Brit. J. Pharmacol. (1958), 13, 444.

OBSERVATIONS CONCERNING THE ACTION OF 5-HYDROXYTRYPTAMINE ON THE PERISTALTIC REFLEX

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(RECEIVED JULY 28, 1958)

In isolated guinea-pig intestine 5-hydroxytryptamine increased the longitudinal muscle contractions in response to acetylcholine while the ganglionic action of nicotine was first facilitated and then blocked. Phenyldiguanide, veratrine, veratridine and protoveratrine, like 5-hydroxytryptamine, depressed the response to nicotine, leaving that to acetylcholine unaffected.

The sensory stimulants, like 5-hydroxytryptamine, facilitated the peristaltic reflex when applied to the mucosa, and abolished it when applied to the serosa. Preceding the block, the initial effect of low concentrations of 5-hydroxytryptamine applied to the serosa was a short stimulation of peristalsis.

Concentrations of 5-hydroxytryptamine which had an approximately equal stimulant action (mucosal 1 to 4 $\times 10^{-6}$, serosal 2 to 8 $\times 10^{-8}$) were tested when various parts of the reflex arc were blocked. During block by procaine introduced into the lumen, mucosal application of 5-hydroxytryptamine re-established peristalsis, but serosal application of 5-hydroxytryptamine had no effect. During block by hexamethonium or atropine present in the bath, 5-hydroxytryptamine restored peristalsis more effectively by serosal application than by mucosal application. During block by serosal application of 5-hydroxytryptamine, morphine, phenoxybenzamine or dihydroergotamine, mucosal application of 5-hydroxytryptamine restored the peristaltic reflex while serosal application had no effect. During block by 2-bromo-lysergic acid diethylamide or lysergic acid diethylamide acting from the serosal surface, 5-hydroxytryptamine had no effect whether acting on the mucosal or on the serosal surface.

It is concluded that 5-hydroxytryptamine facilitates the peristaltic reflex at two sites: when introduced into the lumen it stimulates mucosal sensory receptors; when acting from the serosal surface it sensitizes the muscle to the transmitter acetylcholine. There is also a transient stimulant action on the ganglia which is soon followed by inhibition; this indicates that 5-hydroxytryptamine applied to the serosa abolishes peristalsis by ganglion block.

Two different actions of 5-hydroxytryptamine on the peristaltic reflex have recently been observed on isolated intestine of the guinea-pig. If 5-hydroxytryptamine was applied to the serosal surface, it inhibited peristalsis (Kosterlitz and Robinson, 1957; Ginzel, 1957); if it was applied to the mucosal surface it facilitated the peristaltic reflex (Bülbring and Lin, 1958; Lembeck, 1958). The stimulant action could be explained by the effect of 5-hydroxytryptamine on sensory receptors in the mucosa. The mechanism of the inhibitory action, however, when 5-hydroxytryptamine was applied to the serosa was not clear.

It is known that a variety of substances stimulate not only chemoreceptors but also tension and stretch receptors in various organs (Dawes and Comroe, 1954; Iggo, 1957; Paintal, 1954, 1957a and b). In the present investigation the action of some sensory stimulants, phenyldiguanide and veratrum alkaloids, has been compared with that of 5-hydroxytryptamine. Furthermore, the action of 5-hydroxytryptamine was tested in the presence of various substances which blocked different parts of the peristaltic reflex arc selectively, in an attempt to obtain more information on the site of action of 5-hydroxytryptamine.

METHODS

In one series of experiments the longitudinal contractions of isolated strips of guinea-pig ileum were recorded in the conventional way. In a second series of experiments the peristaltic reflex was studied, using the method of Bülbring and Lin (1958) and also a modification described by Bülbring, Crema and

Saxby (1958). By this method records were obtained of the longitudinal contractions, the intraluminal pressure, the threshold at which the peristaltic reflex was elicited, and of the fluid expelled from the lumen during peristalsis. Normally, no fluid flowed unless there was peristaltic activity. However, in experiments in which peristalsis was blocked and in which it was desired to test the effect of intraluminal application of various substances, the height of the fluid reservoir in relation to the outflow valve was adjusted in such a way that a slow flow of solution was maintained.

We used pure samples of veratridine and protoveratrine, a crude preparation of veratridine (B.D.H.), and veratrine (mixture of alkaloids) [B.D.H.]. Doses of 5-hydroxytryptamine are expressed in weight of 5-hydroxytryptamine creatinine sulphate (Sandoz, Ltd.).

RESULTS

The Effect of 5-Hydroxytryptamine, Phenyldiguanide and Veratrine on the Longitudinal Contractions of Isolated Guinea-pig Ileum Evoked by Acetylcholine and Nicotine

Feldberg and Lin (1949) used small doses of acetylcholine and nicotine to produce contractions of the isolated intestine in order to test the excitability of the muscle and ganglia respectively. We have used their method to investigate the action of 5-hydroxytryptamine and other substances which are known to be sensory stimulants. Acetylcholine in concentrations between 1 and 2×10^{-8} and nicotine between 2 and 8×10^{-6} produced contractions of about equal size in isolated strips of guinea-pig ileum. We found that 5-hydroxytryptamine sensitized the muscle to the action of acetylcholine but reduced the action of nicotine on the ganglia.

Fig. 1 shows the effect of a short exposure to three different concentrations of 5-hydroxytryptamine on (*a*) the response to acetylcholine, and (*b*) the response to nicotine. Both were increased. It may be noted that in Fig. 1 (*a*) the response to acetylcholine was increasingly augmented with higher concentrations of 5-hydroxytryptamine in spite of the tachyphylaxis to 5-hydroxytryptamine itself, the largest dose of which had no effect. Fig. 1 (*b*) shows that the response to nicotine was more increased by 5-hydroxytryptamine 2×10^{-7} than by 2×10^{-8}, but less increased by 2×10^{-6}. Larger doses of 5-hydroxytryptamine caused only depression.

Fig. 2 (*a*) shows the effect of prolonged exposure to 5-hydroxytryptamine 2×10^{-5}. This caused a large contraction, but the muscle relaxed completely while 5-hydroxytryptamine was still

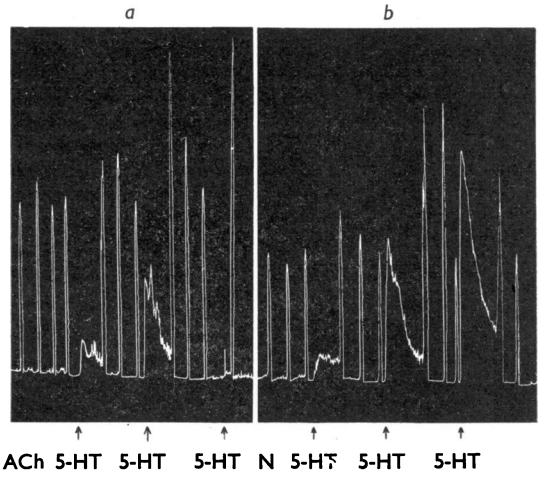

FIG. 1.—Longitudinal contractions of isolated guinea-pig ileum. Effect of 5-hydroxytryptamine (*5-HT*) (1) 2×10^{-8}, (2) 2×10^{-7} and (3) 2×10^{-6} on (*a*) response to acetylcholine (*ACh*) 1×10^{-8}; (*b*) response to nicotine (*N*) 2×10^{-6}.

present. In this condition the acetylcholine response was increased, while the nicotine response was depressed. When 5-hydroxytryptamine was washed out, the acetylcholine response declined and the nicotine response recovered slowly, but complete recovery occurred only after 45 min.

Thus in all concentrations from 2×10^{-8} to 2×10^{-5} 5-hydroxytryptamine increased the action of acetylcholine; it never decreased it. On the other hand, though low concentrations of 5-hydroxytryptamine enhanced the nicotine response, this soon gave way to depression. Higher concentrations of 5-hydroxytryptamine regularly reduced the effect of nicotine.

Phenyldiguanide, tested in the same way as 5-hydroxytryptamine, did not increase the response to acetylcholine or nicotine. In concentrations below 2×10^{-5} it had no effect on the acetylcholine response, but depressed that to nicotine; in doses of 4 and 8×10^{-5} it depressed both, but the response to nicotine was reduced to a greater extent than that to acetylcholine. Moreover, the depression of the acetylcholine response by phenyldiguanide was very transient. This is probably the reason why it was not observed previously by Bülbring and Lin (1958), who only noticed the depression of the nicotine response.

In Fig. 2 (*b*) an experiment is shown in which phenyldiguanide 4×10^{-5} was present for 1 hr. The responses to both acetylcholine and to nicotine were at first reduced by half. But while the nicotine response was progressively further depressed the acetylcholine response soon recovered though phenyl-

diguanide was still present. The nicotine response recovered only 30 min. after washing out the phenyldiguanide.

A few experiments were carried out using veratrine which, in concentrations of 1 and 2×10^{-6}, depressed the response to acetylcholine as well as to nicotine but, like 5-hydroxytryptamine and phenyldiguanide, had a stronger depressant effect on the nicotine contraction.

From the results described so far it appeared that 5-hydroxytryptamine had a selective depressant action on ganglionic excitability but increased the muscular response to acetylcholine. Phenyldiguanide had a similar differential action, depressing the ganglionic response severely but reducing the muscle response only temporarily. Further experiments were therefore carried out in which the peristaltic reflex was used to test the action of sensory stimulants on the nervous mechanism.

The Effect of Phenyldiguanide, Nicotine, and Veratrum Alkaloids on the Peristaltic Reflex

In the method described by Bülbring and Lin (1958) the preparation was suspended isometrically. This method was only used for a few

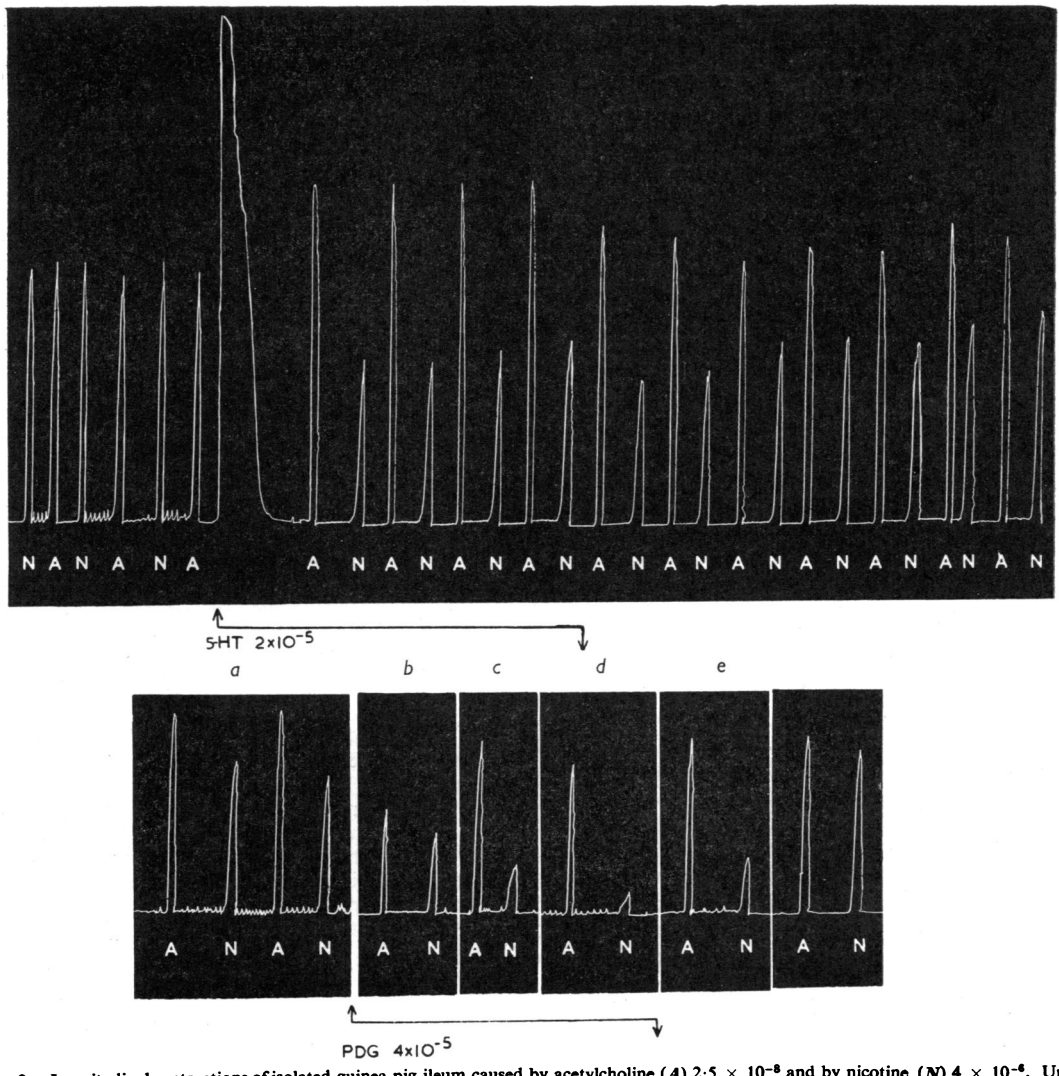

FIG. 2.—Longitudinal contractions of isolated guinea-pig ileum caused by acetylcholine (*A*) $2{\cdot}5 \times 10^{-8}$ and by nicotine (*N*) 4×10^{-6}. Upper tracing: between the arrows 5-hydroxytryptamine (5-HT) 2×10^{-5} was present. Lower tracing: between arrows phenyldiguanide (PDG) 4×10^{-5} present. (*a*) before PDG added; (*b*) 9 min., (*c*) 24 min., (*d*) 51 min. after PDG added., (*e*) 6 min., (*f*) 24 min. after PDG removed.

experiments. It was modified (Bülbring *et al.*, 1958) in order to record longitudinal contractions in isotonic conditions as in the original method described by Trendelenburg (1917). In addition fluid was passed through the lumen so that the intraluminal pressure, the threshold at which the peristaltic reflex occurred, and the fluid transport could also be recorded.

The substances tested all acted like 5-hydroxytryptamine when they were administered into the intestinal lumen. They first caused facilitation of the peristaltic reflex, and this was followed by block. An example is shown in Fig. 3. The record of intraluminal pressure (P) was taken from the oral end of the intestine, showing the slow rise of filling pressure and (at the dot) the steep rise due to the peristaltic contraction. After the injection of 50 μg. protoveratrine the threshold was reduced and thus the frequency of contractions increased. The change of pressure during each contraction became smaller, as there was less back pressure and more fluid was pushed in the caudal direction. This was evident from the lower record (V) in which the vertical strokes, indicating the expulsion of 1 ml. of fluid, occurred at shorter intervals. When the stimulant action gave way to paralysis, the back pressure increased while fluid propulsion diminished and finally stopped. The paralysis which followed the initial stimulation was observed in spite of the fact that in most experiments the substances to be tested were given by injection at a short distance from the point at which the fluid entered the lumen of the intestine (which represented a dead space of 1 ml.). This method allowed the drug to exert its action only for a short time after which it was removed by the constant flow of solution. All the results are summarized in Table I.

Serosal application of the sensory stimulants blocked peristalsis. Phenyldiguanide in doses of 0.2 to 0.4 mg., producing a concentration of 4 to 8×10^{-6} in the bath, depressed the peristaltic reflex. Lower concentrations had no effect, higher concentrations abolished the reflex. Fig. 4 shows the effect of adding to the bath in (*a*) 0.4 mg. phenyldiguanide which reduced the longitudinal muscle contractions and raised the pressure threshold. When 1.0 mg. phenyldiguanide was added to the bath in (*b*) a spasm occurred and peristalsis stopped, except for some irregular waves which occurred at a high threshold.

Veratrine and the pure veratrum alkaloids de-

TABLE I

EFFECTS ON PERISTALTIC REFLEX OF ISOLATED GUINEA-PIG ILEUM

(+ = stimulation, − = depression)

Bath vol. = 50 ml. Intestinal lumen, c. 2 ml., continuously changing during peristalsis at c. 2 ml./min.

Substance	Mucosal Application		Serosal Application	
	Dose Giving Effect (Equivalent to 100 μg. 5-Hydroxytryptamine)	Effect	Dose	Effect
Phenyldiguanide	0·2 mg. injected	+ −	0·2–0·4 mg.	Depression, threshold raised
,,	4×10^{-5} infused	+ −	2·0 mg.	Contracture, reflex abolished
Veratrine complex	150 μg.	+ −	Not tested	
Veratridine crude	100 μg.	+ −	,,	
Veratridine pure	50 μg.	+ −	5–10 μg.	Irregular contractions, reflex impaired
			50 μg.	Peristalsis abolished
Protoveratrine	50 μg.	+ −	5–10 μg.	Irregular contractions, reflex impaired
			50 μg.	Peristalsis abolished
Nicotine	20–30 μg.	+ −	20 μg.	+ −

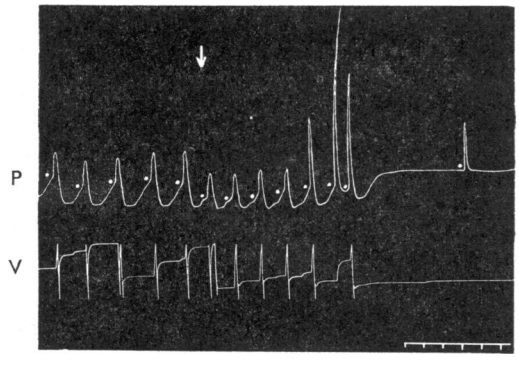

FIG. 3.—Effect of an intraluminal injection (at arrow) of 50 μg. of protoveratrine on peristalsis. Method of Bülbring and Lin (1958). Upper trace: intraluminal pressure (*P*). Threshold is marked by dots. Lower trace: volume (*V*). Each vertical stroke indicates the expulsion of 1 ml. fluid from the isolated gut. Time, 30 sec.

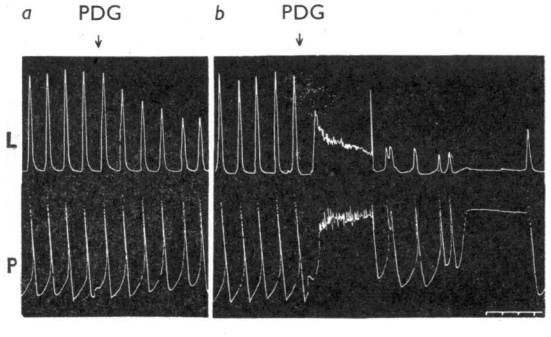

FIG. 4.—Effect of serosal application of phenyldiguanide (PDG) on peristalsis. Method of Bülbring *et al.* (1958). Upper trace: longitudinal contractions (*L*). Lower trace: intraluminal pressure (*P*). At first arrow 0·4 mg. PDG and at second arrow 1 mg. PDG was added to fluid in the 50 ml. bath. Time, 30 sec.

pressed or abolished the peristaltic reflex when they were applied to the serosal surface. They caused irregular, incoordinated muscle contractions, and consequently fluid propulsion was impaired. When veratrine or the pure veratrum alkaloids were washed out, after serosal as well as mucosal application, there followed a prolonged period of increased activity with frequent contractions at very low pressure threshold; yet the volume of fluid expelled was diminished. While this activity persisted, further veratrine doses were ineffective. Furthermore, if veratrine or the pure veratrum alkaloids were given in gradually increasing doses, starting with subthreshold doses, no effect was produced by large doses which otherwise, if applied as the first dose, were effective.

From these experiments it appeared that phenyldiguanide and the veratrum alkaloids, like 5-hydroxytryptamine, caused sensitization followed by desensitization of mucosal sensory receptors when the substance in question was introduced into the lumen of the intestine.

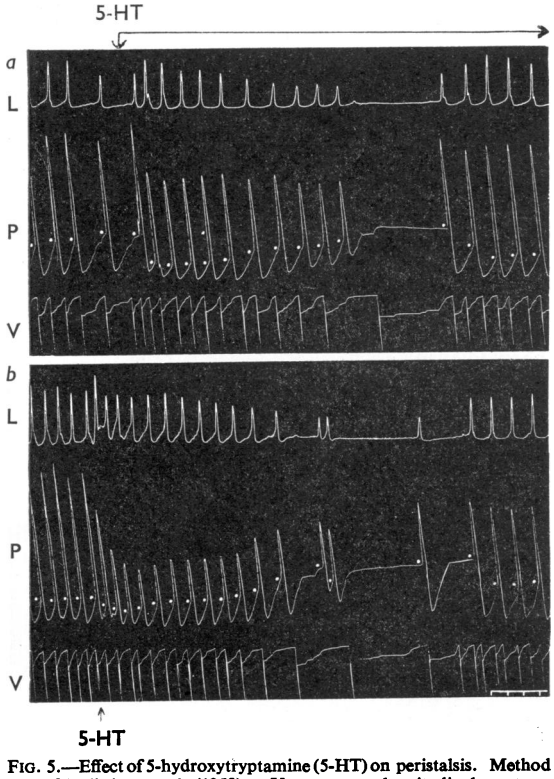

FIG. 5.—Effect of 5-hydroxytryptamine (5-HT) on peristalsis. Method of Bülbring *et al.* (1958). Upper trace: longitudinal contractions (*L*). Middle trace: intraluminal pressure (*P*). Threshold is marked by dots. Lower trace: Volume (*V*). Each vertical stroke indicates expulsion of 1 ml. fluid. (*a*) From arrow, mucosal application of 5-HT: concentration of 4×10^{-6} in solution passing through lumen. (*b*) At arrow, serosal application of 5-HT 2×10^{-7} (10 μg. added to 50 ml. bath). Time, 30 sec.

Serosal application of the sensory stimulants depressed the peristaltic reflex as did 5-hydroxytryptamine; but unlike 5-hydroxytryptamine they produced no initial facilitation.

The Action of 5-Hydroxytryptamine on the Normal Peristaltic Reflex

In the present work, in which the isolated piece of intestine was suspended in isotonic conditions, it was found that 5-hydroxytryptamine, when applied to the serosal surface, could in low concentrations (10^{-8} to 10^{-7}) also stimulate peristalsis. This effect has been described for cooled intestine by Beleslin and Varagić (1958). The stimulation was short-lasting but rapid in onset. The effect of intraluminal application of 5-hydroxytryptamine 4×10^{-6} is shown in Fig. 5*a*, and the response to the addition of 10 μg. 5-hydroxytryptamine to the solution in the bath, producing a concentration of 2×10^{-7}, is seen in Fig. 5*b*. Both caused stimulation followed by paralysis. Acting on the mucosa (*a*), 5-hydroxytryptamine lowered the threshold considerably for a period of 6 min., and after a short pause in activity the threshold remained lower than before. Acting on the serosal surface (*b*), 5-hydroxytryptamine lowered the threshold slightly for about 2 min., and, after a pause, the threshold remained higher than before. It should be noted that as the propulsive efficiency was increased the development of back pressure during each contraction, as measured at the oral end of the intestine, in the middle trace, became less. Fig. 6 shows, for comparison, the effects of two different doses of 5-hydroxytryptamine added to the bath. The contracture of the longitudinal muscle and the diphasic action on the threshold of intraluminal pressure and on the fluid transport are clearly seen.

With 5-hydroxytryptamine, as with veratrine, it was difficult to determine threshold doses because, when gradually increasing doses were administered, desensitization took place and no effect was produced by large doses. Many experiments were carried out giving doses in ascending as well as in descending order. As a result it was found that the concentrations of 5-hydroxytryptamine which had an approximately equal stimulant action on peristalsis were 4×10^{-6} (intraluminal) and 8×10^{-8} (serosal). These concentrations were used for testing the activity of 5-hydroxytryptamine in a series of experiments in which the peristaltic reflex was blocked at different parts of the nervous pathway.

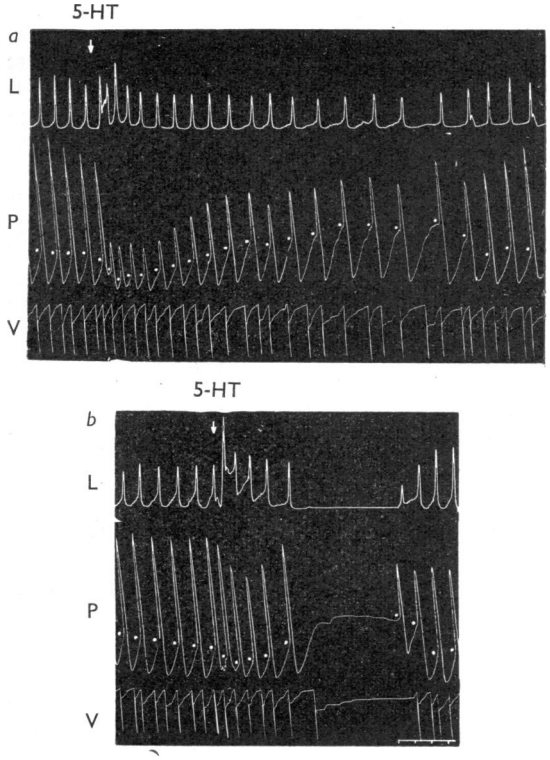

FIG. 6.—Records as in Fig. 5. Effect of serosal application of 5-hydroxytryptamine (5-HT). At arrow, (*a*) 5 μg., (*b*) 20 μg. 5-HT added to 50 ml. bath. Time, 30 sec.

The Action of 5-Hydroxytryptamine on the Peristaltic Reflex in the Presence of Procaine in the Intestinal Lumen

Procaine was used to block the mucosal sensory receptors. It was added to the fluid passing through the intestinal lumen in concentrations of 4 to 8×10^{-5}, which produced partial block of peristalsis, raising the threshold and slowing the contractions. Occasionally complete block of the peristaltic reflex occurred. Fig. 7 shows that, in the presence of procaine, 5-hydroxytryptamine applied to the mucosa was able to overcome the partial block, but that serosal application of 5-hydroxytryptamine had no effect. (Phenyldiguanide 1×10^{-4} applied to the mucosa was similarly active but weaker than 5-hydroxytryptamine.) The concentrations of procaine used for these experiments never exceeded 1×10^{-4}. When complete block occurred, mucosal application of 5-hydroxytryptamine and phenyldiguanide still caused peristalsis to reappear though only after a latent period of 3 to 6 min.

The results suggested that, when procaine was present in the lumen, stimulation of the partially or completely depressed sensory receptors by mucosal application of 5-hydroxytryptamine or phenyldiguanide restored the reflex, but that stimulation of muscles and ganglia did not.

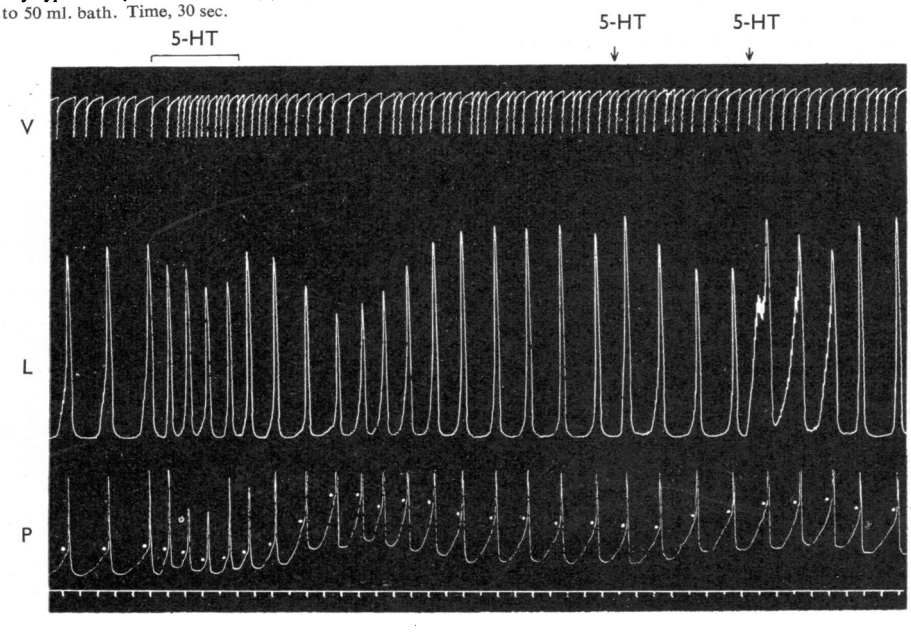

FIG. 7.—Records as in Fig. 5. In presence of procaine 8×10^{-5}, addition of 4×10^{-6} 5-hydroxytryptamine (5-HT) to solution passing through the lumen (indicated by the bracket) lowered threshold and increased volume of fluid expelled. Addition to 50 ml. bath of 4 μg. and 10 μg. of 5-HT (arrows) had no effect on peristalsis. Time, 30 sec.

The Action of 5-Hydroxytryptamine on the Peristaltic Reflex in the Presence of Hexamethonium and Atropine Acting from the Serosal Surface

Hexamethonium was used to block the peristaltic reflex path at the ganglia. A concentration of 3×10^{-6} usually produced partial block and 6×10^{-6} abolished peristalsis. When the block was not complete, 5-hydroxytryptamine, whether applied to the mucosa or to the serosal surface, was about equally active in facilitating the reflex. This is illustrated in Fig. 8. When peristalsis was abolished by hexamethonium, mucosal application restored the reflex only after a latent period of 3 to 5 min. (Fig. 9*a*). Repeated doses of nicotine, reaching a concentration of 4×10^{-7} in the bath, also overcame the blocking action of hexamethonium (Fig. 9*c*). In this record the vigorous pendular activity of the longitudinal muscle should be noted which occurred in the presence of hexamethonium during prolonged distension of the intestine; peristaltic activity, however, occurred only after 5-hydroxytryptamine or nicotine was added.

Atropine was used to block the action of the nervous transmitter acetylcholine on the muscle. Concentrations between 2×10^{-9} to 5×10^{-7} were used to depress peristalsis (Fig. 10); the threshold became higher and the longitudinal contractions became smaller. In these conditions mucosal and serosal application of 5-hydroxytryptamine were about equally effective in lowering the threshold. The addition of methacholine to the bath to produce a concentration of 2×10^{-9} to 1×10^{-8} was followed by a strong contraction and lowered the threshold at which the peristaltic reflex occurred (Fig. 10*b*). When the atropine concentrations were raised and the action of previously effective doses of methacholine was abolished, 5-hydroxytryptamine was still active, both with mucosal and serosal application (Fig. 10*c*). Very high concentrations of atropine, up to 1.2×10^{-5}, were required to abolish the peristaltic reflex. In this condition mucosal application of 5-hydroxytryptamine had no effect, but Fig. 10*d* shows that serosal application of only 4 μg. of 5-hydroxytryptamine was still effective in overcoming the block. The dose of methacholine (Fig. 10*d*), however, had to be increased to 1,000 times that which stimulated peristalsis before atropine was given. It produced a contracture of the longitudinal muscle lasting for $2\frac{1}{2}$ min. before violent contractions of the circular muscle occurred. In general, during complete block of peristalsis by atropine, as with hexamethonium, mucosal application of 5-hydroxytryptamine was less effective than serosal application.

The results obtained in the presence of hexamethonium and atropine suggested that, when the reflex pathway was blocked either at ganglionic or at muscular sites, the stimulation of these sites by serosal application of 5-hydroxytryptamine restored the reflex. When peristalsis was only partially blocked, the stimulation of sensory receptors in the mucosa also restored the reflex; but this was less effective than the action of 5-hydroxytryptamine at ganglionic and muscular sites. Further experiments were therefore carried out to ascertain that the two effects of 5-hydroxytryptamine were in fact due to two distinct actions at two different sites.

The Action of 5-Hydroxytryptamine, Applied to the Mucosa, on the Peristaltic Reflex in the Presence of 5-Hydroxytryptamine Acting from the Serosal Surface

When small doses of 5-hydroxytryptamine were repeatedly added to the bath, the first dose caused, as described above, some stimulation, but subsequent doses caused inhibition, and peristaltic activity ceased. During this block, which was caused by 5-hydroxytryptamine present in the bath (concentration about 5×10^{-7}), it was possible to restore the peristaltic reflex by introducing 5-hydroxytryptamine into the lumen of the intestine.

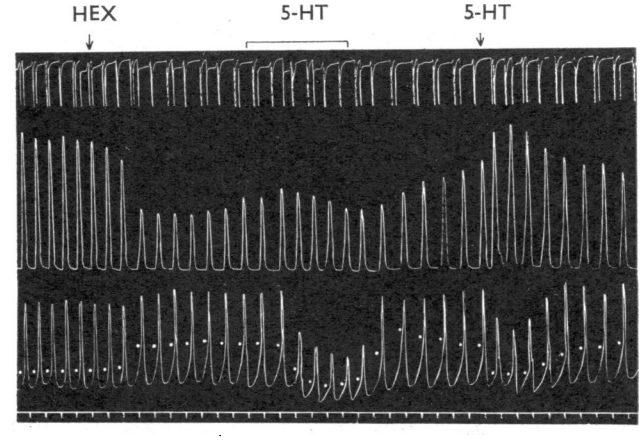

FIG. 8.—Records as in Fig. 5. At first arrow addition of 150 μg. hexamethonium to 50 ml. bath reduced longitudinal contractions (*L*) and raised pressure threshold (*P*). Mucosal application (indicated by the bracket) of 4×10^{-6} of 5-hydroxytryptamine (5-HT) had a stronger effect on threshold and at second arrow serosal application of 8×10^{-8} of 5-HT had a stronger action on longitudinal muscle contractions. Time, 30 sec.

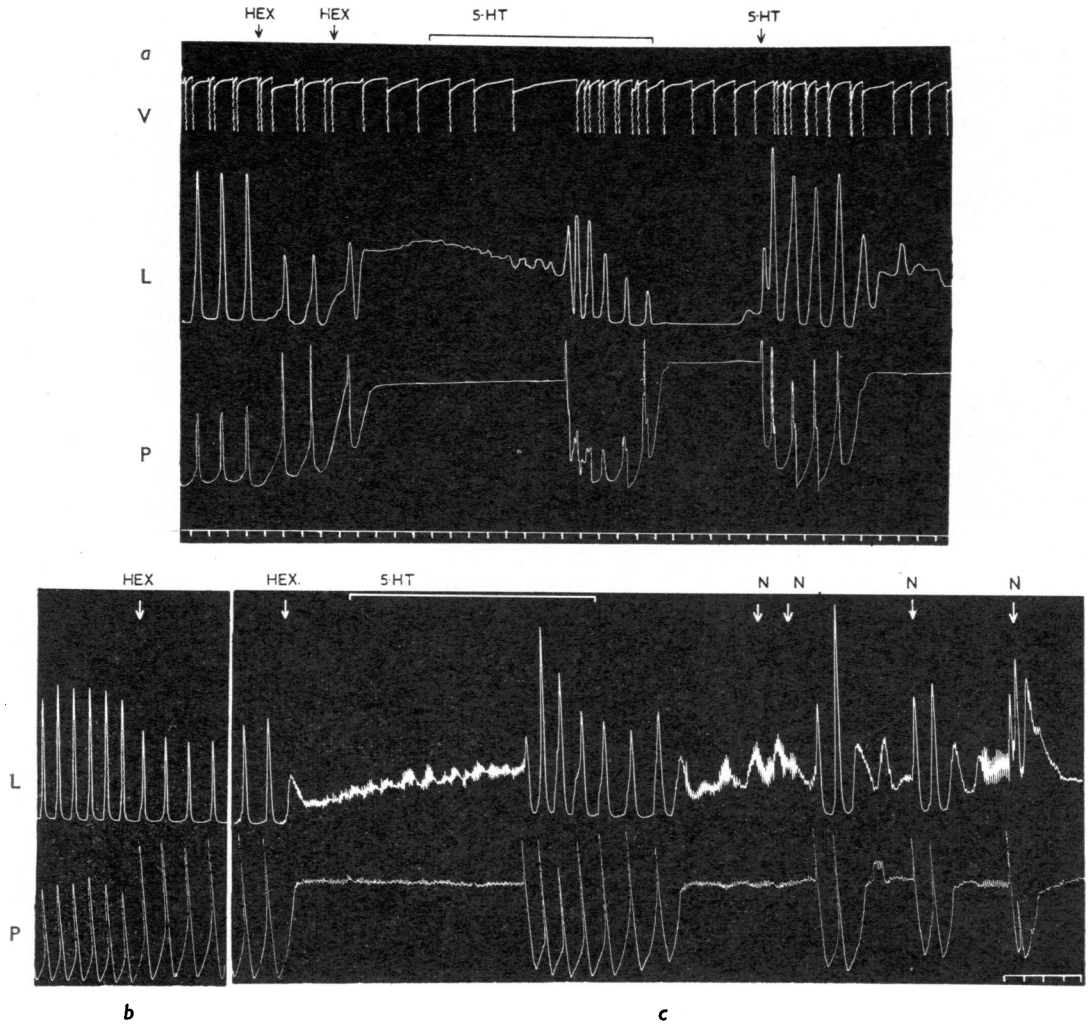

FIG. 9.—Records as in Fig. 5. (*a*) Complete block of peristalsis by two doses of hexamethonium (***HEX***) of 300 μg. each, to 50 ml. bath. Intraluminal 5-hydroxytryptamine (***5-HT***) indicated by the bracket restored reflex after 3 min. latent period, addition to bath (arrow) had immediate effect. Note increased fluid volume expelled. (*b*) Partial block caused by addition of 150 μg. of ***HEX***. (*c*) Complete block caused by further addition of 300 μg. of ***HEX*** to the bath. Reflex restored by mucosal application of ***5-HT*** during the bracket and also by serosal application of nicotine (***N***) at the arrows of 20 μg., 20 μg., 20 μg., and 40 μg. into 50 ml. bath. Time, 30 sec.

Such an experiment is shown in Fig. 11. The first dose of 16 μg. of 5-hydroxytryptamine, applied to the serosa, caused stimulation: subsequent doses of 4 μg. became gradually ineffective and peristalsis stopped. However, mucosal application caused peristalsis to reappear (Fig. 11*a*) after a latent period of several minutes. Peristalsis stopped once more when 5-hydroxytryptamine was washed out by flowing normal solution through the lumen. By adding a further dose of 4 μg. 5-hydroxytryptamine to the bath it was shown that the 5-hydroxytryptamine receptors in muscles or ganglia were still saturated. It was known from the results described in the first subsection of "Results" that this concentration of 5-hydroxytryptamine which caused the peristaltic block did not diminish the muscle response to the nervous transmitter acetylcholine. Therefore it was likely that peristalsis stopped as a result of ganglionic block. It could be restarted by stimulation of mucosal receptors with 5-hydroxytryptamine, or by stimulating the mucosal receptors with acetylcholine (Fig. 11*b*), or by serosal application of acetylcholine or nicotine.

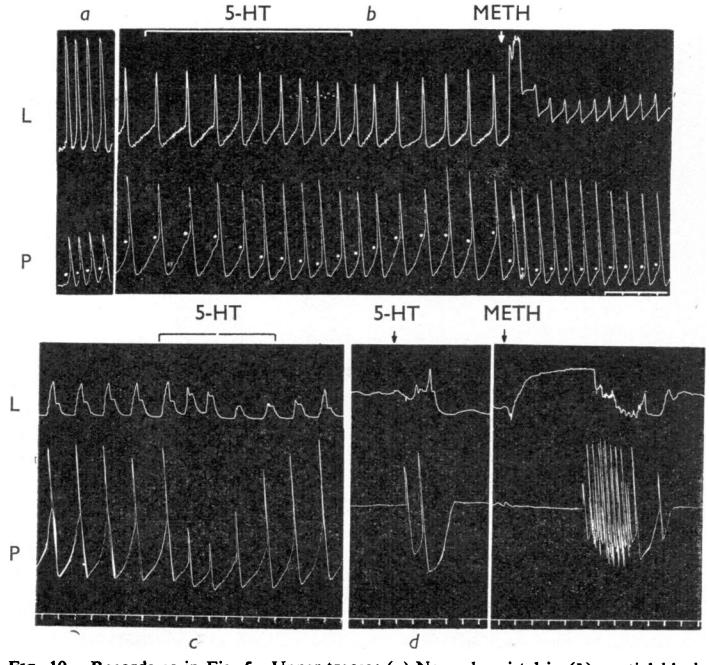

FIG. 10.—Records as in Fig. 5. Upper traces: (*a*) Normal peristalsis, (*b*) partial block in presence of $4{\cdot}8 \times 10^{-7}$ of atropine in bath. Effect produced by mucosal application (during the bracket) of 5-hydroxytryptamine (***5-HT***) 4×10^{-6}, and by addition (at the arrow) of 0·2 μg. of methacholine to the 50 ml. bath. (*c*) Partial block in the presence of 4×10^{-6} atropine in bath; effect of mucosal application (during bracket) of ***5-HT*** 4×10^{-6}, (*d*) complete block by atropine $1{\cdot}2 \times 10^{-5}$. Effect of serosal application (at arrow) of 4 μg. of ***5-HT***, (*e*) of 200 μg. methacholine at arrow. Time, 30 sec.

The Action of 5-Hydroxytryptamine on the Peristaltic Reflex in the Presence of Morphine, Phenoxybenzamine and Dihydroergotamine

Morphine was used to block 5-hydroxytryptamine receptors in nervous structures (Gaddum and Picarelli, 1957). When it was added to the fluid in the bath producing a concentration of 2 to 5×10^{-8}, morphine abolished the preparatory phase as well as the emptying phase immediately. However, while the longitudinal muscle contraction stopped completely, the circular muscle contracted at a very high frequency, but irregularly. When 5-hydroxytryptamine was introduced into the lumen these contractions became regular and soon reverted to co-ordinated peristaltic waves (Fig. 12*a*). The longitudinal contractions were only restored to some extent. Fig. 12 (*b* and *c*) shows that, when peristalsis was blocked by serosal application of morphine, serosal application of 5-hydroxytryptamine was ineffective at a time when mucosal application was still able to co-ordinate the high-frequency circular contractions and restore the reflex.

When morphine was added to the solution flowing through the lumen, 100 times higher concentrations (1 to 2×10^{-6}) were required to block the peristaltic reflex than when morphine was present in the bath. The effect, however, was the same as that of morphine acting from the serosal surface. The longitudinal contractions and the emptying phase were abolished, but the circular muscle showed irregular activity of high frequency. Mucosal application of 5-hydroxytryptamine again restored the reflex.

Phenoxybenzamine (Dibenzyline) and dihydroergotamine were used to block 5-hydroxytryptamine receptors in the muscles (Gaddum and Picarelli, 1957). Serosal application of phenoxybenzamine 3×10^{-7} or dihydroergotamine 3×10^{-6} stopped the peristaltic reflex, abolishing longitudinal as well as circular muscle contractions. 5-Hydroxytryptamine was effective in overcoming the block, but its action was much more powerful when it acted on the mucosa than when it acted from the serosal surface. In the presence of 3×10^{-7} of phenoxybenzamine, however, 5-hydroxytryptamine produced no recovery of longitudinal muscle contractions though it restored the peristaltic waves of the circular muscle. In some experiments it was observed that phenoxybenzamine caused a small contraction and some stimulation of peristalsis before it caused depression. This happened only when the intestine had already been treated with 5-hydroxytryptamine before phenoxybenzamine was given.

In order to block 5-hydroxytryptamine receptors in the muscles as well as in nervous structures, morphine (1×10^{-7}) and either phenoxybenzamine (1×10^{-7}) or dihydroergotamine (1.5×10^{-6}) were administered simultaneously. These concentrations caused at first partial block, but during prolonged exposure complete cessation of peristalsis. Fig. 13*a* and *b* illustrates one of these experiments. Phenoxybenzamine reduced the longitudinal contractions to about half and raised the threshold at which the reflex was elicited (*b*). Morphine abolished the longitudinal contractions but stimulated the circular muscle, which contracted irregularly and

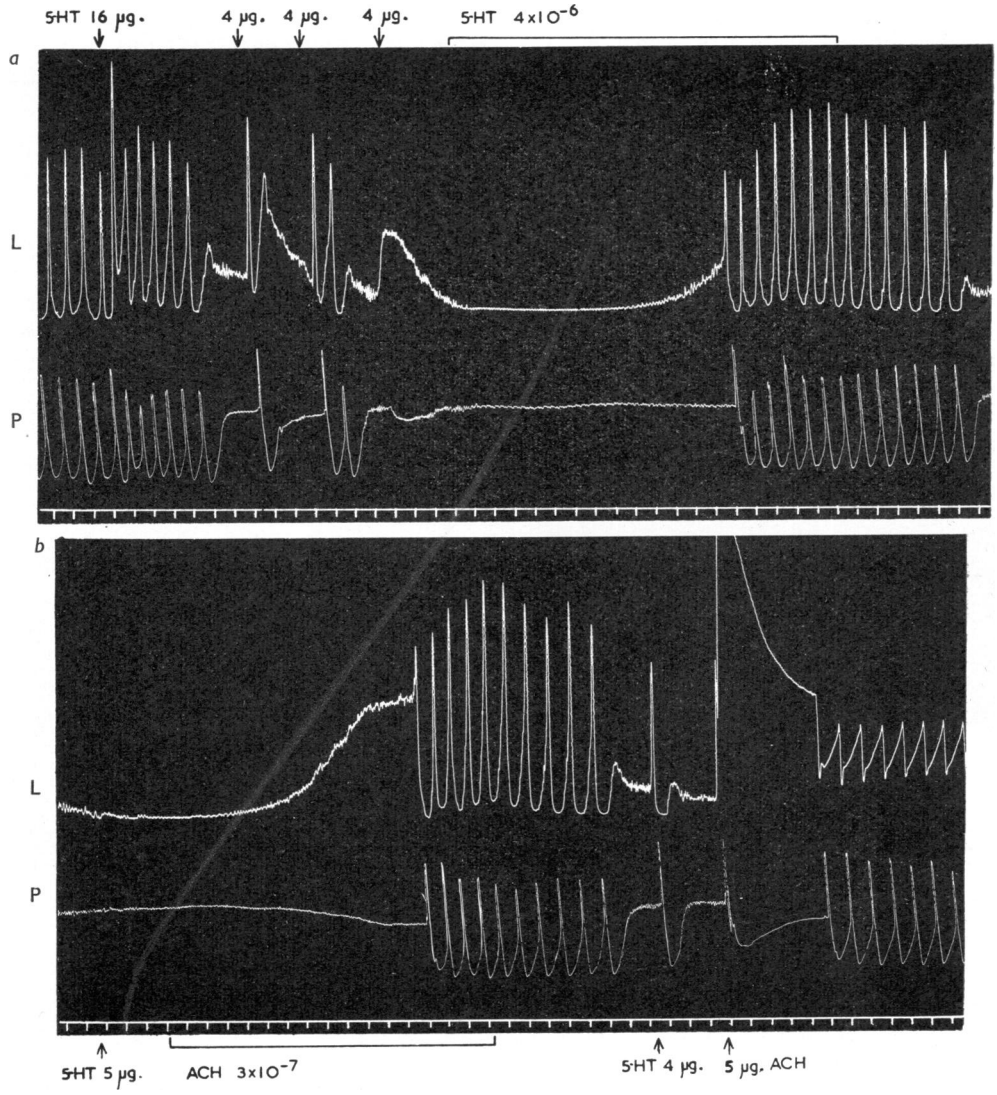

FIG. 11.—Records as in Fig. 5. (*a*) Peristalsis gradually blocked by repeated serosal application (at arrows) of small doses of 5-hydroxytryptamine (*5-HT*) and restored by mucosal application of *5-HT* (during bracket). (*b*) Continuation of record in (*a*), showing (during the bracket) effect of mucosal application of acetylcholine (*ACH*) 3×10^{-7}, and, at arrow, adding at 5 µg. *ACH* to 50 ml. bath. Time, 30 sec.

frequently. The introduction of 5-hydroxytryptamine into the intestinal lumen at once slowed the circular muscle movements, which now became co-ordinated; the longitudinal contractions also recovered to some extent. When 5-hydroxytryptamine was removed from the lumen peristalsis stopped. Serosal application of 5-hydroxytryptamine had no effect at this stage.

The most pronounced effect of 5-hydroxytryptamine, when introduced into the intestinal lumen and thus acting on the mucosa, was its co-ordinating action on the circular muscle contractions. Fig. 13*c* shows a tracing from an experiment in which peristalsis had been depressed by dihydroergotamine 1.5×10^{-6} in the bath. The addition of morphine 1×10^{-7} abolished longitudinal contractions and caused very frequent highly irregular movements of the circular muscle. When 5-hydroxytryptamine was added to the bath, it had no effect. However, for a short

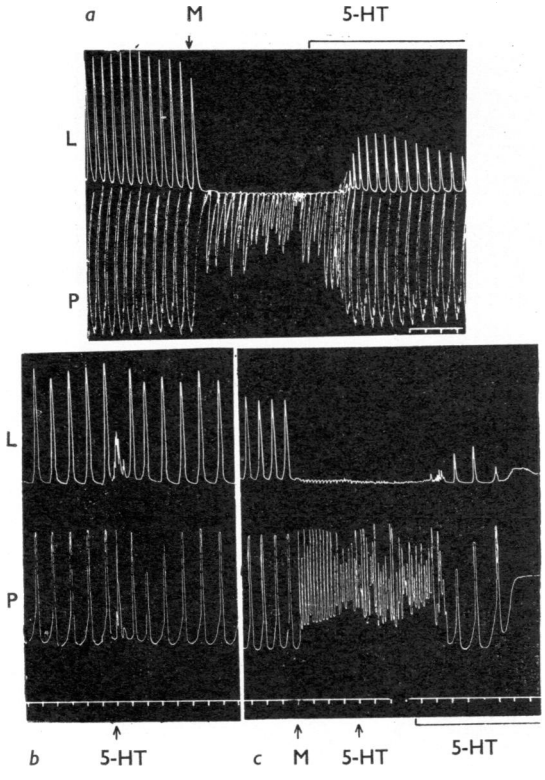

FIG. 12.—Records as in Fig. 5. (*a*) Abolition of peristalsis by adding 1 μg. morphine (*M*) to 50 ml. bath and restoration by presence of 5-hydroxytryptamine (*5-HT*) 4×10^{-6} in intestinal lumen (during the bracket). (*b*) and (*c*): traces from another experiment. (*b*) Effect of serosal application (at arrow) of *5-HT* (4 μg. in 50 ml.) on normal peristalsis. (*c*) Effect of 2·5 μg. morphine (*M*) after which serosal application (at arrow) of 4 μg. of *5-HT* had no action; but mucosal application (during the bracket) of *5-HT* 4×10^{-6} caused reappearance of co-ordinated waves. Time, 30 sec.

TABLE II

ACTION OF 5-HT ON PERISTALSIS IN PRESENCE OF 5-HYDROXYTRYPTAMINE ANTAGONISTS

Blocking Substance and Site of Application	Action of Blocking Substance on Longitudinal and Circular Muscle	5-Hydroxytryptamine: Serosal Application	5-Hydroxytryptamine: Mucosal Application	Comments on 5-Hydroxytryptamine Effect
5-Hydroxytryptamine Serosal	Longitudinal and circular contractions abolished Peristalsis abolished	0	+	Longitudinal and circular contractions restored Peristalsis restored
Morphine Serosal or mucosal	Longitudinal contractions abolished Circular contractions more frequent, irregular and uncoordinated Peristalsis abolished	0 0	+ +	Longitudinal contractions partly restored Circular contractions fully restored, slowed and co-ordinated Peristalsis restored
Phenoxybenzamine or dihydroergotamine Serosal	Longitudinal and circular contractions abolished Peristalsis abolished	(+) or 0	+	Longitudinal contractions partly restored Circular contractions fully restored Peristalsis restored
Morphine + phenoxybenzamine or dihydroergotamine Serosal	Longitudinal contractions abolished Circular contractions more frequent, irregular and uncoordinated Peristalsis abolished	(+) or 0	+	Longitudinal contractions partly restored Circular contractions fully restored, slowed and co-ordinated Peristalsis restored
2-Bromo-lysergic acid diethylamide or lysergic acid diethylamide Serosal	Longitudinal contractions continue Circular contractions abolished Peristalsis abolished	0	0	No effect

period during which 5-hydroxytryptamine was present in the intestinal lumen, regular peristalsis was restored; and this reverted to the irregular high-frequency activity as soon as the 5-hydroxytryptamine was washed out from the lumen.

These experiments, which are summarized in Table II, show that mucosal application of 5-hydroxytryptamine was always effective when 5-hydroxytryptamine receptors had been blocked selectively at nervous structures in the outer wall by morphine, or selectively at the muscle by phenoxybenzamine or dihydroergotamine, or when both kinds of receptors had been blocked. Serosal application in these conditions produced either no effect or a much smaller one than that on the mucosa. These results lent further support to the view that the two sites of action of 5-hydroxytryptamine were different and that serosal application did not reach mucosal sensory receptors; nor did mucosal application reach muscle or nervous structures in the outer wall.

The Effect of 2-Bromo-lysergic Acid Diethylamide and Lysergic Acid Diethylamide

When 2-bromo-lysergic acid diethylamide 8×10^{-6} or lysergic acid diethylamide 1.2×10^{-5} was present in the bath the peristaltic reflex was abolished (see Kosterlitz and Robinson, 1957; Ginzel, 1957). It was noted, however, that longitudinal muscle contractions still occurred at regular intervals, as shown in Fig. 14. In these conditions 5-hydroxytryptamine was entirely without action both from the serosal and the mucosal surface.

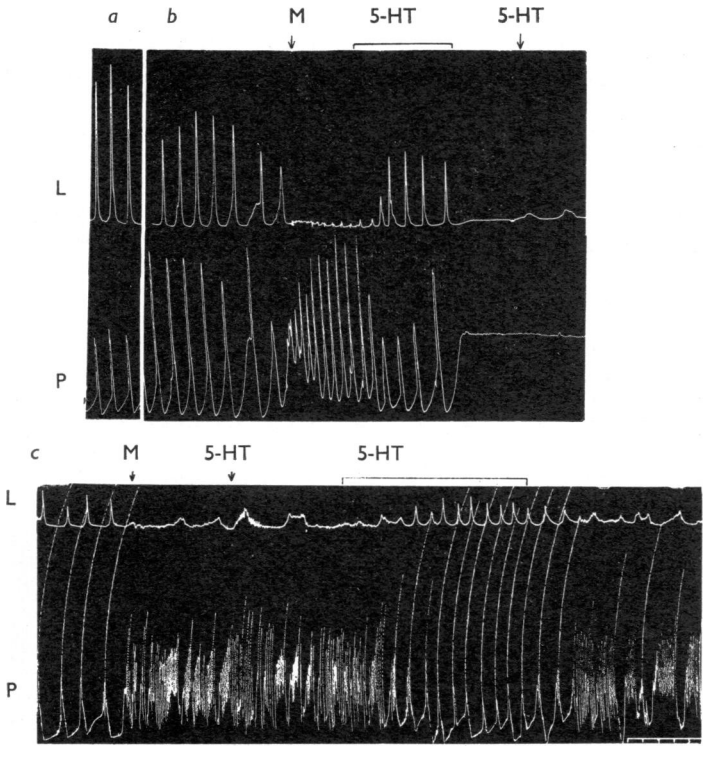

FIG. 13.—Records as Fig. 5. (*a*) before, (*b*) in the presence of phenoxybenzamine 10^{-7} in bath. Morphine 10^{-7} at *M*. Mucosal 5-hydroxytryptamine (***5-HT***) during bracket and serosal ***5-HT*** at arrow. (*c*) Trace from another experiment in presence of dihydroergotamine $1{\cdot}5 \times 10^{-6}$ in bath. Morphine 10^{-7} at *M*. Serosal ***5-HT*** at arrow and mucosal ***5-HT*** during bracket. Time, 30 sec.

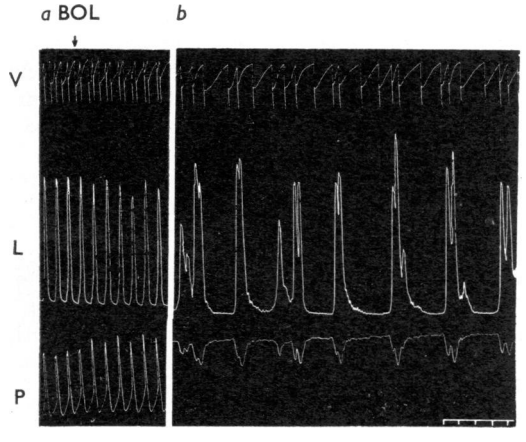

FIG. 14.—Records as in Fig. 5. Effect of serosal application of 2-bromo-lysergic acid diethylamide (BOL). (*a*) Immediate effect of 7×10^{-7} of BOL at arrow. (*b*) Final condition in presence of 8×10^{-6} of BOL in the bath. Time, 30 sec

DISCUSSION

When an organ of such complicated and only incompletely known anatomy as that of the intestine is exposed simultaneously to several pharmacologically active substances, phenomena are produced which are difficult to analyse. Nevertheless the present work has brought out several points which may clarify the action of 5-hydroxytryptamine.

The motor transmitter to intestinal muscle is generally believed to be acetylcholine, and supporting evidence for this view has recently been put forward by Paton (1956). In the present work it was found that 5-hydroxytryptamine, in addition to its own stimulant action, sensitized intestinal muscle to the action of acetylcholine. Moreover, at a time when 5-hydroxytryptamine receptors in the muscle were insensitive to 5-hydroxytryptamine after saturation with 5-hydroxytryptamine itself, the muscle was still hypersensitive to acetylcholine. The short-lasting facilitation of the peristaltic reflex which was observed after serosal application of 5-hydroxytryptamine might therefore be explained by sensitization at muscular sites.

This action of 5-hydroxytryptamine was similar to that in the sympathetic superior cervical ganglion described by Trendelenburg (1956a and b). He found that 5-hydroxytryptamine stimulated the ganglia, facilitated but never blocked ganglionic transmission, and sensitized the ganglia to injected acetylcholine which is the synaptic transmitter substance. The nature of the synaptic transmitter from sensory to motor neurons in the gut is not known. Marrazzi (1953) observed that 5-hydroxytryptamine blocked synapses in the central nervous system. All the evidence which we obtained indicated that in intestinal ganglia 5-hydroxytryptamine caused a transient facilitation of transmission followed by block. This assumption was based on the following observations:

While the longitudinal contractions of an isolated piece of intestine in response to acetylcholine were never depressed by 5-hydroxytryptamine, the nicotine response, after a transient increase, was always depressed by 5-hydroxy-

tryptamine. This indicated that 5-hydroxytryptamine depressed ganglionic excitability.

The peristaltic reflex was blocked by serosal application of 5-hydroxytryptamine. This could be overcome by serosal application of nicotine or acetylcholine. Moreover, when the peristaltic reflex was blocked by serosal application of 5-hydroxytryptamine itself, the block could also be overcome by applying 5-hydroxytryptamine to the mucosa. This last observation was of particular significance because it showed that 5-hydroxytryptamine acted on different sites according to the site of application. When it was added to the fluid in the bath it caused ganglion block. When it was introduced into the lumen it reinforced the sensory impulses arising from the mucosa and re-established the reflex pathway.

Recently Beleslin and Varagić (1958) showed that, when the peristaltic reflex was abolished by cooling the intestine, not only mucosal but also serosal application of 5-hydroxytryptamine restored the reflex. The authors believed that, when ganglionic transmission had been depressed by cooling, it could be stimulated by 5-hydroxytryptamine acting from the serosa and, though low concentrations facilitated transmission, high concentrations produced ganglion block. Furthermore, when in the cooled intestine block was produced after serosal application of a large dose of 5-HT, it was possible, by introducing the drug into the lumen, to overcome the block. In the present investigation results similar to those of Beleslin and Varagić (1958) were obtained at normal temperatures.

The action of some other sensory stimulants which were studied was similar to that of 5-hydroxytryptamine. When tested on the longitudinal muscle contractions in a piece of intestine, they had no depressant effect on the response to acetylcholine but they all depressed the response to nicotine. They all depressed the peristaltic reflex when applied to the serosal surface of a loop of intestine, and they all facilitated the peristaltic reflex when applied to the mucosa.

The experiments in which the peristaltic reflex was blocked by various substances acting specifically on different parts of the reflex arc threw further light on the various sites of action of 5-hydroxytryptamine.

When mucosal sensory receptors had been blocked by introducing procaine into the lumen of the intestine, 5-hydroxytryptamine applied to the mucosa was effective, but 5-hydroxytryptamine applied to the serosa was ineffective in overcoming the block. Thus when the sensory stimuli arising from the mucosa were diminished or absent, no facilitation at ganglionic or muscular sites by 5-hydroxytryptamine could restore peristalsis, but sensory stimulation at the mucosa by 5-hydroxytryptamine re-established the reflex; so did phenyldiguanide.

When ganglionic or neuromuscular transmission had been impaired by hexamethonium or atropine, the facilitation at ganglionic or muscular sites by 5-hydroxytryptamine as well as the reinforcement by 5-hydroxytryptamine of sensory stimuli arising from the mucosa re-established the reflex pathway. However, during complete block by hexamethonium or atropine, 5-hydroxytryptamine applied to the mucosa was less effective when applied to the serosa.

A few experiments were carried out using dihydroergotamine or phenoxybenzamine to block selectively muscular 5-hydroxytryptamine receptors and morphine to block nervous 5-hydroxytryptamine receptors (Gaddum and Picarelli, 1957). Morphine abolished the preparatory as well as the emptying phase (Kosterlitz and Robinson, 1955; Schaumann, 1955) but stimulated the circular muscle to high-frequency activity, which, however, did not lead to fluid propulsion. In this condition mucosal application of 5-hydroxytryptamine had a striking effect: it slowed the excessive activity and transformed the contractions of the circular muscle into well-coordinated peristaltic waves.

It has been shown that morphine diminishes the release of acetylcholine from postganglionic motor nerve endings (Paton, 1957; Schaumann, 1957), which might be part of its blocking action on peristalsis. In these conditions serosal application of 5-hydroxytryptamine can have no effect because morphine blocks its action. If morphine diminished the release of the preganglionic sensory transmitter, the mucosal application of 5-hydroxytryptamine might be capable of re-establishing the reflex pathway through stimulation of mucosal sensory receptors, as in fact it does.

Procaine has been shown by Harvey (1939) to diminish the release of acetylcholine at preganglionic nerve endings. In this connexion it should be pointed out that the action of morphine in stimulating high-frequency activity in the circular muscle was not dissimilar from the effect seen in the presence of local anaesthetics. Here also, 5-hydroxytryptamine applied to the mucosa facilitated the peristaltic reflex, but applied to the serosa it had no effect.

The origin of the circular muscle activity produced by morphine is not explained. Increased muscle tone has been observed by many authors after administration of morphine. The mechanism has been discussed by Vaughan Williams and Streeten (1950), who pointed out that it might be a muscular response when the intestine is kept forcibly dilated. In the experimental conditions of the present investigation the intraluminal pressure remained high unless the fluid was expelled by a wave of peristalsis. It may be that the circular contractions represent the muscular response to the prolonged distension of the wall, which occurs when the nervous control, which normally co-ordinates the contractions to propulsive waves, has been removed by morphine. On the other hand, it may be the result of a direct stimulant action of morphine on the smooth muscle which has been observed by Bülbring and Burnstock (unpublished observations).

This work has been done during the tenure by one of us (A. C.) of a Riker Fellowship for which he wishes to express his thanks to the Riker Laboratories, Ltd. We are indebted to Professor O. Krayer for the samples of pure veratrine and protoveratrine to Smith, Kline and French for the phenoxybenzamine, and to Sandoz, Ltd., for 5-hydroxytryptamine creatinine sulphate.

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