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OBSERVATIONS ON THE SIGNIFICANCE OF 5-HYDROXYTRYPTAMINE IN RELATION TO THE PERISTALTIC REFLEX OF THE RAT

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Peristalsis of normal rats, and of rats fed either on a control diet or on a tryptophan-free diet (5-hydroxytryptamine-depleted rats), was studied *in vitro* and *in situ* to test the hypothesis that 5-hydroxytryptamine functions as a local hormone in the intestine and may be essential for initiation of the peristaltic reflex. A tryptophan-free diet depleted intestinal 5-hydroxytryptamine by a mean value of 90% ; in some rats, the depletion appeared to be complete. Peristaltic responses, even of rats with complete depletion, were qualitatively similar to, and quantitatively not statistically different from those of normal or of pair-fed control animals whose intestinal mucosa contained high concentrations of 5-hydroxytryptamine. Intraluminal and serosal 5-hydroxytryptamine produced effects in 5-hydroxytryptamine-depleted rats similar to those in the normal and in the control animals. Furthermore, the maximal stimulatory effects of 5-hydroxytryptamine on peristaltic performance were not greater than spontaneous variations in performance in any group of animals, except with tryptophan-fed control rats, when the effects of the amine on peristalsis *in situ* were greater than spontaneous variation. It was therefore concluded that 5-hydroxytryptamine is not essential for peristalsis in the rat.

The experiments described in this paper were undertaken primarily to test the hypothesis that 5-hydroytryptamine possesses a physiological function in relation to the peristaltic reflex (Bülbring & Lin, 1958 ; Bülbring & Crema, 1958, 1959a, b). Bülbring and her co-workers proposed that the enterochromaffin cells situated at the base of the mucosal epithelium possess a neurosecretory function. Deformation of these cells, in response to the physiological stimulus of a rise in intraluminal pressure, might cause the release of 5-hydroxytryptamine, which, in turn, might stimulate adjacent mucosal sensory receptors and facilitate the peristaltic reflex. Thus, 5-hydroxytryptamine might be considered to modulate the activity of sensory receptors involved in peristalsis.

In order to determine whether or not the postulated function for 5-hydroxytryptamine was essential for initiation of the peristaltic reflex, it was necessary to observe the reflex in intestine depleted of 5-hydroxytryptamine. For this fundamental experiment, peristalsis was studied *in situ* in guinea-pigs severely depleted of intestinal 5-hydroxytryptamine by reserpine (Bülbring & Crema, 1959b). Though

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a depletion of mucosal 5-hydroxytryptamine greater than 90% was obtained in some instances, peristaltic activity was not only unimpaired, but was abnormally active. This was so even when 98 to 99% depletion was achieved, and meant that either 5-hydroxytryptamine was not essential for peristalsis or, as the amine was presumably still being synthesized in large amounts, these were sufficient to exert the decisive effect. Thus, Bülbring & Crema (1959b) were unable to draw a definite conclusion as to the significance of 5-hydroxytryptamine in peristalsis.

In the present work rats were used, and synthesis of 5-hydroxytryptamine was prevented by feeding a diet free of tryptophan, the ultimate precursor of 5-hydroxytryptamine. This method has the advantage over the use of reserpine (which was also used in a few experiments) that a complete depletion of intestinal 5-hydroxytryptamine can be obtained, and that noradrenaline concentrations are unaltered (Gal, Drewes & Barraclough, 1961, 1962). Moreover, since reserpine increases the acetylcholine content of the ileum (Malhotra & Das, 1962), this effect, which may have contributed to the hyperperistalsis observed in guinea-pigs treated with reserpine, was also avoided.

METHODS

Animals. Male, Sprague-Dawley rats, littermates unless stated otherwise, and aged about 1 month (weight 45 to 60 g), were used for the experiments involving feeding of the tryptophan-free or control diets. Stock rats which received rat cake (diet 41 b ; Bruce, 1950) or a complete synthetic diet (Boullin, 1961) were either weanling littermates as described above or adults (weight 180 to 220 g) ; these are called " normal " rats.

Depletion of 5-hydroxytryptamine. This was achieved by feeding, *ad libitum*, a synthetic diet containing amino acids, but free of tryptophan ; full details are given by Boullin (1963c). Control rats were pair-fed an identical diet with the addition of 0.4% L-tryptophan.

Peristalsis. This was recorded either *in vitro* or *in situ*. The technique for *in vitro* recording was either the isometric method of Bülbring & Lin (1958), which was used in only a few experiments, or the isotonic method of Bülbring, Crema & Saxby (1958).

The methods of recording peristalsis *in situ*, in rats, have not been described previously. The animals were first anaesthetized with urethane (1.25 g/kg, subcutaneously). About 1 hr later the abdominal cavity was opened along a midline incision and immediately bathed with Tyrode solution, gassed with a mixture of 95% oxygen and 5% carbon dioxide, at 37° C. Two methods of recording peristalsis were used.

In the first method, the oesophagus was ligated as far as possible above the cardiac sphincter, and severed immediately below the ligature. A proximal cannula was then passed through the cardiac sphincter into the stomach and secured. Tyrode solution, previously gassed as described above, entered the stomach through the proximal cannula, to which was attached a side-arm for recording intragastric pressure, and passed into the duodenum. It left the intestinal loop through a distal cannula tied in place either immediately beyond the ligament of Treitz, or at the end of the first 2 to 3 cm of proximal ileum, as shown in Fig. 1. This method will be called " stomach perfusion."

In the second method, called " duodenal perfusion," the oesophagus was ligated but not severed. The proximal cannula was fastened into the stomach through an incision in the lower portion of the antrum, and the tip of the cannula was then passed through the pyloric sphincter into the region of the duodenal cap. The distal cannula was then placed as described for stomach perfusion.

With the dissection completed and the cannulae in position, the abdominal cavity was closed with artery forceps, and the proximal cannula was connected to the perfusion apparatus

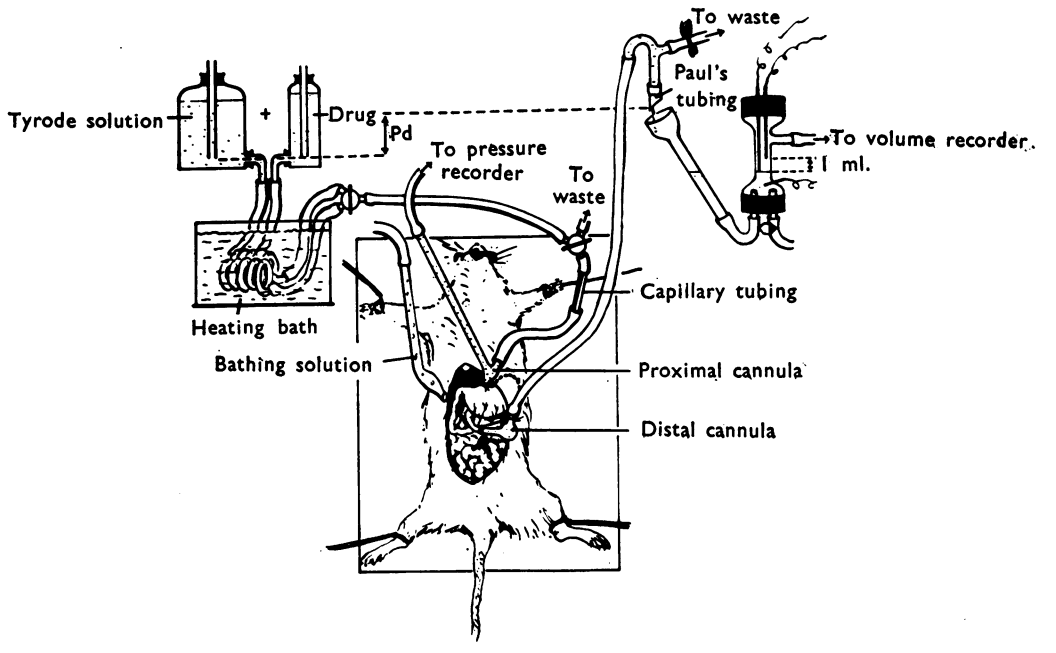

Fig. 1. Diagram of apparatus used for recording peristalsis *in situ* by the stomach perfusion technique; for description see text.

(Fig. 1), which is similar to that described by Bülbring & Lin (1958). The heating bath was kept at 45° C as experience showed that the fluid entering the gut was then at about 37° C. Usually a positive pressure of 20 to 120 mm of water was applied to the proximal end of the stomach by raising the Marriotte bottles to the appropriate height. Further, a slightly greater positive pressure (Pd, Fig. 1) was exerted on the distal end of the intestine by an outflow valve, though this was not always necessary (Lee, 1960).

Assessment of peristaltic performance. This was determined quantitatively by measurement of the " transport rate," defined as the volume of fluid expelled from the gut, by the action of the peristaltic reflex, in a given time (usually 10 to 30 min), expressed as ml./hr (Boullin, 1963b). Changes in transport rate were expressed as positive or negative percentages of the rate immediately preceding that in question. The " transport volume " is defined as the volume of fluid, in ml., expelled from the gut by one peristaltic reflex response.

When studying the effects of intraluminal 5-hydroxytryptamine on the transport rate of tryptophan-deficient and control preparations, comparison was always made between preparations from deficient and control animals which received several concentrations of amine under identical conditions of timing and sequence of administration. In Tables 4, 6 and 7, where the maximal percentage changes in transport rate are shown, preference has been given to positive changes. These are shown rather than any subsequent decrease in transport rate, even if the secondary decrease was much greater.

Drugs. The effects of 5-hydroxytryptamine creatinine sulphate, DL-5-hydroxytryptophan, acetylcholine chloride, expressed as weights of bases, and 2-bromo-D-lysergic acid diethylamide and atropine sulphate, expressed as weights of salts, were studied on peristalsis by serosal or intraluminal application *in vitro,* and intraluminal application *in situ.* The sources of the dietary components used are given by Boullin (1961); amino acids were purchased from L. Light & Co.

Extraction and estimation of 5-hydroxytryptamine. 5-Hydroxytryptamine was extracted from the intestinal mucosa according to the method of Bülbring & Lin (1958), and assayed either biologically, using the rat fundus preparation (Vane, 1957), and/or spectrophotofluorimetrically, using the modification of Boullin (1962).

RESULTS

Effect of tryptophan-free diet on weight and 5-hydroxytryptamine content of the mucosa of the small intestine

In an initial experiment the tryptophan-free diet was fed to thirteen rats for 17 to 32 days. The 5-hydroxytryptamine content of the mucosa of the small intestine was reduced by a mean value of 89%, compared with the value for pair-fed littermate controls (Table 1). Two of the thirteen deficient rats apparently possessed no

TABLE 1

EFFECT OF TRYPTOPHAN-FREE DIET ON 5-HYDROXYTRYPTAMINE CONTENT OF MUCOSA OF SMALL INTESTINE OF TRYPTOPHAN-DEFICIENT AND LITTERMATE CONTROL RATS

5-Hydroxytryptamine was assayed biologically. The depletion (%) relates the 5-hydroxytryptamine content of tryptophan-deficient rats to their controls. * Rats given reserpine, 1 mg/kg. For the means of the control and deficient rats, $P<0.001$

No. of expt.	5-Hydroxytryptamine content (μg/g)		Depletion (%)	Days on deficient diet	
	Control	Deficient		Total	Before reserpine
1	2·96	0	100	30	
2	0·87	0	100	28	
3	2·07	0·05*	97·5	28	27·83
4	2·30	0·11*	95·2	17	15·0
5	2·30	0·19*	91·7	17	15·0
6	1·74	0·15*	91·4	23	22·75
7	1·03	0·12	88·4	24	
8	1·73	0·22	87·3	27	
9	2·81	0·38	86·5	32	
10	1·45	0·26*	82·1	29	28·83
11	2·49	0·48*	80·7	28	27·67
12	2·23	0·44	80·3	23	
13	2·23	0·55*	75·3	24	14
Mean	2·02	0·23	89·0	25·4	
Standard error	0·18	0·05	2·2	1·3	
P		<0·001			

mucosal 5-hydroxytryptamine whatsoever, having been fed the diet for 30 and 28 days (experiments 1 and 2, Table 1). These results were obtained using the bioassay method. In a second experiment, with twenty-two deficient rats, a similar degree of depletion was obtained when intestinal 5-hydroxytryptamine was assayed spectrophotofluorimetrically (Table 2). Here an absolute depletion was obtained in seven experiments ; this was confirmed by bioassay.

During these experiments it was also noted that the weight of the intestinal mucosa of the deficient rats was substantially lower ($P<0.001$) than either that of the controls, or that of rats fed diet 41 b. The latter value was significantly lower ($P<0.05$) than the value for controls (Table 3).

TABLE 2

EFFECT OF TRYPTOPHAN-FREE DIET ON 5-HYDROXYTRYPTAMINE CONTENT OF MUCOSA OF SMALL INTESTINE OF NORMAL, CONTROL AND TRYPTOPHAN-DEFICIENT RATS

5-Hydroxytryptamine was assayed spectrophotofluorimetrically. Normal rats were fed diet 41 b. Control rats were fed tryptophan but were not littermates. Deficient rats lacked tryptophan in their diet

5-Hydroxytryptamine content (μg/g)			Time on experimental diet (days)	
Normal	Control	Deficient	Control	Deficient
2·34	1·97	0	23	18
3·51	2·85	0·12	25	20
2·74	5·59	1·82	25	21
2·64	8·60	1·48	25	21
3·89	6·65	0·77	28	22
6·10	4·49	0·44	31	23
4·09	1·77	0·87	31	23
2·64	7·79	0·78	35	24
4·96	4·58	0·33	35	25
		0		25
		0		27
		0		28
		0·92		28
		0·18		30
		0·14		30
		0		30
		0		31
		0·32		33
		0		33
		0·84		35
		0·84		35
		0·49		35
Mean 3·66	4·92	0·47	28·7	27·3
Standard error 0·42	0·82	0·11		
P	>0·05	<0·001		
Mean depletion (%)		90·4		
% of normal 100	134·4	12·8		
% of control 74·4	100	9·6		

Peristalsis in vitro *in normal rats*

The peristaltic responses of duodenum and ileum from rats fed diet 41 b or the complete synthetic diet with added tryptophan were identical. This was so whether peristalsis was recorded isotonically or isometrically, and the responses with both methods were essentially the same as those described for the guinea-pig (Bülbring & Lin, 1958 ; Bülbring & Crema, 1958 ; Bülbring, Lin & Schofield, 1958 ; Boullin, 1963b). However, as the isometric method was unsuccessful in deficient preparations (see below), and as in normal preparations in the absence of 5-hydroxytryptamine peristalsis continued longer and was more efficient when reflex activity was recorded by the isotonic method, the latter was used for all quantitative studies.

In the duodenum and the proximal ileum, the threshold pressure was in the range 20 to 70 mm of water and reflex activity was continuous (Fig. 2*a*). With mid-ileum the reflex occurred in cycles, periods of activity being interspersed with periods of quiescence when the circular muscle layer remained contracted. Such responses were most pronounced in the terminal ileum (Fig. 2*b*), where the reflex

TABLE 3

EFFECT OF VARIOUS DIETS ON MUCOSAL WEIGHT OF THE SMALL INTESTINE

All rats were of similar body-weight. Normal rats were given diet 41 b. Control rats were given tryptophan-free diet+0.4% L-tryptophan. Deficient rats were given a tryptophan-free diet. Values in each row for control and deficient rats were from littermates

	Mucosal weight (g)		
	Normal	Control	Deficient
	1·45	1·58	1·15
	1·14	1·54	0·90
	1·19	1·31	0·64
	1·48	1·25	0·70
	1·20	1·82	0·91
	1·06	1·35	0·93
	1·30	1·36	1·24
		1·42	0·97
		1·70	0·70
Mean	1·26	1·48	0·91
Standard error	0·06	0·06	0·07
P		<0·05	<0·001
% of normal	100	117·5	72·2
% of control	85·1	100	61·5

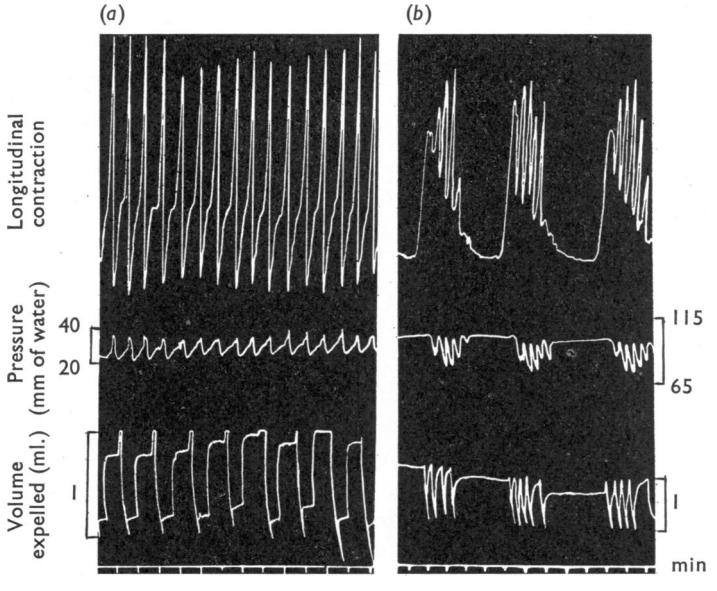

Fig. 2. Peristalsis in normal rat intestine *in vitro*. (*a*) duodenum, (*b*) terminal ileum, using the isotonic method. When the intraluminal pressure reached the threshold pressure the peristaltic reflex was evoked; this is shown by: (1) a contraction of the longitudinal muscle (upwards), (2) an increase in intraluminal pressure, and (3) a "step-rise" in the volume of fluid expelled from the intestine. Each "step-rise" represents the transport volume (see text). Time marker, 1 min.

was not evoked below a pressure of 70 to 90 mm of water, and were similar to those noted in the large intestine by Straub & Schild (1933) and Lee (1960).

Effects of 5-hydroxytryptamine

Intraluminal application. Low intraluminal concentrations of 5-hydroxytryptamine (0.01 to 10 μg/ml.) first stimulated and then inhibited peristalsis; higher concentrations (up to 1 mg/ml.) inhibited or abolished the reflex, often without prior stimulation. Though stimulation was quite pronounced, it was transient (duration 15.2 ± 3.2 min, mean and standard error, nine experiments), and the maximal percentage changes in transport rate that occurred with 5-hydroxytryptamine ($21.0 \pm 11.29\%$, ten experiments, Table 4) were not significantly greater

TABLE 4

EFFECT OF INTRALUMINAL 5-HYDROXYTRYPTAMINE ON PERISTALTIC PERFORMANCE (TRANSPORT RATE) *IN VITRO* (ISOTONIC METHOD) IN TRYPTOPHAN-DEFICIENT AND CONTROL DUODENUM PREPARATIONS

Control rats were fed added tryptophan and were littermates to the deficient rats, which were fed a diet free of tryptophan. Depletion percentages relate mucosal 5-hydroxytryptamine (5HT) contents of tryptophan-deficient rats to their controls. The change in transport rate was produced by 5-hydroxytryptamine and was calculated as described in Methods. The optimal 5-hydroxytryptamine concentration was the last tried which produced the maximal effect on transport rate

		Transport rate				Optimal 5HT concn.	
		Initial (ml./hr)		Change (%)		(μg./ml.)	
No. of expt.	5HT depletion (%)	Control	Deficient	Control	Deficient	Control	Deficient
1	100	102	45	+6	+9	0·01	1·0
2	100	54	28	−33	−20	1·0	1·0
3	97·5	54	78	−19	−29	0·01	0·01
4	91·7	13	38	+67	−100	1·0	0·01
5	88·4	33	32	+50	+69	1·0	5·0
6	87·3	84	42	+78	+43	0·5	0·01
7	82·1	48	33	+18	+41	0·05	0·01
8	80·7	30	25	+20	−20	5·0	5·0
9	80·3	27	48	0	−100	1·0	1·0
10	75·3	48	18	+24	−100	1·0	1·0
Mean	89·0	49·3	38·7	+21·0	−29·3		
Standard error		8·49	5·25	11·29	18·69		
P		>0·05		<0·05			

($P > 0.05$) than similar changes occurring spontaneously in the absence of the amine ($-4.30 \pm 9.91\%$, six experiments). These effects were similar to those seen in the guinea-pig ileum *in vitro* (Bülbring & Lin, 1958; Bülbring & Crema, 1958). In the rat the stimulation produced by 5-hydroxytryptamine was more prominent when peristalsis was recorded isometrically, and in some experiments a high propulsive efficiency was attained. This was evident (Fig. 3*a*) from an increase in force of peristalsis and in transport volume, and by a reduction in back pressure. These changes persisted for more than 1 hr after 5-hydroxytryptamine had been washed out of the lumen. Though the transport rate and frequency of reflex contractions declined, each contraction resulted in expulsion of fluid with greater propulsive efficiency than before the drug was given.

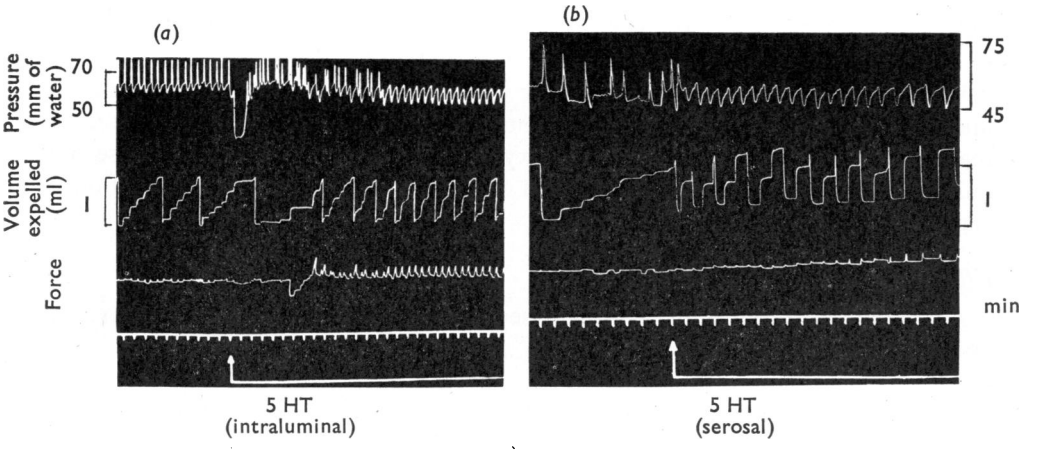

Fig. 3. Effect of 5-hydroxytryptamine on peristaltic reflex activity of normal rat duodenum *in vitro*, using the isometric method. (*a*) effect of intraluminal 5-hydroxytryptamine (5HT, 4 μg/ml. at the arrow) infused into the lumen by lowering the outflow pressure (intraluminal pressure, top trace, fell below 50 mm of water). When the intraluminal pressure rose, peristalsis was re-established. Within 8 min the force, transport volume and transport rate increased, and after 12 min " back-pressure " disappeared and high propulsive efficiency was attained. (*b*) Serosal 5-hydroxytryptamine (5HT, 0.01 μg/ml. at arrow and during line) produced stimulation of peristalsis almost identical with that shown in (*a*).

When peristalsis was recorded isotonically, the most obvious effect of 5-hydroxytryptamine was an increase in the inherent tone of the longitudinal muscle. This increase was transient when separate doses of 5-hydroxytryptamine were applied to the lumen, but was more persistent when the intestine was perfused for some time (Fig. 4, *a* and *b*).

Serosal application. 5-Hydroxytryptamine applied serosally always produced an initial brief stimulation associated with a single large contraction of the longitudinal muscle immediately after the drug had been added to the bath, irrespective of the concentration used (0.01 μg/ml. to 1 mg/ml.) or the method of recording peristaltic activity. This was so even with the highest concentrations which abolished peristalsis immediately after this first contraction.

With regard to the propulsive activity, the responses were indistinguishable from those seen with 5-hydroxytryptamine in the lumen (Fig. 3, *a* and *b*). The response to serosal application of 5-hydroxytryptamine, as with intraluminal application, was more pronounced when the reflex was recorded isometrically than when recorded isotonically. No quantitative estimates were made of the effects of 5-hydroxytryptamine applied serosally.

Effects of 5-hydroxytryptophan

This amino acid produced effects similar to those of 5-hydroxytryptamine, but higher concentrations were required (50 to 1,000 μg/ml.) to evoke any response. Introduced into the lumen, 5-hydroxytryptophan increased the tone of the longitudinal muscle, this effect sometimes lasting until the drug was washed out. Peri-

stalsis was not always stimulated (and any stimulation was always transient) but in some experiments there was immediate inhibition.

With serosal 5-hydroxytryptophan there was an immediate increase in longitudinal muscle tone, which suggested that the amino acid exerted an effect itself, rather than after decarboxylation to 5-hydroxytryptamine. This initial response was followed by a transient stimulation and later by inhibition or even abolition of peristalsis.

Effects of other drugs

Acetylcholine, atropine and 2-bromolysergic acid diethylamide had similar actions to those seen in the guinea-pig.

Peristalsis in vitro *in tryptophan-deficient rats*

The only respect in which *in vitro* preparations from tryptophan-deficient rats differed from control and normal preparations was that, when peristalsis was recorded isometrically, the reflex rapidly died out in the deficient group. This effect was aggravated by intraluminal 5-hydroxytryptamine even when the drug was introduced into the lumen at the very beginning of an experiment. Therefore, most records *in vitro* were obtained with the isotonic method. Then the peristaltic responses and propulsive efficiency in the tryptophan-deficient preparations, including two experiments (nos. 1 and 2, Table 4) in which no 5-hydroxytryptamine was

TABLE 5

EFFECT OF VARIOUS DIETS ON INITIAL TRANSPORT RATE

Transport rate was determined by the isotonic method, *in vitro*. The *P*-values for the first two columns relate to the means of these columns with the mean of column four. Control rats had tryptophan added to the diet, deficient rats were given a tryptophan-deficient diet

	Transport rate (ml./hr) from			
			This paper	
	Boullin (1961)	Bruce (1950)	Control	Deficient
	67·5	26	102	45
	22	42	54	28
	53	60	54	78
	48	42	13	38
	72	51	33	32
	84	120	84	42
	60	90	48	33
	36	60	30	25
	66	30	27	48
	40		48	18
	75			
	75			
	60			
	36			
	108			
	58			
	72			
	172			
	114			
Mean	69·40	57·89	49·3	38·7
Standard error	7·78	10·03	8·50	5·25
P	<0·02	>0·05	>0·05	

detectable in the intestinal mucosa, did not differ either qualitatively or quantitatively from those of control or normal rats. Comparison of transport rates, recorded at the beginning of each experiment before any drugs were given, showed that there was no significant difference ($P>0.05$) between mean values for tryptophan-deficient and control groups, but the value for normal rats was significantly greater ($P<0.02$) than that for the deficient group (Table 5).

Effects of 5-hydroxytryptamine

Generally, 5-hydroxytryptamine produced the same pattern of responses in all groups of animals. Figs. 4,*a* and *b*, show the remarkable resemblance between responses of a littermate control preparation with normal levels of intestinal 5-hydroxytryptamine, and the deficient preparation with no intestinal amine at all. As indicated in Table 4, inhibitory effects predominated in the deficient group. In Tables 4, 6 and 7, a negative value for maximal change in transport rate indicates that only inhibition of peristalsis was seen in that particular experiment (see Methods). Table 4 shows that stimulation occurred in only four of ten deficient experiments, but in seven of ten controls. The doses required to produce maximal effects were variable (Table 4), but it is interesting that, in one of the two experiments where there was an absolute depletion of 5-hydroxytryptamine, the dose required to evoke maximal stimulation (9% increase in transport rate) in the deficient preparation (Fig. 4,*b*) was 100-times greater than in the littermate control (6% Fig. 4,*a*). The duration of the effects of 5-hydroxytryptamine in the deficient group (13.0 ± 2.1 min, seven experiments) was not significantly different ($P>0.05$) from values obtained in control experiments (15.2 ± 3.2 min, nine experiments). Furthermore, the maximal changes in transport rate produced by 5-hydroxytryptamine (Table 4) were not significantly different ($P>0.05$) from those occurring spontaneously in the same deficient animals ($-14.6\pm10.91\%$, five experiments) in the absence of any drug.

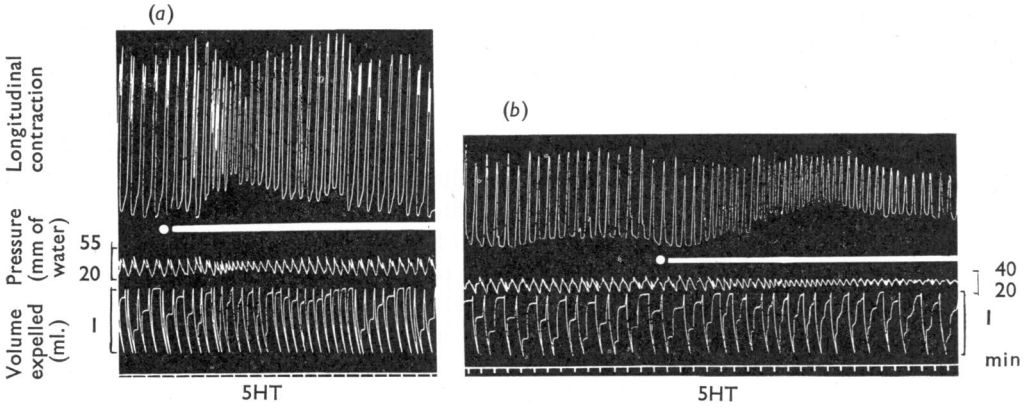

Fig. 4. Isotonic method; records as Fig. 2. Maximal stimulation of the peristaltic reflex, in the duodenum *in vitro*, by intraluminal 5-hydroxytryptamine (5HT, at white dots and during lines). (*a*) Tryptophan-fed control rat. 5-Hydroxytryptamine (00.1 μg/ml.) stimulated peristalsis, increasing the transport rate by 6%. (*b*) Tryptophan-deficient rat (littermate of animal described in *a*). 5-Hydroxytryptamine (1 μg/ml.) increased the transport rate by 9%.

The effects of serosal 5-hydroxytryptamine in tryptophan-deficient rats were not compared quantitatively; they were essentially the same as those in preparations from control and normal rats, except that sometimes, in the deficient animals, 5-hydroxytryptamine had more pronounced actions when applied outside compared with inside the lumen.

Effects of 5-hydroxytryptophan

The precursor exerted similar actions in deficient animals to those seen in control and normal rats, except that the increase in longitudinal muscle tension was often greater, and persisted for as long as the amino acid was present in the lumen. This is shown also in the presence of 5-hydroxyptamine in Fig. 4,*b*. In preparations completely depleted of 5-hydroxytryptamine (for example, experiment 1, Table 4), 5-hydroxytryptophan produced no permanent facilitation of peristalsis, and any peristaltic reflex stimulation that did occur was always succeeded by inhibition.

Effects of other drugs

The responses of 5-hydroxytryptamine-depleted small intestine to atropine, acetylcholine and 2-bromolysergic acid diethylamide were similar to those of normal and control intestine preparations.

Peristalsis in situ

Stomach perfusion

The sequence of events occurring during peristalsis, including the opening and closing of the pyloric sphincter, was determined by observation of the gut in the abdominal cavity. Apart from closure of the sphincter, the changes were also evident from the kymograph records.

At the beginning of an experiment, the pylorus was closed and the duodenum was usually empty. The first sign of impending peristalsis was a very transient relaxation of the pylorus for 2 to 3 sec which was shown on the kymograph records by a fall in intragastric pressure, since fluid then flowed out of the stomach into the duodenum. This relaxation was followed by a series of others until eventually the amount of fluid entering the duodenum after one of them was sufficient to provide adequate distension and raise the intraduodenal pressure above the threshold, so that the peristaltic reflex occurred and fluid was propelled from the lumen (Fig. 5). Immediately before propulsion, when the intragastric pressure recorded on the kymograph was at its lowest level, the pyloric sphincter closed. The only difference between the initiation of peristalsis at the beginning of an experiment and the regular cycle of events was that in the latter only one or two relaxations of the pylorus were necessary to trigger the reflex. Then the characteristic sequence of pressure changes was an initial fall, sometimes preceded by a slight rise, followed by a gradual return of pressure to the original value.

It was obvious from these results that the ultimate factor controlling peristaltic performance was the movement of the pylorus, since this regulated the flow of fluid into the duodenum. Consequently, as sphincter movements were particularly

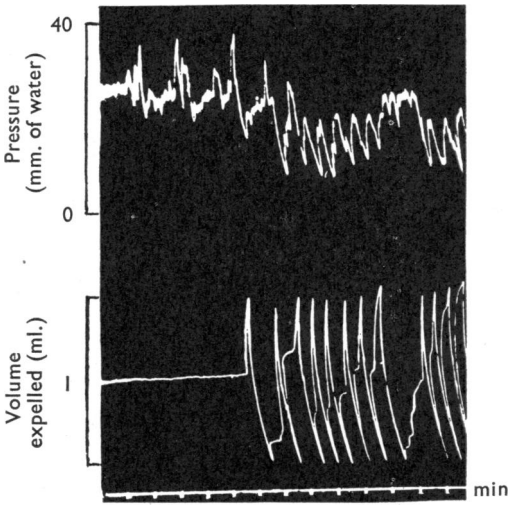

Fig. 5. Peristalsis *in situ* in the duodenum of a tryptophan-fed control rat perfused through the stomach. Uppermost trace, intragastric pressure. Middle trace, volume of fluid expelled. Lowest trace, time in min. The record shows the initiation of peristalsis at the beginning of an experiment. The peristaltic reflex was preceded by several relaxations of the pyloric sphincter (shown by a rise in pressure, followed by a fall) as fluid entered the duodenum. After 5 min the peristaltic reflex occurred, and fluid was expelled into the volume recorder.

susceptible to changes in depth of anaesthesia, being inhibited during light anaesthesia, peristalsis did not occur with the regularity seen in *in vitro* experiments when the sphincter could not interfere.

Normal peristalsis. Stock rats fed diet 41 b showed normal peristalsis. In such animals, with stomach perfusion, the pyloric sphincter was usually competent and closed, resulting in high intragastric pressures. In the flaccid stomach, the pressure was 40 to 60 mm of water; in the distended organ, up to 160 mm of water. The threshold pressure was also very high, in the range 60 to 120 mm of water, and when these high pressures were used the peristaltic reflex was evoked and fluid was expelled from the gut lumen. In these experiments, the initial transport rate varied between 20 and 76 ml./hr (mean, 38 ml./hr, Table 6) and often increased with time; in one experiment the rate doubled in 1 hr to 76 ml./hr.

The peristaltic performance of pair-fed control rats (not littermates to tryptophan-deficient animals) was also studied, and in eight experiments the initial transport rate did not differ significantly, at the 5% level, from that of stock rats. Values varied from 12 to 72 ml./hr (mean, 42 ml./hr, Table 6).

Effects of 5-hydroxytryptamine. The responses of normal and control animals have been considered together as they were similar. Intraluminal 5-hydroxytryptamine in concentrations from 0.1 to 100 μg/ml. caused the following responses: increased frequency and duration of relaxation of the sphincter muscle (indicated on the kymograph records by increased frequency and amplitude of pressure changes); decreased intragastric pressure, increased peristaltic performance, and the regular occurrence of the movements of the pylorus. In some experiments only

TABLE 6

PERISTALTIC PERFORMANCE IN SITU WITH STOMACH PERFUSION

C=Tryptophan-fed control rats. D=Tryptophan-deficient rats. (Deficient and control rats were not littermates.) N=rats fed diet 41 b. n=number of rats. * Mucosa not assayed for 5-hydroxytryptamine (5HT). † Mean, standard error (s.e.) and n refer to total number of rats examined (see Table 2), including all those shown in Tables 6 and 7. Changes in transport rate due to 5-hydroxytryptamine are maximal responses; those due to time refer to spontaneous variation. Optimal 5-hydroxytryptamine concentrations are the least concentrations which produced maximal effects. The probability value for the initial transport rates applies to all pairs from the three means; probability values for the changes in transport rate apply to the pairs of means for 5-hydroxytryptamine and time, for each diet

				Transport rate											
							Change (%) due to								
	Mucosal 5HT† content (μg/g)			Initial (ml./hr)			5HT			Time			Optimal 5HT concn. (μg/ml.)		
No. of expt.	C	D	N	C	D	N	C	D	N	C	D	N	C	D	N
1	*	0·12	2·34	22	75	76	+250		−26·7	−48·2		+52·6	1	—	5
2	*	0·77	3·51	12	60	36	+172·6	−36·5	+302·0	−43·8	+25·0	−38·9	10	5	10
3	*	0·32	2·74	60	24	20			+25·0	−22·2		−30·0	—	—	5
4	4·58	1·82	2·64	54	34	39		+33·3	+33·3	−17·5	−20·6	−12·5	—	10	1
5	*	1·48	3·89	36	16	36	−33·3	+120·0	−6·7		+50·0		10	1	1
6	1·97	0·87	6·10	36	40	45	+66·6				−37·5		1	—	—
7	4·49	0	4·09	72	36	21	−25·0			+25·0	−20·0		5	—	—
8	6·65	0	2·64	41	21	34	+169·5	+47·3		+12·5	+60·0		10	10	—
Mean	4·92	0.47	3·66	41·63	38·25	38·38	+100·07	+41·03	+65·38	−15·70	+9·48	−7·20			
s.e.	0·82	0·11	0·42	7·00	7·13	6·17	47·28	32·08	60·13	12·03	16·75	20·67			
n	9	22	9	8	8	8	6	4	5	6	6	4			
P	See Table 2			>0·05			<0·05	>0·05	>0·05						

propulsion was modified, but usually 5-hydroxytryptamine affected both sphincter movements and propulsion simultaneously. Then responses were characterized by a large and persistent fall in intragastric pressure, due to the increased frequency of sphincter movement permitting fluid to enter the duodenum at a faster rate. This had the effect of increasing the transport rate, as shown in Fig. 6,*a*, where 0.1 μg of 5-hydroxytryptamine increased the transport rate from 36 to 97 ml./hr. After this initial increase, which lasted for 8 min, the intragastric pressure rose again and the transport rate diminished slightly to 72 ml./hr, remaining at this value for 15 min.

Though the duration of these responses was comparatively short it seems probable that they persisted for a longer time than that required for the elimination of 5-hydroxytryptamine from the gut lumen. Thus, in the experiment shown in Fig. 6,*a*, 24 ml. of fluid was expelled in 18 min after administration of 5-hydroxytrypamine in only 0.2 ml. of Tyrode solution. Furthermore, though the increased peristaltic performance was transient, the alteration in the movements of the pylorus described above persisted for the remainder of the experiment in one of five normal and three of six control preparations.

The responses in the experiments with tryptophan-fed control rats were the only instances in which the maximal effects of 5-hydroxytryptamine on the transport rate were significantly greater, at the 5% level, than the spontaneous variations in transport rate that occurred with time in the absence of drugs (Table 6).

Effects of other drugs. The effects of acetylcholine were not studied. 2-Bromolysergic acid diethylamide (10 to 100 μg/ml.) did not alter peristaltic activity, but atropine (5 μg/ml.) abolished peristalsis. The pyloric sphincter became permanently relaxed and fluid passed passively through the gut at a high rate (up to 65 ml./hr). 5-Hydroxytryptophan (50 to 400 μg/ml.) produced similar effects to 5-hydroxytryptamine, and increased the transport rate for short periods. Contrary to the effects seen with 5-hydroxytryptamine, with the amino acid there was no permanent change in the pattern of pyloric sphincter movement.

Peristalsis in tryptophan-deficient rats. Normal peristaltic activity and performance were observed in nine experiments with tryptophan-deficient rats whose mucosal 5-hydroxytryptamine levels were lowered by a mean value of 90%. The initial transport rate in these experiments did not differ significantly at the 5% level from tryptophan-fed control animals, though the value was lower ($P<0.05$) than that for preparations *in situ* from normal rats. Qualitatively responses in the deficient rats were indistinguishable from those of control or even stock animals. This was so even when 5-hydroxytryptamine depletion was absolute (Fig. 6,*b*).

Effects of 5-hydroxytryptamine in tryptophan-deficient rats. The responses of tryptophan-deficient rats to 5-hydroxytryptamine were also normal both qualitatively and quantitatively. The maximal changes in transport rate produced by the amine were not significantly greater ($P>0.05$) than spontaneous variations in rate. The maximal effects were in fact quantitatively smaller than those in most experiments on normal and control preparations. In addition, the change in the pattern of pyloric sphincter movements seen after 5-hydroxytryptamine in some control and normal rats was evident in only one tryptophan-deficient animal.

TABLE 7

PERISTALTIC PERFORMANCE IN SITU WITH DUODENUM PERFUSION

Presentation as for Table 6, except that the probability values for the initial transport rates refer to the columns shown, and the probability value for the change in transport rate refers to all pairs of corresponding means

				Transport rate											
							Change (%) due to								
	Mucosal 5HT content (μg/g)			Initial (ml./hr)			5HT			Time			Optimal 5HT concn. (μg/ml.)		
No. of expt.	C	D	N	C	D	N	C	D	N	C	D	N	C	D	N
1	7·79	0	*	24	13	84	+33·3	+49·6	+62·2	−50·0	+23·1	−50·0	10	100	10
2	8·60	0·84	4·96	41	50	34	−64·2	—	+34·2	+21·6		+25·4	10	—	10
3	2·85	0	*	62	7·5	72	+11·0	+33·3	−33·3	+11·4	−37·5	−41·8	5	1	1
4	5·59	0·33	*	28	17	63	+121·0	—	−9·4	−30		−31·8	10	—	10
5	1·77	0		13	30		+25·0	+100·0					1	1	—
6		0·78			22			−38·8			+50·0		—	5	—
7		0·18			22			−56·8					—	1	—
8		0·49			42			+36·3			−45·3		—	1	—
9		0·92			15			0			−46·7		—	10	—
10		0·44			25			−12·0			−12·0		—	50	—
11		0			67			+50·0					—	10	—
12		0			16			−11·1					—	5	—
Mean	4·92	0·47	3·66	33·60	27·21	63·25	+25·22	+15·05	+13·43	−11·75	−11·40	−24·55			
s.e.	0·82	0·11	0·42	7·13	5·04	10·66	29·53	14·95	21·44	16·94	16·37	17·06			
n	9	22	9	5	12	4	5	10	4	4	6	4			
P	See Table 2			>0·05	<0·05			>0·05							

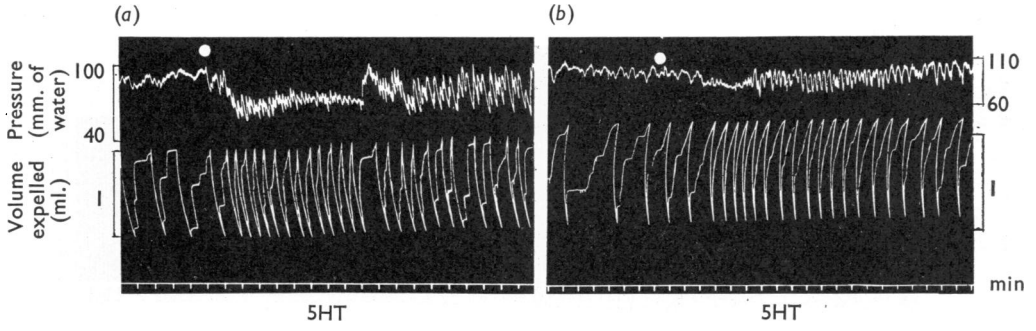

Fig. 6. Increase in frequency of pyloric sphincter movement and peristaltic performance *in situ* (stomach perfusion), produced by a single intraluminal dose of 5-hydroxytryptamine (5HT). Records as Fig. 5. (*a*) Tryptophan-fed control rat. At the signal (white dot) 0.1 μg of 5-hydroxytryptamine in 0.2 ml. of Tyrode solution was injected into the perfusion fluid entering the stomach. There was a spectacular stimulation of peristalsis: the intragastric pressure fell, pyloric sphincter movements occurred at increased frequency, and the transport rate also increased. The stimulation was maximal for 8 min and then declined, but the altered pattern of sphincter movement persisted. (*b*) Tryptophan-deficient rat completely depleted of intestinal 5-hydroxytryptamine. Before injection of 5-hydroxytryptamine (10 μg at white dot) the pattern of peristalsis and pyloric sphincter movement was irregular. 3 min after injection of 5-hydroxytryptamine, the pylorus relaxed for about 3 min (shown by a fall in pressure), and the transport rate was greatly augmented. Then the pressure rose, and regular pyloric sphincter movements appeared and persisted, as in (*a*).

Duodenum perfusion

Peristalsis in stock rats. Four experiments were performed. Peristalsis resembled that seen with isometric recording *in vitro*. The threshold pressure was very low (5 to 20 mm of water) compared with stomach perfusion (60 to 120 mm of water). The reflex was of low propulsive efficiency, large increases in intraluminal pressure being associated with each reflex response (Fig. 7,*a*). The transport volume was also smaller, though there was no significant difference ($P>0.05$) between the mean initial transport rate of this group of rats and of tryptophan-fed controls of comparable body weight, whether perfused with this method or through the stomach (Tables 6 and 7).

Effects of 5-hydroxytryptamine. While 5-hydroxytryptamine produced a transient increase in peristalsis in two experiments (nos. 1 and 2, Table 7), in two others the reflex was inhibited, the transport rate being reduced by up to one-third. 5-Hydroxytryptamine did not produce any alteration of the propulsive efficiency comparable to that seen *in vitro* with isometric recording.

Peristalsis in tryptophan-deficient and control rats. True peristaltic contractions were not evident. While some small nonpropulsive waves appeared to pass in an oral direction, other similar waves passed in the opposite direction for short distances, then died out and were replaced. The latter waves were propulsive. In both groups of animals the cannulated loops of duodenum showed intense activity, but, instead of the fluid being expelled in intermittent bursts as in typical peristalsis, it was propelled along continuously and appeared drop-by-drop.

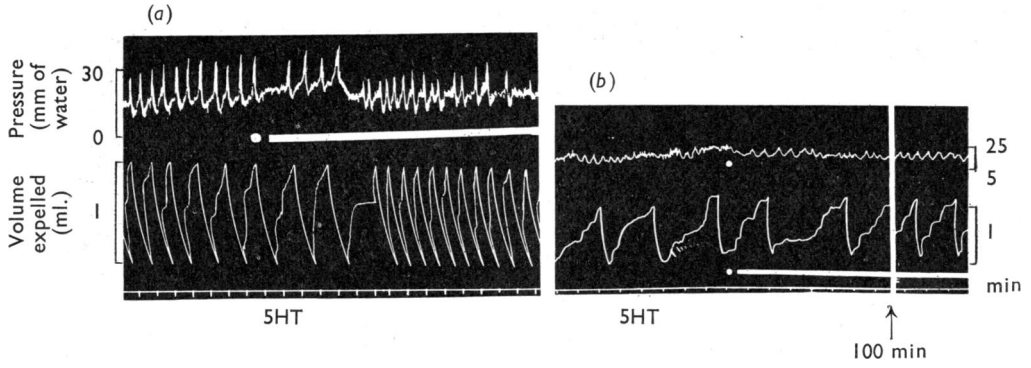

Fig. 7. Peristalsis *in situ*, and the effect of intraluminal 5-hydroxytryptamine perfusion, using the duodenal perfusion technique. Uppermost trace, intraducdenal pressure; middle trace, volume of fluid expelled; lowest trace, time in min. 5-Hydroxytryptamine perfusion (5HT, 100 μg/ml. at white dots and during lines) produced the following effects: (*a*) of normal adult rat (180 g), initial slight inhibition followed by transient stimulation; (*b*) of a tryptophan-deficient rat (30 g), prolonged improvement in peristaltic performance, equal to an increase of 49.6% (transport rate) 100 min later.

Consequently, though the transport volume was less than in stomach perfusions, the initial transport rates were similar (Tables 6 and 7).

Effects of 5-hydroxytryptamine in tryptophan-deficient and control rats. In contrast to experiments using stomach perfusion, with the duodenal perfusion technique 5-hydroxytryptamine (0.1 μg/ml. to 0.5 mg/ml.) in the lumen exerted very little effect. In the tryptophan-deficient rats inhibitory responses predominated in four of ten experiments (Table 7), whereas such effects were predominant in only one of five control rats. Nevertheless, the maximal quantitative effects of 5-hydroxytryptamine were not significantly different ($P>0.05$) in normal, control and deficient animals. The amine failed to cause any pronounced stimulation even in rats showing complete depletion, except in one experiment (no. 1, Table 7) where prolonged perfusion of 100 μg/ml. of 5-hydroxytryptamine did improve the peristaltic performance by 49.6% in 100 min (Fig. 7,*b*), the improvement lasting for about 30 min.

DISCUSSION

This work was carried out to try to answer the following question: is the presence of 5-hydroxytryptamine obligatory for the normal stimulation of sensory receptors, impulses from which initiate the peristaltic reflex? The results indicate that its presence is not obligatory, though the amine may have an ancillary, physiological effect, both on the peristaltic reflex itself and on the movements of the pyloric sphincter.

In the normal rat, peristalsis *in vitro* and *in situ* was similar to that described by Bülbring and co-workers in the guinea-pig and rabbit (Bülbring & Lin, 1958; Bülbring & Crema, 1958, 1959b) and also by Lembeck (1958) in the guinea-pig. The typical transient stimulation of peristalsis by 5-hydroxytryptamine, followed

by inhibition, previously reported in man (Hendrix, Atkinson, Clifton & Inglefinger, 1957 ; Haverback & Davidson, 1958 ; Schmid & Kinzlmeier, 1959), dogs (Haverback, Hogben, Moran & Terry, 1957 ; Ludany, Gati, Szabo & Hideg, 1959), rabbits (Bülbring & Lin, 1958 ; Georges & Herold, 1958) and guinea-pigs (Bülbring & Lin, 1958 ; Bülbring & Crema, 1958), was usually seen in the rat. Thus, 5-hydroxytryptamine behaved as a typical stimulant of sensory receptors: stimulation, though often pronounced, was invariably transient, and, as the sensory receptors adapted and became desensitized, stimulation was followed by inhibition. Though this was the general picture, there was an important exception: the longitudinal muscle of rat gut appears to be exquisitely sensitive to 5-hydroxytryptamine, so that large maintained contractions were produced by serosal, as well as intraluminal, 5-hydroxytryptamine. This sensitivity was less evident in the tryptophan-deficient rats, and may account, in part, for the comparative failure of the amine to stimulate deficient intestine.

The present experiments show clearly that 5-hydroxytryptamine exerted no more stimulatory effect on the peristaltic reflex of preparations apparently completely depleted of amine than it did on those showing a lesser degree of depletion, or on preparations containing normal levels of 5-hydroxytryptamine. Such results are important additional evidence that 5-hydroxytryptamine is not essential for peristalsis, but they are not conclusive.

More critical is the evidence which showed that peristalsis, *in vitro* and *in situ*, was normal in tryptophan-deficient rats *before* any 5-hydroxytryptamine had been given. Since Gal *et al.* (1961, 1962) were unable to detect any synthesis of 5-hydroxytryptamine *in vitro* in brains of tryptophan-deficient rats, actual synthesis of amine was probably also abolished in the intestine and other tissues of my deficient animals after 1 month's deprivation of L-tryptophan, even though brain 5-hydroxytryptophan decarboxylase activity *in vitro* was normal in these animals (Boullin, 1963a).

The normal peristaltic responses seen in deficient rats were all the more surprising in view of the highly significant lowering of the mucosal weights of these animals, compared with control and normal rats. The difference can probably be attributed to erosion of the mucosa, as Cole & Scott (1954) observed this phenomenon in adult tryptophan-deficient rats, and noted that it extended as far as the crypts of Lieberkühn in the duodenum and ileum.

It is possible that the actual sensory receptors necessary for evoking the peristaltic reflex, and commonly thought of as being in the deep layer of the mucosa as well as in close proximity to the enterochromaffin cells adjacent to the basal membrane (Masson, 1928 ; Bülbring & Crema, 1959b ; Verity, Mellinkoff, Frankland & Greipel, 1962), are not there, but in some slightly deeper layer of the intestinal wall. It is known that the number of enterochromaffin cells, measured by the intensity of a histochemical reaction, diminishes in tryptophan deficiency (Zbinden, Pletscher & Studer, 1958), so that the presence of the mucosal layer may not even be essential for initiation of peristalsis (Ginzel, 1959). Removal of this layer in other species does not invariably abolish the peristaltic reflex (Bülbring *et al.*, 1958 ; Ginzel, 1959). In addition, it has been found that the mucosal layer became detached in

normal rats during studies of peristalsis *in vitro*, so that occasionally the entire layer sloughed off and blocked the outlet cannula, preventing further fluid transport. When the blockage was removed peristalsis continued, yet at the end of the experiment it was not possible to scrape off more than a few mg of mucosa to assay for 5-hydroxytryptamine (Boullin, unpublished).

Whatever the precise location of sensory receptors which trigger peristalsis, the present experiments strongly suggest that 5-hydroxytryptamine is not obligatory as a sensory stimulant or essential for peristalsis in any way.

I should like to express my grateful thanks to Dr. Edith Bülbring, who encouraged me to begin this work, for her great interest in its progress. It is also a pleasure to thank Dr Philip Marshall for his help and advice, Professor Robert Hunter for provision of facilities, Messrs Thomas Black, Alec Soutar, Kenneth Didcock, the late Alan O'Neill and Miss Anne Cattermole for technical assistance, and Miss Margaret MacKenzie for drawing Fig. 1.

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