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ON THE NATURE OF INHIBITION IN THE INTESTINE.

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In his recent Croonian Lectures Dale [1929] has indicated that there are good grounds for the belief that the action of the parasympathetic part of the autonomic nervous system is intermediated by the peripheral release of acetyl choline. Little evidence has been brought forward in favour of the humoral action of the sympathetic half of the autonomic nervous system, although in 1904 Elliott had suggested that a mechanism is developed from the plain muscle cell in response to its union with the synapsing sympathetic nerve fibres, the function of which is to receive and transform each nervous impulse, possibly by the liberation of adrenaline. It appeared to the present writer that if it were possible to obtain an isolated preparation of smooth muscle with its inhibitory nerves, experiments might be performed to test this theory of inhibition.

McSwiney and Robson [1929] have shown that a mammalian nerve-muscle preparation will survive for some time in suitable conditions. Because of its power of regular rhythmical contraction, rabbit intestine was chosen as a suitable tissue for the investigation of inhibition. The splanchnic inhibitory nerves to the muscle run as fine fibres in close proximity to the blood vessels supplying the tissue. No attempt was made to isolate the nerves; instead, the blood vessels supplying the intestine were isolated, and with them the nerves were freed.

It was found that inhibition of the rhythmical contractions of the plain muscle could be produced by stimulating the freed nerves with a rapid [20–40 per sec.] series of induction shocks. The experiments here described were commenced with the idea that it might be possible to demonstrate some essential difference between the inhibition produced by adrenaline and that due to nerve stimulation. The result, however, has been to bring the writer to the view that the two forms of inhibition are intimately related.

Preparation.

The abdomen of a freshly killed rabbit is opened, and that part of the duodenum is selected which has the most mobile mesenteric attachment. This is separated from the rest by two transverse cuts about 5 cm. apart. The cuts are continued along the mesentery on each side of the blood vessels supplying the part, which are freed for 8 or 10 cm. and are then cut across. Great care is exercised not to stretch or otherwise damage the mesentery and its contents. The preparation is kept in warm Ringer's solution until required. A similar procedure can be carried out with the ileum, so that from one animal two preparations can be obtained, each

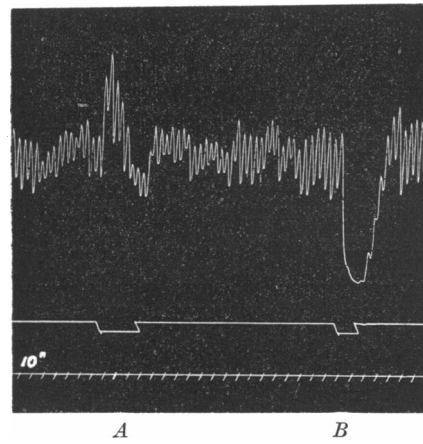


Fig. 1. *A.* 3 stimuli per second cause contraction of the muscle.
B. 40 stimuli per second cause relaxation.

consisting of a small piece of gut with about 8 cm. of attached mesentery in which run the nerves supplying the plain muscle.

To prevent contamination of the Ringer's solution with intestinal contents, the two ends of the piece of gut are ligatured. A loop of thread is tied to the end which is to be attached to the glass hook at the bottom of the bath, while to the other end is tied a length of thread for connection with the writing lever. For convenience in manipulation a length of thread is also fastened to the free end of the mesentery. The preparation is now placed in a bath containing Dale's modification of Ringer's solution which is oxygenated and kept at 36° C.

When the muscle is executing regular and uniform rhythmical contractions, the free end of the mesentery is gently pulled out of the fluid

and laid across the electrodes. To prevent stretching, these are placed just above the surface of the fluid and, if care be taken, the beat is in no way disturbed by this. When the mesentery and its contained nerves are stimulated by means of a series of shocks from an induction coil the effect on the gut is most marked. The muscle ceases to contract rhythmically, and its tonus is reduced. The degree of relaxation varies with the initial tonus of the muscle. Where this is high, as is usual in preparations from duodenum, there is a striking relaxation. Often the preparation from the ileum in which tonus is less marked will cease its rhythmical contractions, on stimulation, but cannot be further relaxed. In some cases the duodenal preparation had initially a low tonus, and stimulation caused only inhibition of the rhythmical contractions. As time went on the tonus increased, and then stimulation of the splanchnic fibres caused also relaxation.

It might be expected that stimulation of the mesentery should tend also to produce contraction of the gut, from the presence along with the splanchnic sympathetic fibres, of some parasympathetic fibres. Only occasionally was it possible, when the preparation was first set up, to demonstrate some slight contraction by stimulating the mesentery with a series of slow, weak induction shocks (2–4 per sec.). This effect could not be long produced, and the explanation seems to be that, as Gaskell [1916] has suggested, the parasympathetic nerve supply to the intestine is intermediated by peripheral ganglia, which one would expect to be rapidly put out of action by the conditions of comparative oxygen lack which obtain in the experiment. The splanchnic fibres, which are post-ganglionic—the ganglia lying around the aorta—continued to give effects, often for 4 hours or more. Though bubbling nitrogen through the Ringer's solution rapidly weakened the muscular contractions, this did not prevent the inhibitory action, which could be elicited on stimulation as long as the muscle continued to contract.

Comparison of adrenaline and nervous inhibitions.

It was found that the inhibition of rhythmical contractions and the relaxation produced by stimulation of the inhibitory nerve preparation could be imitated by the addition to the fluid in the bath of adrenaline in a suitable concentration. This varied with different preparations between 5 and 20 $\times 10^{-8}$. When, as was usually the case with the duodenum, the tonus was high, stimulation of the nerves or addition of adrenaline produced an equal relaxation as well as a total or almost total inhibition of the rhythmical movements; with the preparation of the

ileum only inhibition of the pendular movements could be obtained, either by stimulation or the addition of adrenaline.

The effects of electrical stimulation were almost immediate, and passed off quite rapidly on cessation of stimulation. The addition of adrenaline to the Ringer's solution produced its effects more slowly, and the effects, in the main, persisted until the fluid was replaced by fresh Ringer's solution.

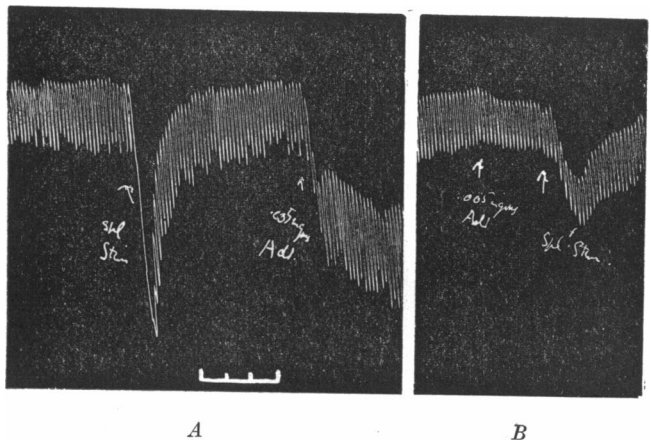
Desensitization by ephedrine.

Curtis [1929 *a*] has shown that ephedrine antagonizes the inhibitory action of adrenaline on smooth muscle. Lately [1929 *b*] he has found that ephedrine in concentrations which do not alter the rhythm or tone of the smooth muscle of rabbit gut so affects the muscle that concentrations of adrenaline which previously inhibited the muscle are now without action. It was thought that, by the use of this drug, it might be possible to differentiate between the action of the nerves and adrenaline inhibition. If the nerve acted directly on some receptive mechanism in the muscle cell, it might be possible to find a concentration of ephedrine, in which nervous inhibition could still take place while the addition of an equivalent concentration of adrenaline was without effect.

Such a differentiation was never observed in any of the fifty experiments that were performed to test this point. Ephedrine seemed to antagonize equally the inhibition by adrenaline and that by electrical stimulation. The method of experiment was as follows. The inhibition produced by stimulating the preparation was compared with that produced by adding 0·005 to 0·025 mg. of adrenaline to the 100 c.c. of Ringer's solution in the bath, giving a concentration of 5 to 25 $\times 10^{-8}$. To the fluid of the bath, in which the preparation was executing regular pendular movements, from 5 to 10 mg. of ephedrine hydrochloride were added in solution, giving concentrations of 5 to 10 $\times 10^{-5}$ ephedrine. Inhibition was again elicited by stimulating the preparation electrically, and by adding the same concentration of adrenaline as before. It was found that both were equally antagonized by ephedrine, so that although the reduction of tone and inhibition of rhythmical contractions were much reduced, the relation between the magnitude of the inhibitions produced by the two methods remained the same.

A concentration of adrenaline, giving slightly less inhibition than that produced by stimulating the preparation, would be without effect at a concentration of ephedrine that still permitted some inhibition with nerve stimulation and with an equivalent concentration of adrenaline.

The inhibition produced by nervous stimulation and that produced by an equivalent concentration of adrenaline were antagonized by the same concentration of ephedrine, while inhibition could in some measure be produced by increasing two—or fourfold—the concentration of adrenaline. (See Fig. 2.)



A *B*

Fig. 2. *A.* Nerve stimulation causes a greater degree of relaxation than that produced by the addition of 0·005 mg. adrenaline. *B.* After the addition of 10 mg. ephedrine the addition of 0·005 mg. adrenaline produces little relaxation, while nerve stimulation still causes some inhibition. In this and in subsequent tracings the time intervals represent minutes.

Other points of similarity between nervous and adrenaline inhibition presented themselves. When the ephedrine was added in smaller quantities at a time, it was found that there was a proportionate reduction of the nervous inhibition. This agrees exactly with the findings of Curtis [1929 *b*] as to the quantitative antagonism between adrenaline and ephedrine.

This evidence seems to suggest that the nerves act through the intermediation of the same receptive mechanism as that on which adrenaline acts to produce its inhibitory effect on the muscle; this receptive point being so influenced by ephedrine as to be unresponsive both to nervous and to chemical stimuli.

Adrenaline desensitization.

Occasionally one finds a preparation of rabbit gut which, on addition of adrenaline, gives but a transient instead of the usual prolonged inhibitory response. It readily resumes its normal rhythmical contractions,

and returns to its original tone without the Ringer's solution being changed. If very small concentrations of adrenaline are added to the bath and gradually increased, it is possible so to desensitize the tissue that it continues to contract regularly and with its original tone in the presence of a concentration of adrenaline which, if added suddenly, would be sufficient to inhibit it completely.

In the tracing (Fig. 3) a piece of duodenum is beating normally in a

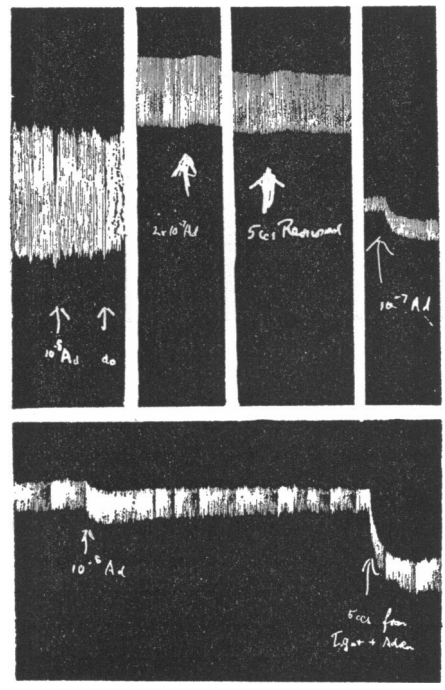


Fig. 3. Adrenaline added in small but gradually increasing concentrations, so desensitizes the muscle that t beats normally in a concentration of about 10^{-6} adrenaline; 5 c.c. of the fluid added to 100 c.c. of fluid in another bath causes marked inhibition of the muscle therein (lower tracing). On washing out, the muscle recovers its normal sensitivity to adrenaline.

concentration of the order of 10^{-6} adrenaline. 5 c.c. of this fluid, on addition to the 100 c.c. of Ringer's solution in another bath, produced a marked inhibition of the gut therein.

As Wiltshire [1930] has recently shown, the presence in the bath of tissues prevents the oxidation of adrenaline, which, in the alkaline warm Ringer's solution, would otherwise rapidly take place.

When a muscle preparation was made from such a piece of intestine, normal inhibition of the pendular movements was produced by stimulating it electrically. On adding adrenaline to the fluid in the bath, at first in very small concentrations, and then in gradually increasing amounts, the nervous inhibition grew less as the concentration of adrenaline was increased, and, finally, almost disappeared. On replacing the fluid by fresh Ringer's solution, stimulation of the preparation again produced marked inhibition (Fig. 4).

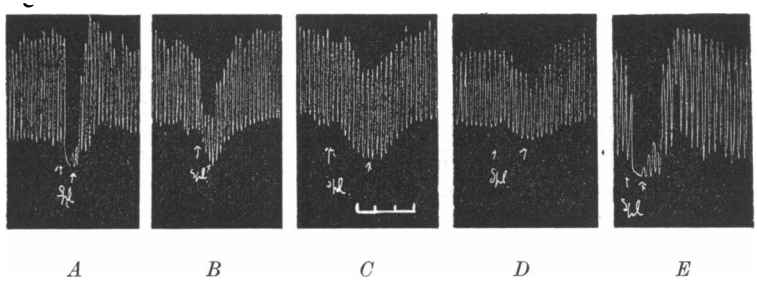


Fig. 4. *A*. Relaxation caused by nerve stimulation in normal Ringer's solution. Between *A* and *B* 0·017 mg. adrenaline was added. Between *B* and *C* 0·025 mg. adrenaline was added, and between *C* and *D* 0·04 mg. adrenaline was added. With increasing concentrations of adrenaline the inhibitory response to nerve stimulation becomes less. On washing out with normal Ringer's solution, as in *E*, normal inhibition is again observed.

The difficulty in the experiment was to find a suitable piece of gut. The experiment was then tried of partially desensitizing a preparation to adrenaline with ephedrine. Stimulation of the preparation would still cause inhibition though less markedly than before. On adding adrenaline in small but gradually increasing amounts, the effect of stimulating the nerves grew less and was finally almost abolished as the concentration of adrenaline was increased. On washing out with fresh Ringer's solution nervous inhibition was once more elicited.

These experiments suggested to the writer that the nerves acted by liberating an inhibitory substance peripherally. It was sought to obtain some more direct indication by attempting to demonstrate on a second preparation that inhibitory substances are liberated during inhibition.

The first method tried was to set up in a bath of Ringer's solution the nerve-muscle preparation described, and an isolated preparation of smooth muscle without any mesenteric attachment. It was thought that if the inhibitory substance were liberated on stimulating the nerves,

enough might diffuse from the nerve endings into the fluid of the bath to inhibit the movements or tone of the other piece of muscle. No such effect could be demonstrated. An attempt was then made to increase the concentration of the inhibitory substance in the bath by using a larger nerve muscle preparation. From 20 to 25 cm. of the ileum of a rabbit were isolated, maintaining in connection the inhibitory nerve supply. This was placed in the bath together with a test piece of plain muscle; records of the contractions of the preparation were taken from a part of the muscle of the preparation, some 5 cm. in length. Using this method, no sign of humoral transference was found. This is not surprising, since it is found that the inhibitory action of the nerve can be paralleled by a concentration of 2×10^{-7} adrenaline. Only a very small amount of inhibitory substance would need to be liberated at the surface of the muscle cells to produce locally an equivalent of this concentration of adrenaline.

If any of the inhibitory substance diffused out into the bath it would be so diluted by the 100 c.c. of fluid present that it would have no effect. Hence it appeared that, to have any chance of success, some method must be devised whereby the volume of the transferring fluid was kept as low as possible.

The method finally adopted consisted of keeping the muscles in a chamber containing warm moist air with warmed Ringer's solution dripping over the surface of the innervated piece of gut and then on to the test piece of gut. Thus the volume of the transferring fluid was kept extremely low, less than 1 c.c. passing over the surface of the pieces of gut during the period of stimulation. Using this method it was finally possible to demonstrate humoral transference.

Methods and results.

Some difficulty was experienced initially in keeping the air of the chamber warm and moist. At first, steam was blown in through a fine jet, but this method was soon abandoned owing to the difficulty of keeping the temperature inside the chamber constant. It was later found that the air could be kept sufficiently warm if there were a layer of water at the bottom of the chamber, heated to between 50° and 60° C. The chamber used was a beaker of 800 c.c. capacity containing water to the depth of half-an-inch, and was heated by a carbon filament lamp underneath, in series with a rheostat. This could be adjusted so that the temperature of the air in the chamber was 36° C. To keep the air in

the chamber saturated with water vapour, oxygen was bubbled through the water at the bottom.

The nerve-muscle preparation was fastened by one end to the special glass hook (see Fig. 5) which terminated in a straight portion 1½ inches

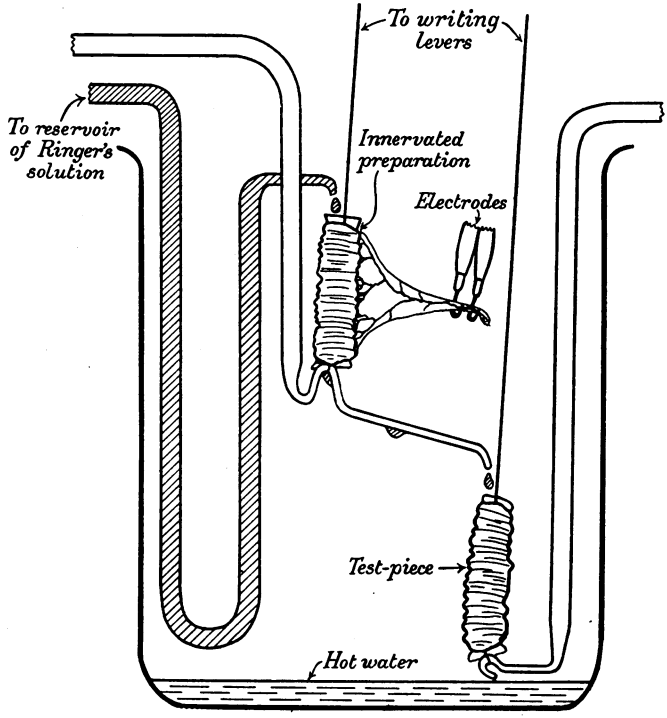


Fig. 5. Diagram of the apparatus. Explanation in text.

long sloping downwards; this was so arranged that Ringer's solution which had trickled over the surface of the muscle would collect at the bottom and run down the sloping portion in the form of separate drops. The other end of the preparation was connected to a writing lever, and the glass hook so adjusted that the muscle occupied the upper half of the chamber. An isolated piece of rabbit gut was fixed to an ordinary glass hook so that it occupied the lower half of the chamber, the other end of the gut was connected by a thread to a second writing lever. Ringer's solution from a reservoir, warmed by passing through the U-shaped tube shown in Fig. 5, was made to drip slowly over the upper piece of gut. The second piece of gut was so arranged that the fluid which had passed over the surface of the innervated piece of intestine fell in drops on it from

the end of the sloping portion of the glass hook. The nerve of the preparation was laid across a pair of electrodes, and everything was then left undisturbed until both pieces of muscle were contracting regularly.

When the test piece of plain muscle was executing regular contractions and had attained a steady level of tone the nerve of the other preparation was stimulated electrically, by means of a rapid series of induction shocks. Inhibition of the innervated preparation at once followed and, under favourable circumstances, inhibition of the test piece of plain muscle followed. This did not occur simultaneously with the commencement of inhibition in the uppermost piece of gut, but after a period varying from 1 to 2 minutes in different experiments. Dale's modification of Ringer's solution was used with the addition of 5 p.c. of serum which, as Wiltshire [1930] has shown, delays the oxidation of adrenaline. The inhibition of the second piece of gut was best seen when it was in a very high state of tonus. The rhythmical pendular movements were not greatly reduced but the tone was diminished. If the nerve were stimulated again after a few minutes the result on the second piece of gut was not so marked although the upper piece of gut was again inhibited almost as well as before (see Fig. 6). This may be explained in

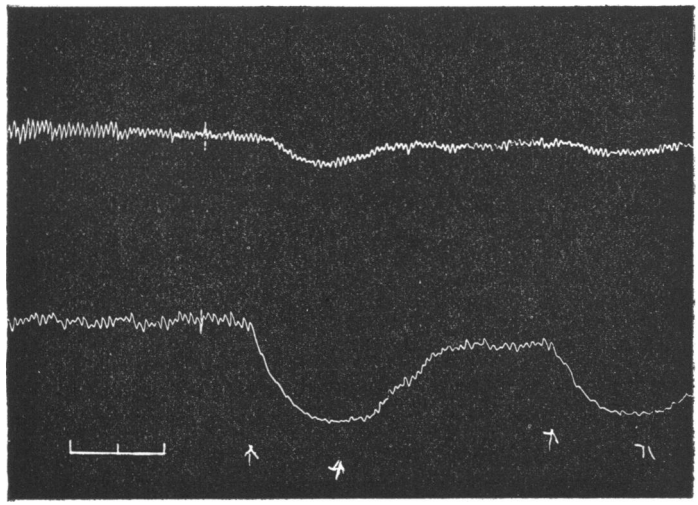


Fig. 6. The lower tracing is from the innervated piece of gut. The upper tracing is from the lower test piece of gut, over which drips fluid from the upper piece. On stimulating the nerve (shown by arrows) inhibition of the innervated preparation takes place almost immediately. After over half a minute, a relaxation of the lower piece of gut also takes place. The time intervals represent minutes.

two ways: Firstly that the second piece of gut will only detect the low concentration of inhibitory substance when its sensitivity is at a maximum, and that the sensitivity is reduced after its inhibition. The other explanation is that under the conditions of the experiment some time is required before a sufficient store of inhibitory substance can again become available; in support of this it has been noticed that the second inhibition was less marked than the first, and that it tended to pass off spontaneously while the nerve was still being stimulated. It was also the case that the inhibition obtained on stimulating the preparation for the first time after it had been set up was markedly greater than any that could subsequently be elicited. Consequently in demonstrating the humoral transference of inhibition the integrity of the preparation was never tested at the outset of the experiment. Everything was arranged and allowed to remain undisturbed until conditions had become perfectly steady, and the test was then applied. No serious embarrassment was caused by this for the preparation is extremely reliable.

Discussion.

The inhibitory nerve fibre may be conceived of as acting on the smooth muscle cell in three different ways.

(1) The nerve may act directly on the cell.

(2) The nerve may terminate in a receptor mechanism at the surface of the cell.

(3) The nerve may terminate in close proximity to the cell and produce its effect by liberating some inhibitory substance, which then acts on the cell.

If the nerves acted directly on the cell, or without the intermediation of the receptive mechanism on which adrenaline acts, it might have been expected that some concentration of ephedrine could be found, which would permit of nervous inhibition, while preventing the inhibition produced by adding adrenaline to the fluid in the bath. Ephedrine did not have this effect but antagonized equally both types of inhibition. While this does not rule out the possibility that the inhibitory nerve acts directly on the muscle cell, it is, at any rate, an argument against this explanation.

If the nerve terminated at some special receptive mechanism on the surface of the cell, adrenaline might be expected to produce its effects by acting on this receptor site. This is not incompatible with the results of the experiments with ephedrine, which might act by paralysing the receptor mechanism. This would prevent both nervous and adrenaline

inhibition, and in a parallel degree. It does not, however, seem possible to reconcile this explanation with the fact that nervous inhibition is antagonized by the gradual addition to the Ringer's solution of adrenaline, for it seems inconceivable that the receptor mechanism should be paralysed to nervous impulses by adrenaline in concentrations which, if added suddenly, would stimulate it and produce effects similar to those produced by the arrival of nerve impulses.

We are, therefore, left with the more probable explanation that the nerve ending lies in close proximity to the muscle cell, and that, on the arrival of nerve impulses, an inhibitory substance is liberated, which diffuses to the muscle cell and there produces inhibition in the same way as does adrenaline. Ephedrine would then act on the receptor mechanism of the muscle cell, rendering it insensitive to adrenaline and to this inhibitory substance. Sollman [1928] points out that the intensity of the action of adrenaline depends on its concentration outside, not inside, the muscle cells. If, therefore, the preparation be in equilibrium with a fairly high concentration of adrenaline, the addition of further amounts of adrenaline will produce no marked effects, since the relative change in the concentration will be small. If the inhibitory substance is of an adrenaline-like character it might be expected that, when the gut was in equilibrium with a fairly high concentration of adrenaline, the relative change in the concentration of inhibitory substance produced by stimulating the nerves would be too small to give much effect.

This explanation is borne out by the demonstration of humoral transference of inhibition described above.

Under the conditions of the experiment it does not seem likely that the result obtained could be a secondary effect of the inhibition, for nothing is altered when the nerve is being stimulated. The rate of flow of the Ringer's fluid is constant, and so the fluid does not remain in contact for a longer time with the first piece of muscle, an objection that has been urged against other methods of demonstrating humoral transference, notably that of Brinkman and Van Dam [1922]. It has been suggested that the results might be due to some leakage of the stimulating current, but this seems impossible since the second piece of gut is not even in contact with the end of the glass rod, but is just below it so that the fluid falls on to it in drops. Further, an inhibition due to spread of current could be repeated again and again. There remain two possibilities; that the nerves liberate at their terminations some inhibitory substance, or that when plain muscle is in a state of inhibition some substance escapes from it which has the power of producing inhibition elsewhere.

Under the conditions of the experiment it has not been possible to decide as yet which of these two theories is the true explanation. In the light, however, of the large body of evidence advanced by Dale [1929] in favour of the humoral transference of autonomic activity, and in view of the experiments reported here it would seem that the inhibitory nerves to the plain muscle of the gut act by liberating peripherally some inhibitory substance. This substance is of an adrenaline-like character in that its effects are in all ways similar to those produced by adrenaline, and that it is without action on the gut in the presence of ephedrine and in the presence of large concentration of adrenaline.

SUMMARY.

1. An inhibitory nerve-smooth muscle preparation is described, and on this, strictly parallel effects are produced by nervous and chemical inhibition.

2. Ephedrine antagonizes both nervous and adrenaline inhibition.

3. When the preparation is brought into equilibrium with a fairly high concentration of adrenaline, neither nervous nor adrenaline inhibition can be elicited.

4. A method is described for obtaining pendular movements of isolated pieces of rabbit gut which are not immersed in Ringer-Locke. When the movements of such a piece of gut are inhibited by stimulating the nerve, a substance appears in the fluid passing over the surface, which has the power of inhibiting a second piece of gut.

5. Hence it is argued that the inhibitory nerves to plain muscle act by liberating peripherally an inhibitory substance.

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