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THE LONGITUDINAL MUSCLE COMPONENT OF THE PERISTALTIC REFLEX IN THE GUINEA-PIG ISOLATED ILEUM

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The peristaltic reflex of the guinea-pig isolated intestine was first described by Trendelenburg (1917). The reflex is elicited by raising the pressure within the lumen. It consists of repeated waves of contraction of the circular muscle layer which pass from the oral to the aboral end of the segment of intestine. Each wave of contraction of the circular muscle layer is preceded by a contraction of the longitudinal muscle layer. Both muscle layers relax simultaneously. The reflex can be recorded either by the interrupted method as described by Trendelenburg (1917) or by the continuous method (Bülbring, Crema & Saxby, 1958). With the former method the reflex usually becomes exhausted after 1 to 2 min, but with regularly interpolated rest periods may be repeated for many hr. With the latter method the reflex may be recorded continuously for several hr without intervening rest periods. Although all workers agree that the circular muscle component of the reflex is governed by cholinoceptive autonomic ganglia there still exists considerable controversy regarding the mechanism of the longitudinal muscle contractions. On the strength of experiments with various nervous blocking agents Feldberg & Lin (1949) claimed that the longitudinal muscle contractions were not nervous in origin, but were a direct response of the muscle fibres themselves. Paton & Zaimis (1949), using hexamethonium, claimed that the longitudinal muscle response was unaffected by this agent and therefore the contractions were not ganglionically controlled. Paton, who showed in 1957 that the response of the longitudinal muscle of the guinea-pig isolated intestine to coaxial electrical stimulation was mediated by a postganglionic cholinergic mechanism, claimed that this response corresponded to the longitudinal muscle contraction of the peristaltic reflex initiated by distension (Paton, personal communication).

According to Kosterlitz (1959) the longitudinal muscle contraction of the reflex can be subdivided into two phases, the first from the resting level until the beginning of the circular muscle contraction, the second from then on until the peak of contraction. This author claimed that the first phase is mediated by an unknown "non-cholinoceptive" mechanism, but the second phase by a ganglionic "cholinoceptive" one, the first being insensitive to the ganglion blocking agent, hexamethonium. All the above-mentioned authors used an interrupted method of recording the reflex.

From the tracings of Bülbring & Crema (1958), however, it appeared that the ganglionic blocking agent, hexamethonium, had a pronounced inhibiting effect also on the longitudinal muscle component of the peristaltic reflex of the guinea-pig isolated ileum.

The present paper is concerned with a more detailed analysis of this component of the reflex.

METHODS

Peristaltic Reflex. The continuous method of recording used was that described by Bülbring, Crema & Saxby (1958), the interrupted method that of Trendelenburg (1917).

Cholinesterase Activity

In vitro. The technique is based on the work of Jensen-Holm (1959) and uses a " Radiometer " (Copenhagen) automatic titration assembly. The method for cholinesterase activity in serum is described in the instruction leaflet. This method was adapted for use with tissue homogenates (Johnson, personal communication). The intestine was first homogenized dry in a mortar. Solution of 0.9% w/v sodium chloride (" physiological saline ") was then added in a proportion of 3 ml./g wet weight. Final homogenization was carried out in a glass hand homogenizer. The substrate used was a 10% solution of acetylcholine bromide. Three ml. homogenate were diluted with 20 ml. " physiological saline " ; to this 2 ml. acetylcholine solution were added to make a final volume of 25 ml. The blocking agents were included in the 20 ml. saline.

On isolated smooth muscle. Preparations of the ileum of the guinea-pig were suspended in Tyrode's solution at 37° C. A submaximal spasmogenic dose of acetylcholine bromide was incubated with a cholinesterase-containing preparation, either haemolysed red cells or an homogenate of small intestine of the guinea-pig, before it was added to the organ bath. This completely abolished the spasmogenic effect of acetylcholine. Threshold amounts of the cholinesterase preparations were determined by dilution. The blocking agents were added in increasing amounts to such threshold doses of cholinesterase. Incubation time for haemolysed red blood cells was 5 min at room temperature and for small intestinal homogenates 7 min at 37° C.

Release of spasmogens during peristalsis

A segment of guinea-pig isolated ileum was allowed to undergo peristalsis for 1 to 2 min in 100 ml. Tyrode's solution at 37° C. An aliquot of 10 ml. was then removed and, without allowing it to cool, tested for spasmogenic activity on a second segment of guinea-pig isolated ileum.

Drugs used: Hexamethonium bromide, tubocurarine chloride, cocaine hydrochloride, neostigmine methyl sulphate, atropine sulphate, acetylcholine bromide.

RESULTS

Continuous method of recording the peristaltic reflex

The three blocking agents that had been used by previous authors, hexamethonium (Paton & Zaimis, 1949), tubocurarine and cocaine (Feldberg & Lin, 1949), were studied with this method and all had similar effects. A dose that inhibited the circular muscle response also depressed the longitudinal muscle contraction. In one case the longitudinal muscle response was even more sensitive to the effect of hexamethonium than was the circular muscle response. In most cases the longitudinal muscle was arrested in spasm. Occasionally, however, it was arrested during relaxation, see Fig. 1.

Interrupted method of recording the peristaltic reflex

Following the method used by previous authors the preparation was incubated for 3 min with the blocking agents before the intraluminal pressure was raised. With all

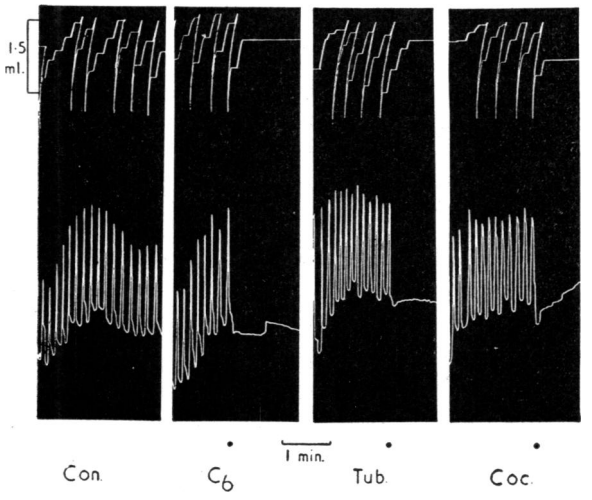

Fig. 1. Guinea-pig isolated ileum. Continuous recording of the peristaltic reflex. Upper tracing: volume of fluid expelled. Lower tracing: contraction of the longitudinal muscle. Con: control tracing; C6: hexamethonium bromide 10 μg/ml.; Tub: tubocurarine chloride 17 μg/ml.; Coc: cocaine hydrochloride 17 μg/ml.

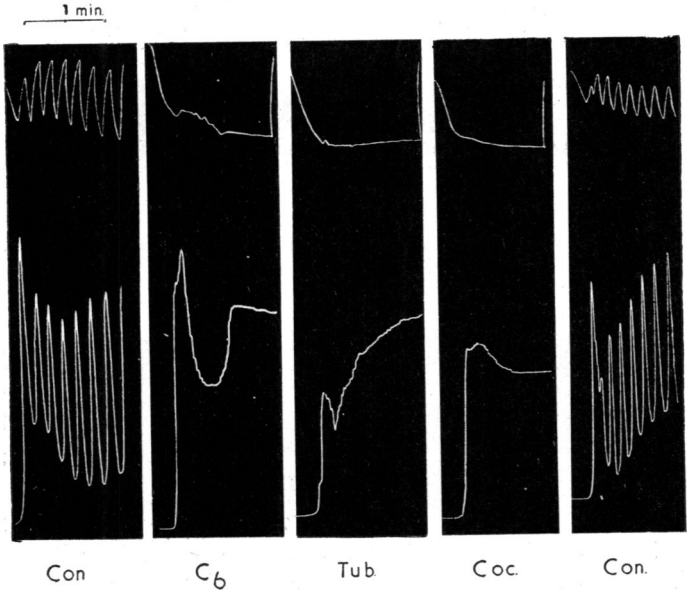

Fig. 2. Guinea-pig isolated ileum. Intermittent recording of the peristaltic reflex. Upper tracing: contractions of the circular muscle. Lower tracing: contractions of the longitudinal muscle. Con: control tracings; C6: hexamethonium 12 μg/ml.; Tub: tubocurarine chloride 20 μg/ml.; Coc: cocaine hydrochloride 12 μg/ml. All three blocking agents were added 3 min before the pressure was raised.

three blocking agents there was a brisk initial contraction of the longitudinal muscle layer (Fig. 2). With hexamethonium the height and type of the contraction differed little from the control, but with the other two agents the height of the contraction was smaller than with the control. In every case the muscle did not relax, but remained in spasm till the end of the experimental period.

Continuous method of recording the peristaltic reflex
Hexamethonium administration at varying time intervals after starting the reflex

The results of several experiments suggested that the response of the longitudinal muscle layer to hexamethonium might be affected by the timing of the administration of the blocking agent. Fig. 3 shows that in this experiment the degree of depression of the longitudinal muscle response was related to the number of contractions that had occurred, before the blocking agent was given. Hexamethonium was therefore given at six different time intervals after the start of the reflex (Fig. 4). When the drug was

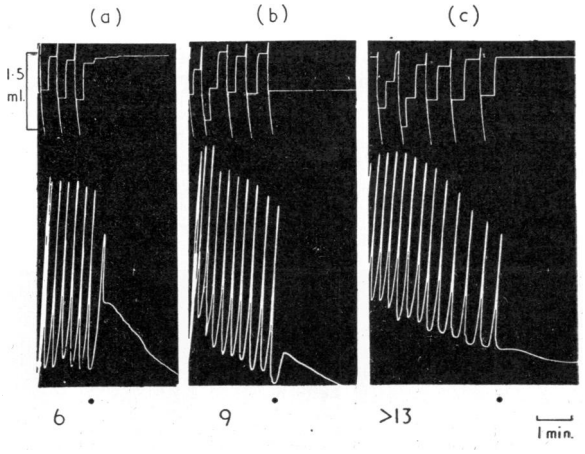

Fig. 3. As Fig. 1. At each dot hexamethonium 10 μg/ml. was added to the bath.

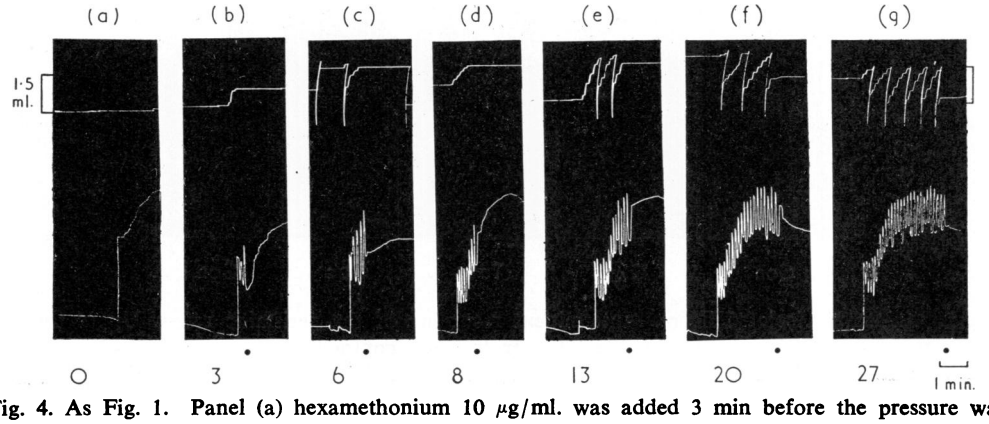

Fig. 4. As Fig. 1. Panel (a) hexamethonium 10 μg/ml. was added 3 min before the pressure was raised. In the remaining panels hexamethonium 10 μg/ml. was added at the black dots.

given 3 min before the reflex was started, the longitudinal muscle contraction was unaffected and behaved as in the experiments in which interrupted method was used. When the blocking agent was given after three, eight and 13 contractions had occurred the longitudinal muscle was again stopped in spasm and the contraction remained sustained, after 20 contractions the longitudinal muscle response was reduced and the spasm immediately relaxed and after 27 contractions the muscle was arrested in relaxation. There was one exception, after six contractions the longitudinal muscle was arrested in relaxation.

Continuous method of recording. Response of the hexamethonium-induced spasm to atropine and neostigmine

Fig. 5 shows the response of the longitudinal muscle arrested in spasm by hexamethonium to atropine and neostigmine. Atropine produced an immediate relaxation of the spasm, and neostigmine immediately arrested the relaxation of the spasm, which usually occurs spontaneously after 60 to 80 sec.

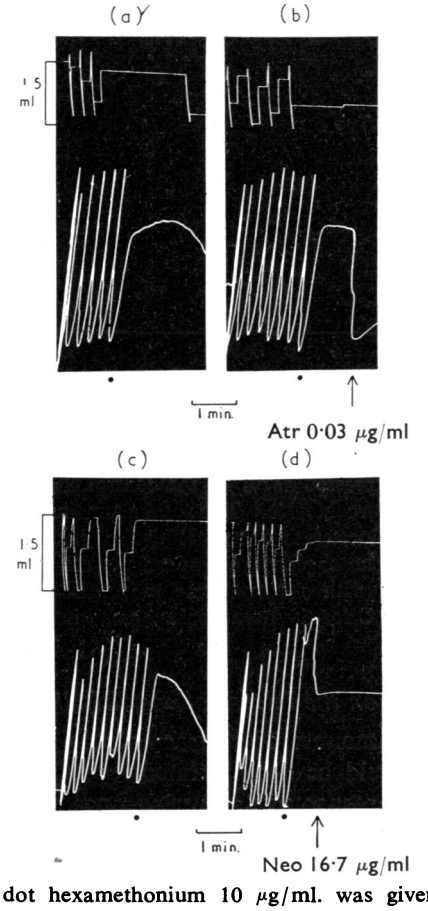

Fig. 5. As Fig. 1. At each dot hexamethonium 10 μg/ml. was given. Panel (b) at the arrow atropine sulphate 0.03 μg/ml. was added to the bath. Panel (d) at the arrow neostigmine methyl sulphate 16.7 μg/ml. was added to the bath.

The effect of the three blocking agents on the hydrolysis of acetylcholine by cholinesterases
(a) Automatic titration technique

An homogenate of guinea-pig small intestine was used as the source of cholinesterase. All three blocking agents were shown to have anticholinesterase activity. The pI50 values calculated from the concentration/action curves were: cocaine, 4.10; tubocurarine, 2.15; hexamethonium, 1.58.

(b) Guinea-pig isolated ileum. Effect on the depression of the acetylcholine-induced spasm by cholinesterases

In the first series of these experiments a solution of haemolysed red cells from a guinea-pig was used as the source of cholinesterase. Increasing amounts of the blocking agents produced an increase in the response of the isolated ileum to acetylcholine in the presence of red cell cholinesterase (Fig. 6).

In similar experiments an homogenate of guinea-pig small intestine was used as the source of cholinesterase (Fig. 6).

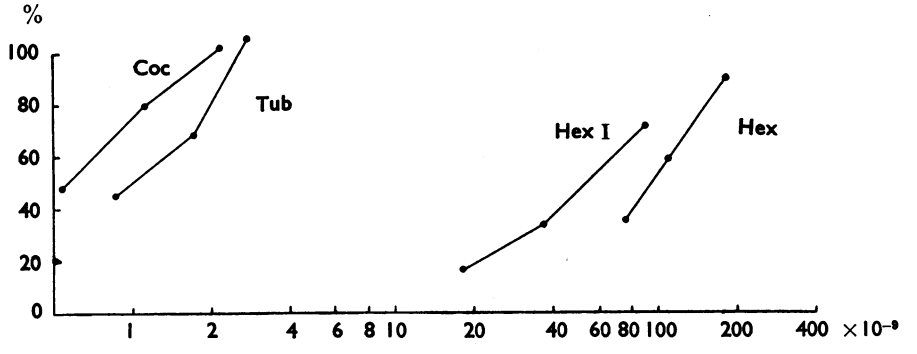

Fig. 6 Anticholinesterase activity of the three blocking agents measured by their effect on the response of a segment of guinea-pig isolated ileum to a submaximal spasmogenic dose of acetylcholine in the presence of a cholinesterase-containing preparation. Ordinate: height of longitudinal muscle contraction expressed as % of the control. Abscissa: dose expressed as 10^{-6}M, log scale. Coc: cocaine hydrochloride; Tub: tubocurarine chloride; Hex: hexamethonium bromide. The source of cholinesterase was a solution of haemolysed guinea-pig red cells, apart from curve Hex.I, when an homogenate of guinea-pig small intestine was used. Period for incubation of blocking agent with cholinesterase preparation was 5 min at room temperature for red cells and 7 min at 37° C for the homogenized small intestine.

The effect of hexamethonium on the release of acetylcholine during peristalsis

Fig. 7 shows that small amounts of a spasmogenic substance are released during peristalsis. Although this could be demonstrated repeatedly, the resulting contractions were within the range of spontaneously occurring ones and those produced by adding hexamethonium directly to the second bath. However, when peristalsis was arrested by hexamethonium a very much larger contraction was obtained (Fig. 7, panels b and c).

Panel (d) shows that the resulting contraction was completely abolished by atropine and therefore presumably caused by acetylcholine.

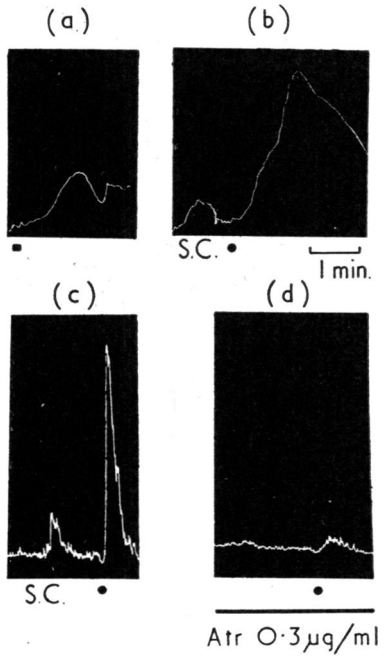

Fig. 7. Guinea-pig isolated ileum in Tyrode's solution at 37° C. Bath volume 15 ml. Panel (a) at the square the bath fluid was replaced by Tyrode's solution from a 60 ml. donor bath in which a segment of guinea-pig isolated ileum had been allowed to peristalse for 1 to 2 min. Panel (b) as (a) but peristalsis in the donor bath had been arrested by adding hexamethonium (10 μg/ml.). Panel (c) as (b). Panel (d) repeat of experiment (c) in the presence of atropine (0.3 μg/ml.) in the receiving bath. S.C.: spontaneous contractions.

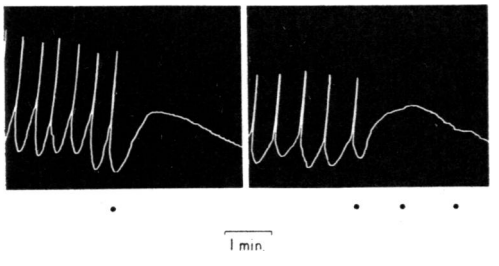

Fig. 8. As Fig. 1. Longitudinal muscle tracing only. At each dot hexamethonium 10 μg/ml. was added to the bath.

The effect of a second dose of hexamethonium on the longitudinal muscle contraction arrested by a previous dose

Continuous recording

The spasm of the longitudinal muscle arrested by hexamethonium gradually declined and eventually the base line was reached. Further doses of hexamethonium retarded this decline of the spasm (Fig. 8) and a very large dose even caused a renewed sharp increase of the longitudinal muscle contraction (Fig. 9).

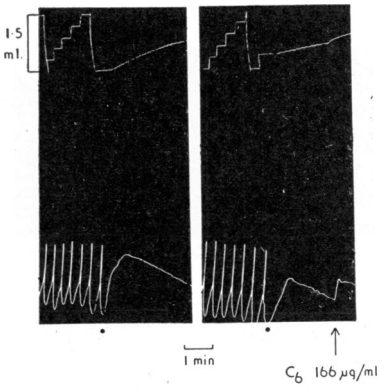

Fig. 9. As Fig. 1. At each dot hexamethonium bromide 10 µg/ml. was added to the bath. At the arrow in the second panel hexamethonium bromide (166 µg/ml.) was added.

DISCUSSION

The present results indicate that the longitudinal muscle contraction which forms part of the peristaltic reflex of the guinea-pig isolated intestine is sometimes sensitive to nervous blocking agents such as hexamethonium, tubocurarine and cocaine and at other times not. It may be partially or even completely inhibited. According to the view previously held this part of the reflex is completely (Feldberg & Lin, 1949; Paton & Zaimis, 1949) or partly (Kosterlitz, 1959) insensitive to such blocking agents.

In the present investigation the continuous recording method of Bülbring, Crema & Saxby (1958) was used, whereas previous workers had used modifications of the interrupted method originally described by Trendelenburg (1917). With this latter method the drugs to be investigated were usually given before the intraluminal pressure was raised, whereas with the continuous method the pressure remains raised throughout; the intestine undergoes peristalsis for long periods, and the drugs may be given at any time. In order to determine whether inhibition of the longitudinal muscle contraction could also be shown with the interrupted method the drugs were given after the reflex had been in operation for 10 to 30 sec. It could be shown that even with this method of recording inhibition of the contraction could be found, when hexamethonium or tubocurarine but not cocaine were used. It seemed possible that the accurate timing of the administration of the drug might affect the response. The drugs were therefore administered at varying stages of the reflex, sometimes before, sometimes after and sometimes at the height of the longitudinal muscle contraction. These modifications, however, did not affect the response. Another possibility seemed to be the effect of the number of times the muscle had contracted, before the drug was administered. This indeed was of importance. Apart from one exception the degree of depression of the longitudinal muscle contraction increased with the number of contractions before the drug was administered. After 27 contractions complete depression of the longitudinal muscle response occurred. When the drug was given 1 min before the pressure was raised little or no depression was observed.

The cholinergic nature of the spasm was confirmed in the present investigation by the rapid relaxation of the spasm with atropine and its enhancement by the anticholinesterase, neostigmine. It was further confirmed that the spasm was a response to distension of the intestinal wall, as lowering of the raised intraluminal pressure caused a prompt relaxation of the longitudinal muscle.

The very slow relaxation of the spasm in some experiments (Fig. 2 and 4), and the continuing slow rise after the initial rapid contraction (Fig. 2 ; and Fig. 4, panels b, c, d) suggested that the blocking agents might have anticholinesterase activity.

Anticholinesterase activity was therefore determined by the radiometer technique. The amounts of the blocking agents necessary for the 50% depression of the small intestinal cholinesterase activity, when measured by this technique, were: hexamethonium 26.4 mg/ml., tubocurarine 7.0 mg/ml. and cocaine 80.0 μg/ml. The potencies of hexamethonium and tubocurarine as anticholinesterases measured by the radiometer technique were similar to those obtained by Todrick (1954), who used a manometric technique. These values are high, but so was the amount of substrate used (the titrator was set for one international cholinesterase unit, i.e., the inactivation of 1.0 μ mol of acetylcholine (or 226 μg of the bromide)/min). According to Jensen Holm (1961) the effect of pseudocholinesterase increases with rising substrate concentration and the effect of "reversible" inhibitors becomes more pronounced the lower the acetylcholine concentration. The amount of acetylcholine involved, when the peristaltic reflex was stopped by the three blocking agents, though not accurately known, was obviously much smaller. The release of acetylcholine from a similar strip of intestine as a response to coaxial stimulation was 30 ng/min (Schneider & Bishop, 1960), i.e., approximately 1/7,500 of the above amount. Assuming that the amount of acetylcholine released by distension of the small intestine is of the same order as that released by coaxial stimulation, then $\frac{26.4 \text{ mg/ml.}}{7,500} = 3.5$ μg/ml. of hexamethonium should have affected the enzymatic hydrolysis of the acetylcholine involved. The actual amount used in the organ bath experiment was 10 μg/ml. The corresponding amounts for the other blocking agents were: tubocurarine 0.93 μg/ml. required and 16 μg/ml. actually used, and cocaine 30 ng/ml. required and 16 μg/ml. used. Thus the anticholinesterase effect of the blocking agents though weak, was well within the range of the doses used in the experiments on the peristaltic reflex.

In order to demonstrate the possible anticholinesterase effect of the three blocking agents under conditions more closely related to those in the experiments on the peristaltic reflex the inhibition of the spasmogenic effect of acetylcholine by cholinesterase preparations was used. In these experiments haemolysed red cells were used as a source of true cholinesterase and an homogenate of small intestine as a source of pseudocholinesterase. The amount of acetylcholine (3.3 to 33 ng/ml.) that produced a submaximal contraction was therefore much smaller than that used in the above experiments. Under these conditions anticholinesterase activity by the three blocking agents could be shown with doses well within the range of those used in the experiments on the peristaltic reflex.

The importance of the anticholinesterase action of hexamethonium was confirmed by further experiments. Testing of the perfusion fluid in contact with the serosal side of the small intestine during peristalsis on a second segment of intestine showed a small

spasmogenic effect only. The same experiment carried out while the intestine was arrested by hexamethonium resulted in a marked increase in the spasmogenic effect. This enhanced spasm was atropine sensitive and therefore presumably due to acetylcholine. Addition of further doses of hexamethonium to a segment of intestine that had been arrested during peristalsis by a previous dose of hexamethonium led to a retardation of the subsequent relaxation or even a renewed spasm if the second dose was sufficiently large.

The conclusion was therefore drawn that the hexamethonium insensitive contraction of the longitudinal muscle of the guinea-pig isolated intestine is not an integral part of the reflex but is superimposed on it. It can be separated from the periodic sequence of contraction and relaxation of the longitudinal muscle, which then becomes freely sensitive to this ganglionic blocking agent and is therefore under cholinergic ganglionic control. The spasm of the longitudinal muscle, which can frequently be observed, when the peristaltic reflex is arrested by nervous blocking agents, is probably due to stimulation of postganglionic cholinergic nerves, and is enhanced by the anticholinesterase action of the nervous blocking agents.

The term " preparatory phase " was intentionally omitted in the present paper, as it has been used by different authors to denote different phenomena. According to Trendelenburg (1917), who coined it, it was defined as " preparatory tonus changes in the longitudinal and circular muscle layer " that preceded the second phase, " the emptying peristaltic waves," which were described as " regular ordered movements of the circular muscles as well as of the longitudinal muscles." According to subsequent authors (Feldberg & Lin, 1949 ; Schaumann, 1955 ; Kosterlitz, 1956), the term " emptying phase " is used to denote the wave of circular muscle contraction and the term " preparatory phase " to denote only the longitudinal muscle contraction that precedes it.

SUMMARY

1. The longitudinal muscle contraction of the peristaltic reflex of the guinea-pig isolated ileum is generally regarded as not being under cholinergic ganglionic control, as it is supposed to be insensitive to nervous blocking agents, including hexamethonium.

2. Under certain experimental conditions, however, this part of the reflex could be completely blocked by hexamethonium, under others it remained resistant.

3. These findings suggest that two independent types of mechanism are involved that are superimposed on each other: (1) a rhythmic sequence of contraction and relaxation regulated by cholinergic ganglia, and (2) a response to stimulation of postganglionic cholinergic nerve endings.

4. All the three nervous blocking agents used had anticholinesterase activity which may well have modified the blocking effect of these substances.

5. The hexamethonium resistant element of longitudinal muscle contraction is not found in all species. It has been demonstrated in the guinea-pig and in the rabbit but is entirely absent in the rat.

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