J. Physiol. (1959) 146, 369–379

REFLEX CONTRACTIONS OF THE LONGITUDINAL MUSCLE COAT OF THE ISOLATED GUINEA-PIG ILEUM

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(*Received* 31 *October* 1958)

It has been shown recently that during the peristaltic reflex elicited by intestinal distension, the longitudinal muscle coat contracts by two independent mechanisms which differ in their physiological and pharmacological characteristics (Kosterlitz, Pirie & Robinson, 1956). The first or type I contraction occurs during the preparatory phase and the second or type II during the emptying phase of the peristaltic reflex. The absence of inhibition by ganglion-blocking agents and cocaine suggested to Feldberg & Lin (1949) that the type I contraction might be purely myogenic; however, later findings, which showed that this contraction was inhibited by morphine and atropine (Schaumann, 1955; Kosterlitz & Robinson, 1955, 1957), made this concept rather unlikely. This paper deals mainly with a further analysis of the type I contraction; preliminary accounts of some of the results have already been published (Kosterlitz, Pirie & Robinson, 1955; Kosterlitz & Robinson, 1955).

METHODS

Type I contractions of the longitudinal muscle coat were studied either on fresh guinea-pig ileum in the presence of hexamethonium (Paton & Zaimis, 1949) or on ileum which had been stored at 4° C for 24 hr (Ambache, 1946). Type II contractions were absent in both preparations. When the two types of contractions were compared, gut from a newly killed animal was employed without the addition of a ganglion-blocking agent; use was made of the fact that the threshold of intestinal distension was lower for type I than for type II contractions.

Trendelenburg's method (1917) was used for isotonic recording of the contractions of the longitudinal muscle coat and the methods described by Kosterlitz *et al.* (1956) and by Innes, Kosterlitz & Robinson (1957) for isometric recording.

The detailed experimental technique, bath fluid and the doses of drugs were the same as those described previously (Kosterlitz *et al.* 1956; Kosterlitz & Robinson, 1957; Innes *et al.* 1957).

RESULTS

The possible mechanical component in the shortening of the distended ileum

When the lumen of a distensible tube is filled with fluid under pressure, the specific compliances of the component structures will determine in what direction distortion will take place. Visual observation of an isolated piece of guinea-pig ileum suggests that raising the intra-intestinal pressure causes radial distension, without affecting the length of the piece of gut appreciably until reflex shortening occurs. This impression was confirmed by direct experiment on guinea-pig ileum stored at 4° C for 24 hr. In such preparations type II contractions were permanently absent but type I contractions recovered slowly after the gut was placed in the organ bath at 35° C. Thus, immediately after setting up the preparation, raising the intra-intestinal

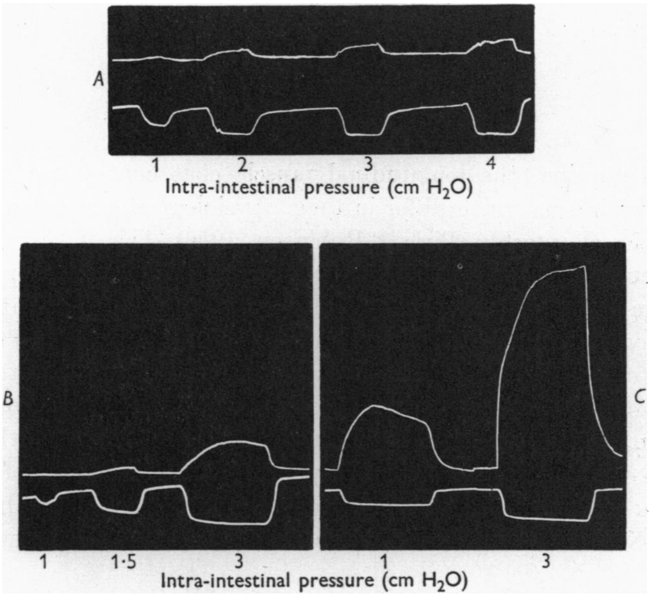

Fig. 1. The possible mechanical component in the shortening of the gut on distension. Ileum, stored at 4° C for 24 hr. Upper tracing, isotonic record of longitudinal muscle coat, 0·75 g tension, shortening upwards. Lower tracing, distension of lumen, increased filling downwards. *A*, immediately after setting up in the organ bath at 35° C; *B*, 30 min after *A*; *C*, 30 min after *B*.

pressure caused filling of the lumen, with only an insignificant shortening of the gut (Fig. 1*A*). Half an hour later a slightly better response was obtained (Fig. 1*B*), and 1 hr later the contractions appeared to have become maximal (Fig. 1*C*). The mechanical effect of distending the lumen was never a lengthening of the gut but rather a small amount of shortening which, however, was only a negligible fraction of the shortening which occurred when the gut had been in the organ bath for some time.

The adequate sensory stimulus for the contraction of the longitudinal muscle coat

If type I contractions were myogenic, we should expect them to be elicited by a stretch in the direction of the long axis of the muscle cells. It was found, however, that sudden lengthening of the gut did not lead to a contraction of the longitudinal muscle coat. The experimental arrangement (Fig. 2*A*) was similar to that described by Winton (1930). Initially the isotonic writing lever

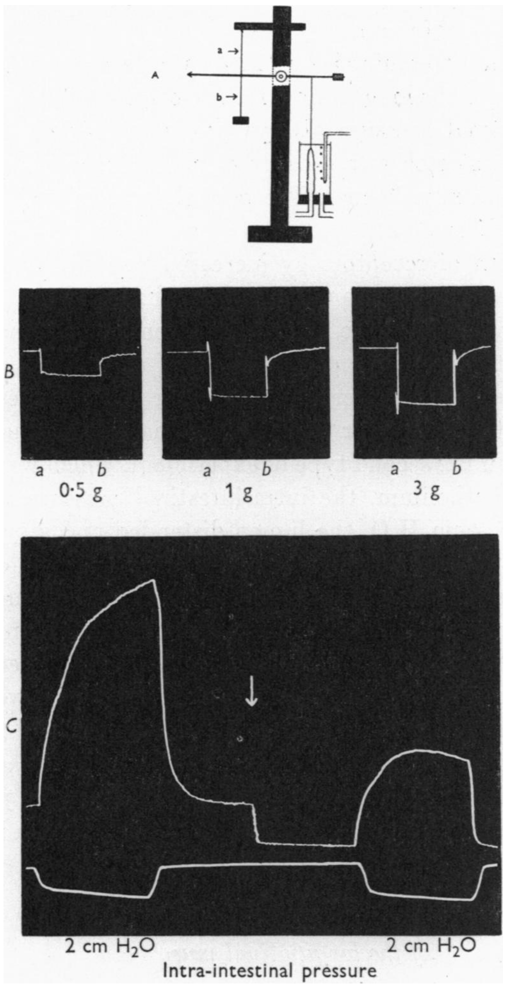

Fig. 2. The effect of sudden stretching on the longitudinal muscle coat. Ileum, stored at 4° C for 24 hr. *A*, Experimental arrangement; *B*, effects of sudden application and release of weights on the length of the ileum; *C*, upper and lower tracings as in Fig. 1. Tension, initially 0·75 g, increased to 1·75 g at arrow.

exerted a tension of 0·75 g on the ileum. When the thread *a* supporting the lever was severed by applying a lighted match, a weight varying between 0·5 and 3 g was suddenly applied; the ileum was stretched and remained at the new length. Half a minute later the thread *b* was burned and the weight released; the gut shortened quickly at first and then more slowly (Fig. 2*B*). The addition of 0·5 or 1 g to the original tension of 0·75 g was not too large to prevent shortening of the gut when the lumen was distended (Fig. 2*C*).

Similar results were obtained whether stored ileum was used, capable of type I contractions only, or ileum from a newly killed animal, in the presence or absence of hexamethonium.

Thus, longitudinal stretching is excluded as the sensory stimulus responsible for the contraction of the longitudinal muscle coat. On the other hand, raising of the intra-intestinal pressure always produces marked radial distension, which is bound to stretch any sensory elements arranged in parallel to the fibres of the circular muscle coat. The possible sites of such receptors will be considered in the discussion.

That deformation of receptors by increased pressure is not the adequate stimulus may be shown by two observations. First, when a glass or Perspex tube was slipped over the gut to reduce or prevent the distension of the lumen on raising the intra-intestinal pressure, type I contractions obtained in the presence of hexamethonium were diminished or absent. These findings are in general agreement with similar observations made by Yanagiya, Ohkubo & Shimada (1958). In the second type of experiment, which was also done in the presence of hexamethonium, the intra-intestinal pressure was increased by a given value, say 2 cm H_2O, the lumen distended and a type I contraction of the longitudinal muscle coat was obtained. Then the level of the fluid of the organ bath in which the gut was suspended was raised by 2 cm, with the result that the gut collapsed as fluid left the lumen, and the longitudinal muscle relaxed. Though all structures in the wall of the intestine were now subjected to the same water pressure as before, the difference between the pressure exerted on the mucosa and that exerted on the peritoneum had fallen to zero and the gut was no longer distended. This indicated that the adequate stimulus for the longitudinal muscle contraction was not the water pressure in itself, but the distension caused by the pressure gradient between intraluminal and external pressure.

The relationship between intestinal filling and contraction of the longitudinal muscle coat

It has already been shown that the two types of reflex contractions of the longitudinal muscle coat are independent of each other, that type I requires less intestinal distension than type II and that type II, which occurs during the emptying phase, is reversibly inhibited by hexamethonium and irreversibly suppressed by storage of the ileum at 4° C for 24 hr (Kosterlitz *et al.* 1956; Kosterlitz & Robinson, 1957). When the degree of distension was near the threshold of the emptying phase, a type I contraction was often present during the early stages of intestinal distension and followed by a type II contraction as soon as the emptying phase came into play; after that there were rhythmic contractions and relaxations and it became impossible to distinguish between the two types of contractions (Fig. 3*A*). These were not separable when the intestinal distension was well above threshold (Fig. 3*B*).

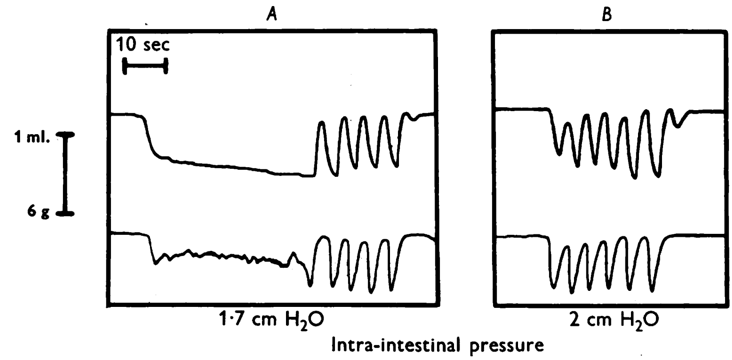

Fig. 3. Type I and II contractions of the longitudinal muscle coat; fresh ileum. Upper record, distension of lumen, increased filling downwards; lower record, isometric record of longitudinal muscle coat, increased tension downwards.

When the increase in intra-intestinal pressure led to type I contractions only, the degree of intestinal filling was closely correlated with the height of intra-intestinal pressure. Hexamethonium did not have any certain effect on intestinal filling (Fig. 4).

The magnitude of the contractions of the longitudinal muscle coat was dependent on the degree of intestinal filling; however, the quantitative relationship between filling and longitudinal tension differed for the two types of contraction (Fig. 5). Below the threshold for type II contractions, or in the presence of hexamethonium, the tension in the longitudinal muscle coat increased proportionally to the degree of filling. However, when type II contractions were superimposed at near-threshold values of filling, there was always a sudden discontinuous rise in longitudinal tension.

Hexamethonium in low concentrations (5–12 μg/ml.) raised the threshold of intestinal distension at which type II contractions were triggered off, e.g. from 0·3 to 0·9 ml. in the experiment shown in Fig. 5*A*; the tension developed was, however, not affected. At the same time the tension during type I contractions was raised from about 1·5 g at 0·3 ml. filling to 2·5–3 g at 0·9 ml. filling. When the concentration of hexamethonium was sufficiently high (25 μg/ml.) to suppress the type II contractions of the emptying phase even at high degrees of intestinal distension, the rise in the tension developed during type I contractions was still proportional to the degree of filling (Fig. 5*B*).

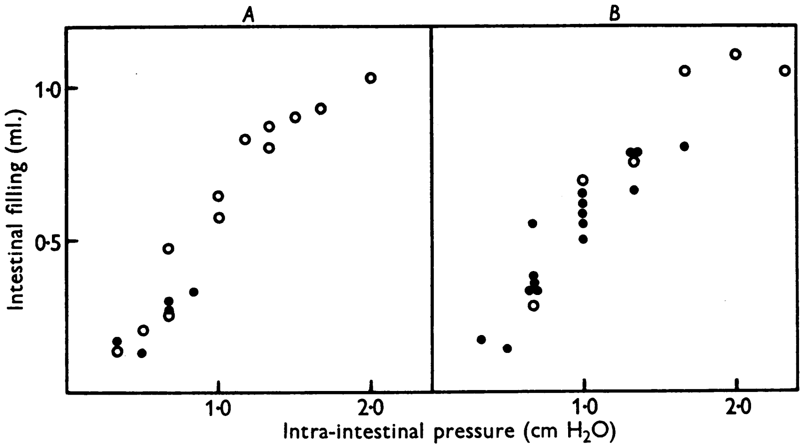

Fig. 4. The correlation of intra-intestinal pressure and intestinal filling; two fresh ilea ●—● without, ○—○ with hexamethonium, 12·5 μg/ml. in *A* and 25 μg/ml. in *B*.

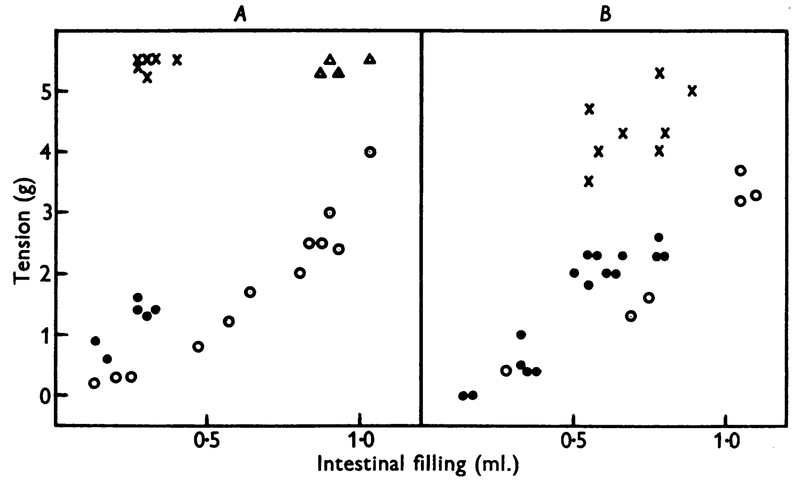

Fig. 5. The effects of different degrees of intestinal filling on type I and II contractions of the longitudinal muscle coat. Same preparations as in Fig. 4. Tension developed during type I contractions: ●—● without, ○—○ with hexamethonium. Tension developed during combined type I and II contractions: × — × without, △—△ with hexamethonium, 12·5 μg/ml. in *A* and 25 μg/ml. in *B*.

The effect of resting length on the tension developed during type I contractions

Although stretching of the ileum does not cause a contraction of the longitudinal muscle coat, the resting length influences the response of the gut to intestinal distension. The maximum maintained response was found when the resting length was adjusted so that there was a small resting tension (Fig. 6).

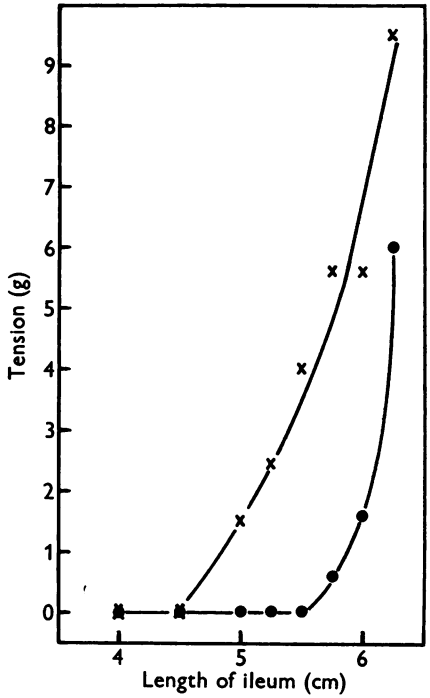

Fig. 6. The effect of resting length on the tension developed during type I contractions of the longitudinal muscle coat. Fresh ileum, with hexamethonium, 60 μg/ml. ●—● Resting tension, ×—× maintained tension at an intra-intestinal pressure of 2 cm H_2O.

Similar results were obtained with histamine as stimulus. This is in agreement with the results of Brocklehurst (1926) on the cat ileum stimulated by histamine and of Winton (1926) on the electrically stimulated retractor penis of the dog and on the guinea-pig uterus stimulated by pituitrin.

When the resting length was below the value which produced a resting tension, intestinal filling caused the appearance of longitudinal tension which, however, declined rapidly to a lower value. This phenomenon will be described in more detail in a later paper.

Pharmacological characteristics of the reflex arc of type I contractions

It has already been stated that the absence of an inhibitory effect of cocaine and ganglion-blocking agents led Feldberg & Lin (1949) to assume that the type I contraction is myogenic, whereas Schaumann (1955) and Kosterlitz & Robinson (1955, 1957) concluded from its inhibition by atropine and morphine that it is caused by a reflex. Further evidence for the latter view was found in the observation that 0·33 mM sodium cyanide reversibly depressed type I contractions (Fig. 7). This concentration was too low to affect contractions due to acetylcholine.

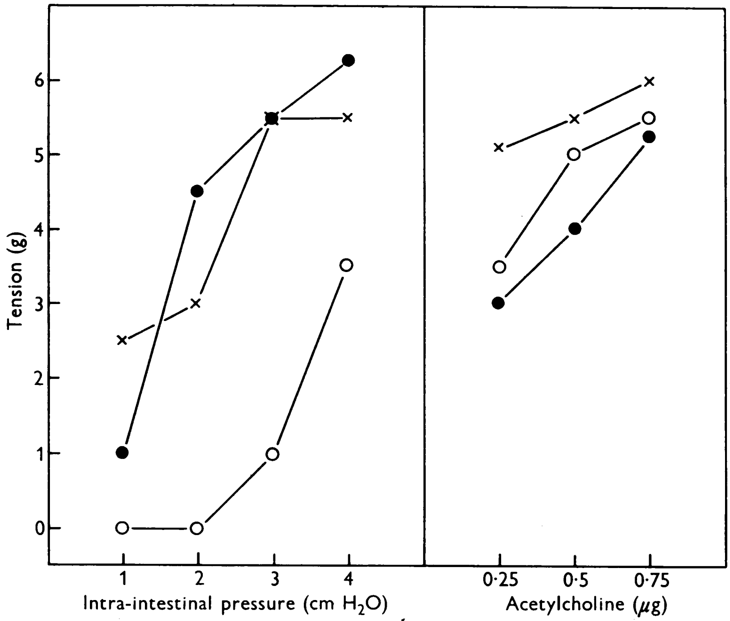

Fig. 7. The effect of 0·33 mM sodium cyanide on type I contractions of the longitudinal muscle coat; 40 ml. bath. Fresh ileum, with hexamethonium, 60μg/ml. ●—● Controls, ○—○ 60–80 min after addition of NaCN, ×—× 40–60 min after washing out.

In order to obtain more information about the nature of the reflex arc underlying type I contractions, the effects of blocking the tryptamine and substance P receptors were examined. Saturation of the tryptamine receptors with large doses of 5-HT added to the bath fluid had only a transient inhibitory effect on type I contractions, which recovered rapidly although the tryptamine receptors remained blocked (Fig. 8). When the action of 5-HT on the longitudinal muscle responded to the inhibitory action of dibenamine, which did not happen in every preparation, type I contractions remained unaffected. Saturation of substance P receptors by high concentrations of substance P was more difficult to investigate since the available preparations were only partly purified and often led to unspecific inhibitions. However, in the experiment shown in Fig. 9 the block produced by adding to the bath fluid a preparation chromatographed once and containing 13 Euler units/mg had no effect on the contractions produced by intestinal distension or by acetylcholine. Mepyramine did not inhibit type I contractions.

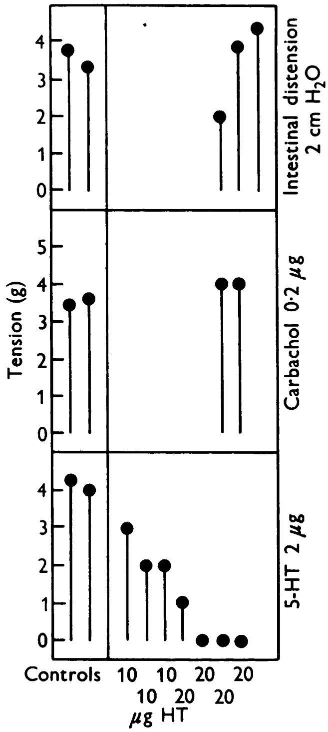

Fig. 8

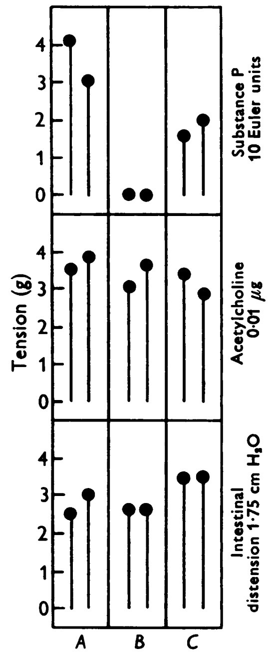

Fig. 9

Fig. 8. The effect of saturation of the tryptamine receptors on type I contractions of the longitudinal muscle coat; 40 ml. bath. Ileum stored at 4° C for 18 hr. Order of tests: intestinal distension, carbachol, 5-HT, at intervals of 3 min. The tryptamine receptors were saturated by the addition of the indicated quantities of 5-HT to the bath fluid.

Fig. 9. The effect of saturation of the substance P receptors on type I contractions of the longitudinal muscle coat; 15 ml. bath. Fresh ileum, with hexamethonium, 33 μg/ml. Order of tests: substance P, acetylcholine, intestinal distension, at intervals of 3 min. *A*, Controls; *B*, 4 mg of substance P (52 Euler units) was left in bath for 6 min; after washing out, tests were done in the presence of 39 Euler units, which did not cause contraction; *C*, 10 min after washing out substance P.

DISCUSSION

There is little doubt that the contractions of the longitudinal muscle during the preparatory phase (type I concentrations) are reflex in nature, although they are not inhibited by cocaine or ganglion-blocking agents. This view is supported by the following evidence. They are inhibited by morphine and atropine and are more sensitive to the depressing action of adrenaline, of lowering the bath temperature (Kosterlitz & Robinson, 1957) and of sodium cyanide than contractions caused by acetylcholine or histamine.

Radial distension and not longitudinal stretching is the sensory stimulus for the type I contractions. The stretch receptors show little adaptation, and isotonic shortening or isometric increase in longitudinal tension lasts as long as the lumen is distended. The tension increase during type I contractions varies directly with the degree of intestinal filling; the mechanism underlying this gradation is unknown.

Bülbring, Lin & Schofield (1958) showed recently that removal of the mucous membrane containing sensory nerve endings abolished the contractions of the circular muscle of the emptying phase but not the type I contractions of the longitudinal muscle. These findings would indicate that the postulated sensory stretch receptors for the type I contractions are not situated in the mucous membrane.

The reflex arc of the emptying phase is made up of at least a sensory and a motor neurone and has one or more cholinoceptive synapses. On the other hand, the failure of ganglion-blocking agents to inhibit type I contractions suggests that the reflex arc is either non-synaptic or possesses a non-cholinoceptive synapse. So far no evidence for a chemical transmitter other than acetylcholine has been obtained. Experiments with saturation of the respective receptors from the serosal side of the ileum have failed to implicate 5-HT or substance P in type I contractions of the longitudinal muscle, at least as far as a possible synapse and the efferent side of the reflex are concerned. It should, however, be pointed out that such experiments do not exclude a possible role of 5-HT and substance P on the sensory side, such as has recently been shown for the reflex arc of the emptying phase (Bülbring & Lin, 1958; Beleslin & Varagić, 1958).

SUMMARY

1. The contraction of the longitudinal muscle coat during the preparatory phase of the peristaltic reflex of the isolated guinea-pig ileum (type I contraction) is a reflex contraction, the purely mechanical component being a negligible part of the total tension developed.

2. The adequate sensory stimulus is not lengthening of the gut but radial distension. The stretch receptors are not situated in the mucous membrane.

3. The tension developed in the longitudinal muscle coat varies directly with the degree of intestinal filling which, in turn, is directly proportional to the intra-intestinal pressure. The tension increase during contraction is affected by the resting length, the maximum response being obtained when a small resting tension is present.

4. The pharmacological characteristics of the type I contractions support

the hypothesis of their neurogenic nature. The contractions are inhibited by concentrations of sodium cyanide, morphine, atropine and adrenaline which do not affect contractions due to acetylcholine or histamine. Substance P and 5-hydroxytryptamine do not seem to play a part on the efferent side of the reflex arc.

5. Since ganglion-blocking agents do not inhibit type I contractions, a reflex arc with a non-cholinoceptive synapse or without a synapse is postulated.

We wish to thank Messrs W. J. Davidson and J. McConnachie for valuable technical assistance.

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