various solvents. No separation could be demonstrated.

From these results it seemed clear that the phenol present in F_{4-6} was indeed 5-hydroxytryptamine and larger scale paper chromatographic fractionation was therefore used to find if the biological activity of fraction 4–6 on the rat

colon preparation was entirely due to this phenol or whether other substances not capable of giving colour reactions with our reagents were responsible for part of the activity.

TABLE 4. R_F values of fraction F_{4-6} from counter-current fractionation of enteramine concentrate compared with the behaviour of 5-hydroxytryptamine and related compounds

Solvent ...	Phenol–water*	Butanol†–acetic acid	Butanol†–acetic acid	Pyridine‡–amyl alcohol	§Propanol‖–ammonia
Paper ...	Whatman no. 4	Whatman no. 4	Whatman no. 1	Whatman no. 4	Whatman no. 4
5-Hydroxytryptamine	0·69	0·40	0·33	0·68	0·77
Tryptamine	0·92	0·66	0·58	—	0·91
Adrenaline	0·37	0·39	—	—	—
Noradrenaline	0·32	0·30	0·20	—	—
Fraction F_{4-6}	0·69	0·40	0·33	0·69	0·77

* Phenol–water 5 : 2 (v/v).
† Butanol, acetic acid, water 4 : 1 : 5 (v/v) (Partridge, 1946).
‡ Pyridine, amyl alcohol, water 35 : 35 : 30 (v/v) (de Verdier & Ågren, 1948).
§ Ascending chromatogram, all others descending.
‖ Propanol, 0·88 NH_3, water 60 : 30 : 10 (v/v) (Hanes, Hird & Isherwood, 1950).

TABLE 5. Colours produced by spraying paper chromatograms of fraction F_{4-6} from counter-current fractionation of enteramine concentrate with various reagents and comparison with colour produced by 5-hydroxytryptamine and related compounds

	Reagents				
Compounds	Ninhydrin	$AgNO_3$	Ehrlich	Pauly	Folin-Denis
5-Hydroxy-tryptamine	Weak grey	Rapid reduction	Pink going through purple to grey	Immediate brick red	Blue before alkaline spray, increasing in intensity afterwards
Tryptamine	Weak grey	—	Magenta becoming grey	—	—
Noradrenaline	—	Immediate reduction	—	Salmon pink	Pale blue before alkaline spray. Blue afterwards
Adrenaline	—	Immediate reduction	—	Salmon pink	
Fraction F_{4-6}	Weak grey	Rapid reduction	Purple becoming grey	Immediate brick red	Blue before alkaline spray, increasing in intensity afterwards

About one-fifth of the remaining material of fraction F_{4-6} was deposited on a 75 × 75 cm sheet of Whatman no. 4 paper as a strip about 1 × 50 cm parallel to and 10 cm from one edge of the paper. Synthetic 5-hydroxytryptamine and tryptamine were used as markers on the strips of paper remaining on either side of the 50 cm line of F_{4-6}. The sheet was irrigated overnight with butanol–acetic acid, dried and the two reference strips with the markers sprayed with Ehrlich's reagent. The sheet was cut into eight parallel strips, the cuts being made at 10, 23, 26, 31, 34, 42 and 50 cm from the starting line. Each strip was eluted separately and the eluate assayed on the atropinized rat colon. The material from the 26–31 cm strip (about 12 mg) gave a strong contraction at a dose of 20 μg. None of the other strips yielded active material. Reference to the markers showed that the material in the 26–31 cm strip had

the same R_F as 5-hydroxytryptamine. This material was also tested for its effect on a guinea-pig ileum preparation and on the arterial blood pressure of the rabbit. Table 6 shows the results of these tests and of comparison with 5-hydroxytryptamine. Although our material was still impure, it showed, qualitatively, the same action as synthetic 5-hydroxytryptamine.

TABLE 6. Comparison of the pharmacological activity of 5-hydroxytryptamine and eluate from strip 26–31

	Amounts giving equal response		
Test*	5-OH-tryptamine† (μg)	Eluate (μg)	Activity ratio
Rat colon	1·2	20	17 : 1
Guinea-pig ileum	0·44	10	23 : 1
Rabbit blood pressure	20·0	450	23 : 1

* For details of tests see text, p. 299.
† As creatinine sulphate complex.

The results given in Table 3 had shown that two smooth-muscle stimulating substances could be distinguished from one another by their different degrees of activity in different types of biological test. These tests were now applied to the active eluate from the paper. It was found (Table 6) that the ratio of the activity of pure 5-hydroxytryptamine to activity of eluate was constant within the limits of experimental error.

Butanol fractions: unidentified active principle

Preliminary chromatograms of the butanol fractions from the countercurrent distribution (F_{13-15}, F_{16-18}, F_{19-21} and F_{22-24}), made by the methods already described, showed a weak spot in the position occupied by 5-hydroxytryptamine and, in addition, a second spot (R_F 0·77) further from the origin but giving much the same colour reactions. As judged by intensity of colour, this substance was mainly concentrated in F_{16-18} and F_{19-21}. Reference to Table 2 shows that a second peak of biological activity might be expected in this region. The whole of the material in F_{16-18} and F_{19-21} was transferred to a sheet of Whatman no. 4 paper (75 × 75 cm) as a narrow band 50 × 1 cm and developed with butanol–acetic acid as described above. Markers of 5-hydroxytryptamine and tryptamine were used as before. The sheet was cut into five strips, the cuts being made at 20, 30, 45 and 51 cm from the origin. Strip 20–30 contained the 5-hydroxytryptamine marker and strip 45–51 the unknown phenol which we shall refer to as substance C. Each strip was eluted and the eluate tested on the isolated guinea-pig ileum in the presence of atropine and mepyramine. The eluate from strip 0–20 was inactive, the eluate from strip 20–30 had activity equivalent to about 1 μg 5-hydroxytryptamine. Samples of eluates from strips 30–45 and 45–51, particularly the latter, caused considerable smooth-muscle contraction. Although tryptamine

could not be detected in these eluates by paper chromatography, it seemed just possible that the observed activity of strips 30–45 and 45–51 was due to this base; observation of the qualitative nature of the contraction showed, however, that this was not so. Both 5-hydroxytryptamine and tryptamine produced in the mepyraminized, atropinized guinea-pig ileum an immediate contraction which is not maintained beyond 45 sec, even if either substance is left in the bath. When the eluate from strip 45–51 was added to the bath, contraction occurred after a latent period of 20–60 sec and the contraction was maintained, with a smaller superimposed rhythmic contraction, as long as the eluate was kept in the bath.

We have not yet had enough of this material to make a full examination of its pharmacological properties. It gives positive colour reactions with Ehrlich's, Folin-Denis's and Pauly's reagents similar to 5-hydroxytryptamine.

DISCUSSION

When this investigation was begun, little was known as to the nature of the two smooth-muscle stimulating substances enteramine (Erspamer, 1940) and substance P (Gaddum & Schild, 1934). Recent work (Fischer & Vogt, 1950) has suggested that substance P may be a mixture of peptides.

The notes of Erspamer & Asero (1951, 1952), in which they reported the isolation of 5-hydroxytryptamine and suggested that it was identical with enteramine, were of great assistance to us in the final identification of one of the active principles concentrated from extracts of mammalian intestine. This principle had the same R_F value as 5-hydroxytryptamine in four different solvent systems, showed the same colour reactions with five colour reagents and gave the same pharmacological responses as 5-hydroxytryptamine. Thus, although we did not isolate a crystalline specimen, there is no doubt that one of our concentrates was mainly 5-hydroxytryptamine.

Our results also make it clear that acetone extracts of small intestine contain a second smooth-muscle stimulating substance and we therefore suggest that the name enteramine should now be dropped in favour of 5-hydroxytryptamine. The nature of the second active component present in acetone extracts of intestine is not clear but it seems likely from its behaviour with various colour reagents that it is chemically related to 5-hydroxytryptamine. We suggest that no name should be given to this substance until its nature is known, and meanwhile we shall refer to it as fraction C. Under the conditions of extraction used in these experiments it is a relatively minor component.

5-Hydroxytryptamine (serotonin) has previously been obtained from mammalian blood (Rapport, 1949; Rand & Reid, 1951), and the present work suggests that it may be rather widely distributed in animal tissues. Although it is characterized pharmacologically by its smooth-muscle stimulating effects, it cannot be concluded that this is its main function.

SUMMARY

1. A technique using the isolated rat's atropinized colon is described for assaying the smooth-muscle stimulating activity of acetone extracts of the small intestine.

2. By submitting the acetone extracts of small intestine to counter-current fractionation and assaying the fractions on the rat's atropinized colon, it is shown that there are two active principles.

3. One of these active principles is identified as 5-hydroxytryptamine by means of paper chromatography.

4. The other active principle has not been isolated but is related to 5-hydroxytryptamine.

5. Substance P is distinct from these indole derivatives.

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