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THE SITE OF ACTION OF SOME DRUGS CAUSING STIMULATION OF THE CIRCULAR COAT OF THE RABBIT'S INTESTINE

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Observations on suprarenalectomized rabbits had shown that abnormal responses to certain drugs were sometimes exhibited by the circular coat of the small intestine. For a correct analysis of the results, however, too little was known of the circular muscle's normal behaviour. Experiments on the pharmacology of the circular coat were, therefore, carried out in the normal rabbit, and the results are presented in this paper.

Methods

The rabbits were killed by a blow on the neck, the jejunum excised and immersed immediately in glucose-free Tyrode's solution. Two lengths of gut measuring 5–6 cm. were suspended in a double organ bath for observation, and the remaining piece of intestine kept at room temperature for periods up to 5 hr. to supply more strips if required. It was found that the results on different strips were only insignificantly changed by keeping the intestine at room temperature for a few hours, whereas they were strongly modified by cooling it down in the refrigerator for the same length of time. The gut was, therefore, only placed in the ice chest if the object of the experiment was observation of the changes produced by cold.

In all experiments, simultaneous record was made of the movements of the longitudinal and the circular coat by means of the method devised by Trendelenburg [1917]. In this technique, the activity of the circular coat is measured by changes in intestinal volume. One end of the strip of gut is tied over a piece of glass tubing connected to an aspirator bottle partly filled with Tyrode's solution and leading to a volume recorder. The other end is ligated and attached to the lever recording the activity of the longitudinal coat. The volume recorder registers movements of fluid in the bottle produced by circular muscle activity; it consisted of either a piston recorder or a small Krogh chamber. Details about the interpretation of the records can be found in the paper by Feldberg & Solandt [1942]. The organ tank contained 2 baths of equal volume (75 ml.); levers for the longitudinal muscle and volume recorders were also duplicated,

so that two strips of intestine could be observed simultaneously under identical conditions. The aspirator bottles were so placed that the hydrostatic pressure in the lumen of the gut was about 2 cm. water.

The intestine was suspended at temperatures between 35 and 37° C. in glucose-free Tyrode saturated with oxygen. As shown by Feldberg & Solandt [1942], the reactions of the two muscular coats are affected very differently by the immersion in a glucose-free medium: whereas the spontaneous activity of both is much reduced or abolished, response to drugs is strongly inhibited in the longitudinal but hardly diminished, and occasionally even enhanced, in the circular muscle. The condition of 'exhaustion' through lack of carbohydrate seemed, accordingly, particularly suited for an investigation of the responses of the circular coat.

Results

Potassium chloride

Powerful contraction of the circular coat is obtained, as described by Feldberg & Solandt [1942], by the addition to the glucose-free Tyrode of small quantities of muscarine. Very similar effects were seen in the present investigation with KCl.

Smaller doses (25–60 mg. in 75 ml. bath) usually produce a rhythmical outburst of activity (Fig. 1), whereas larger quantities (75–100 mg.) may cause a prolonged tonic contraction on which very small rhythmical movements are superimposed (Fig. 2). The figures also show how weak, in comparison with the circular muscle's responses, are the reactions to KCl of the longitudinal coat after exhaustion through lack of glucose. Whereas 20μg. of atropine are sufficient to prevent the action of muscarine on the circular coat, 20, 40 and 100μg. do not abolish, though they may slightly reduce, the contractions elicited by KCl. Fig. 1 illustrates the effect of two consecutive doses of 60 mg. KCl: the first one is on the untreated preparation, the second 5 min. after 40μg. atropine. The effect on the circular coat is unchanged, that on the longitudinal coat even enhanced. A further dose of KCl was given 84 min. later, following 100μg. atropine, and there was still a powerful response of the circular muscle.

Nicotine was found to be equally incapable of abolishing the effect of KCl, although doses were employed which paralysed the circular muscle to further additions of nicotine. Successive additions of four doses of 2 mg. nicotine acid tartrate, for instance, had rendered the strip entirely insensitive to the third and fourth dose of nicotine, whereas a large response followed the addition to the bath of 40 mg. KCl.

The persistence of the potassium chloride effects in the presence of paralysing doses of nicotine strongly suggests that their action is on the muscle itself. The quantities of KCl necessary to elicit a response are not sufficiently large

to explain the effect as being caused merely by increased tonicity of the organ bath, since stimulation by hypertonicity could only be expected with doses

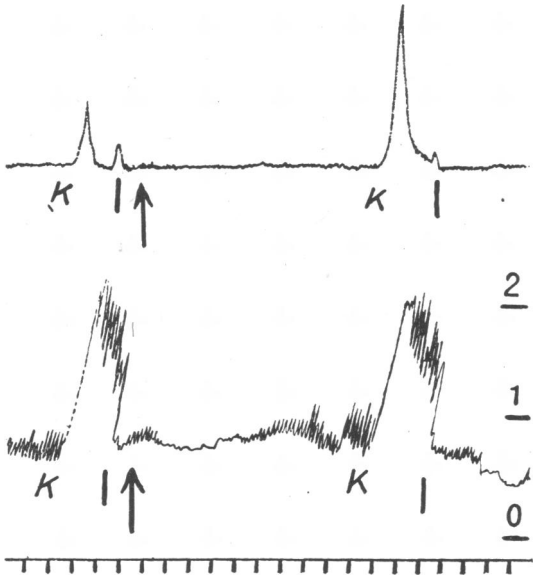

Fig. 1.

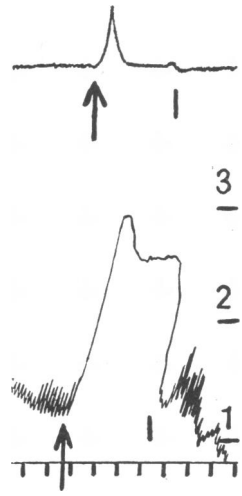

Fig. 2.

Fig. 1. Four-months-old rabbit, 0·93 kg. Piece of intestine (length 4 cm.) suspended in warm, oxygenated, glucose-free Tyrode. Record starts 35 min. later, when the pendulum movements have ceased. Upper tracing: movements of longitudinal muscle, amplification 4·5. Lower tracing: intestinal volume recorded with Krogh chamber. Scale in ml. Time: 30 sec. Size of bath: 75 ml. 60 mg. KCl are added to the bath at *K* and washed away at the strokes. At the arrows, 40μg. atropine sulphate are added to the bath.

Fig. 2. Same rabbit as that of Fig. 1. Piece of jejunum, 4 cm. long, suspended for 45 min. in warm, oxygenated, glucose-free Tyrode. Tracings and scale as in Fig. 1. 100 mg. KCl are added to the bath at the arrows, and washed away at the strokes.

at least five times the size of those used in the experiments just described. The effect is evidently analogous to the stimulation observed by Brown [1937] on normal or denervated striated muscle.

Sodium chloride

The addition of sodium chloride to the intestinal strip suspended in glucose-free Tyrode proved unexpectedly to have a potent stimulating effect on the circular coat. The quantities used were such as to increase the tonicity of the bath by about 20 %. An example is given in Fig. 3. (The movements of the longitudinal coat are not reproduced as they were negligible.) Tracing *a* represents the effect of two doses of 160 mg. salt (one dose had already been given before the record started). After a latent period of about a minute, during which there was a slight rise in tone, rhythmic activity developed

superimposed on a strong tonic contraction. In the interval between tracings *a* and *b*, 20μg. of atropine followed by 10μg. of eserine were given: the eserine had no effect. (This dose of eserine, in the absence of atropine, invariably caused stimulation of the circular coat. Examples of the action are given in Figs. 4 and 5 of the paper by Feldberg & Solandt [1942].) The eserine was

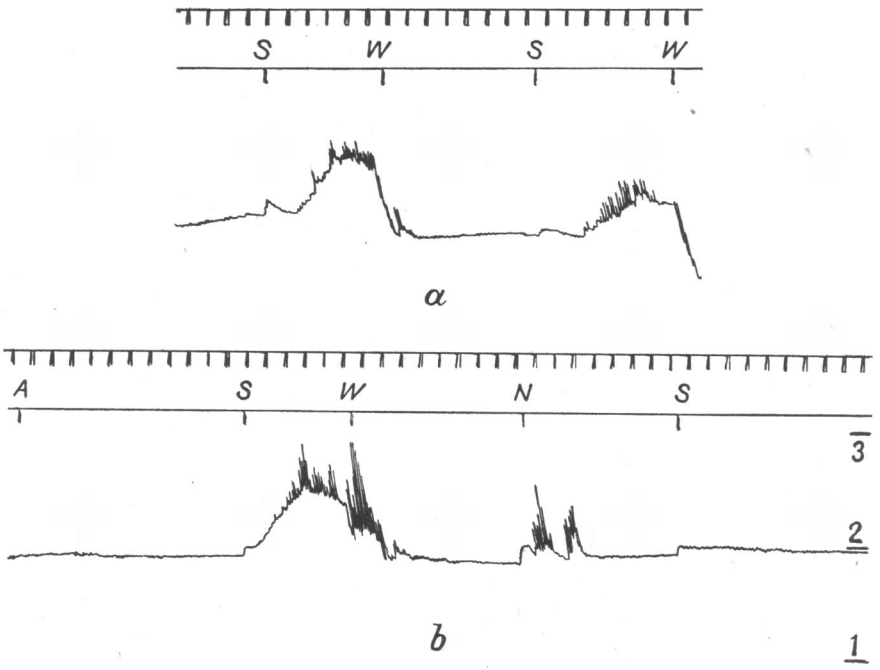

Fig. 3. 4. v. 42. Male rabbit, 1·7 kg. Strips of jejunum kept for 3½ hr. in Tyrode at room temperature, then suspended in warm, oxygenated, glucose-free Tyrode. Pendulum movements have nearly subsided 30 min. later, when a first dose of 160 mg. NaCl is given. Record starts 7 min. later. Intestinal volume recorded with Krogh chamber. Calibration in ml. Time: 30 sec. Volume of bath: 75 ml. Tracing *a*: *S*, 160 mg. NaCl; *W*, strip washed. Interval of 23 min., during which a first dose of 20μg. atropine sulphate followed by 10μg. eserine sulphate is given. The action of the eserine is prevented by the atropine. Tracing *b*: *A*, second dose of 20μg. atropine sulphate; *S*, 160 mg. NaCl; *N*, 2 mg. nicotine acid tartrate.

washed out and another dose of 20μg. atropine added at *A* (tracing *b*). This was followed by 160 mg. NaCl (*S*), and it is obvious that the effect was in no way diminished. At *N*, 2 mg. of nicotine were added to the bath. They caused an evanescent outburst of activity. Four minutes later a dose of NaCl was completely ineffective.

Inhibition of the sodium chloride stimulation was, however, also caused by larger amounts of atropine. Thus 40μg. atropine, for instance, nearly abolished the response in a strip of intestine which had reacted vigorously to the salt after treatment with 20μg. atropine.

Hence, stimulation by sodium chloride can be abolished by nicotine but is unaffected by atropine in doses which prevent the action of eserine or muscarine. This result was confirmed in other experiments. The sensitivity of different intestinal strips, however, to the action of salt is rather variable, as also is the latent period between addition of the drug and response. Furthermore, successive doses of NaCl do not always elicit similar responses, as in some preparation the effect gradually disappears.

The next question which arose was whether the stimulation by salt resulted from a specific effect of the sodium ion or whether it was merely a consequence of the hypertonicity of the solution surrounding the gut. The answer could easily be obtained by seeing whether solutions of inert substances of the same

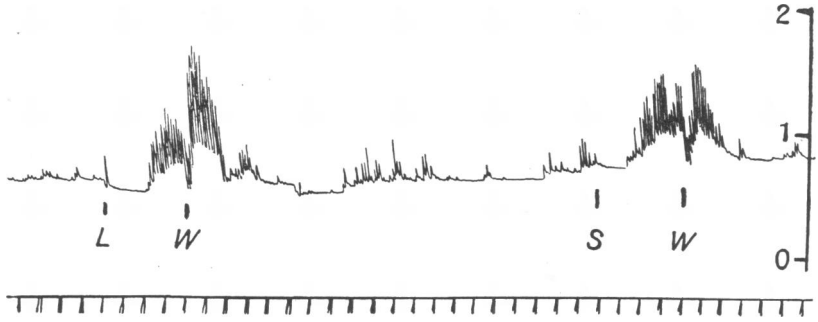

Fig. 4. 20. v. 42. Four-months-old rabbit. Piece of intestine kept for 2 hr. 50 min. in Tyrode at room temperature, then suspended in warm, oxygenated, glucose-free Tyrode. Record starts 35 min. later, when the pendulum movements have almost ceased. Record of circular coat with Krogh chamber. Scale in ml. Time: 30 sec. Volume of bath: 75 ml, *L*, 1·85 g. lactose; *W*, bath washed out; *S*, 1·85 g. sucrose.

tonicity would produce similar effects. From experiments by Rona & Neukirch [1912] and Feldberg & Solandt [1942], it was known that disaccharides have no effect on the longitudinal muscle. It was, therefore, likely that they could be used for our purpose.

The amount of disaccharide equivalent to 160 mg. NaCl is 1·85 g. A 50 % solution of lactose and a 90 % solution of sucrose were prepared and the required volumes added to the bath. Fig. 4 illustrates the result: after a latent period of about a minute, during which any activity of the circular coat previously present ceases, there is an outburst of rhythmic contractions very similar to that shown in Fig. 3 to follow the addition of NaCl. The longitudinal muscle (not reproduced here) was inhibited during the whole period of contact with the sugar solution. There is little doubt, therefore, that the stimulation observed when 160 mg. salt were added to the bath is an effect of hypertonicity. The probable site of this stimulation will be discussed later.

Sodium lactate

Feldberg & Solandt [1942] have shown that large doses of sodium lactate, though they are not utilized as carbohydrate, stimulate the circular coat. The effect is most obvious in the preparation depleted of its energy stores by prolonged activity in a glucose-free medium. The present experiments were carried out with a view to finding out which structures are affected by the lactate.

The amounts of lactate required in order to obtain regular effects are so large that they would increase the tonicity of the bath by 20 % if added to the ordinary Tyrode solution. As demonstrated in the last paragraph, an increase in tonicity of that magnitude will usually in itself stimulate the circular coat. The lactate was therefore given in a sodium chloride-free Tyrode, in which equimolecular amounts of lactate replaced the NaCl. In order to give a dose of 300 mg. lactate, for instance, 20 ml. of that Tyrode solution, in which the chlorine ion was substituted by the lactate ion, were warmed and added to the partly emptied bath. An example is represented in Fig. 5. The tracing is the record of the circular coat. At the first *L*, 300 mg. of lactate were added to the bath and caused a considerable rhythmic activity while the longitudinal muscle remained quiescent. The activity persisted for some time after the drug had been washed out (at *W*); this after-effect is frequently observed, and is more clearly seen in the figure after the second and third dose than after the first one, as this was very soon followed by a dose of 5 mg. nicotine acid tartrate (N_5). That this was not a paralysing dose is shown by the persistence of the activity in the circular muscle during the 7½ min. following its application. The nicotine was then washed out, and a second dose of lactate added to the bath: the effect was somewhat larger than the previous one. Ten mg. of nicotine were then given, which succeeded in diminishing, though not in abolishing, the contractions of the circular muscle after an initial powerful stimulation. The nicotine was left in the bath and lactate was again given 6½ min. later; a further increase of the effect resulted. The gut was then frequently washed and, 11 min. later, treated with another 10 mg. of nicotine. This time a short outburst of rhythmic contractions was followed by nearly complete paralysis of the circular coat. The nicotine was washed away and immediately followed by a fourth dose of sodium lactate. There was no response whatever; in a state of nicotine paralysis the strip had become insensitive to lactate. These results are reflected to a small extent in the longitudinal muscle: no response was obtained from the first two doses of lactate, but a slight activity was seen to result from the third dose which caused a very strong response in the circular coat. After the fourth application no response was seen in either coat. It may be mentioned that the doses of nicotine required to produce paralysis were unusually high in this preparation.

Atropine had the same influence on the stimulation by lactate as it had on the effects of hypertonic sodium chloride. Twenty μg. of atropine sulphate, which abolished the response to eserine, did not alter the effect of lactate, 60μg. atropine, however, diminished it. Care has to be taken in carrying out these

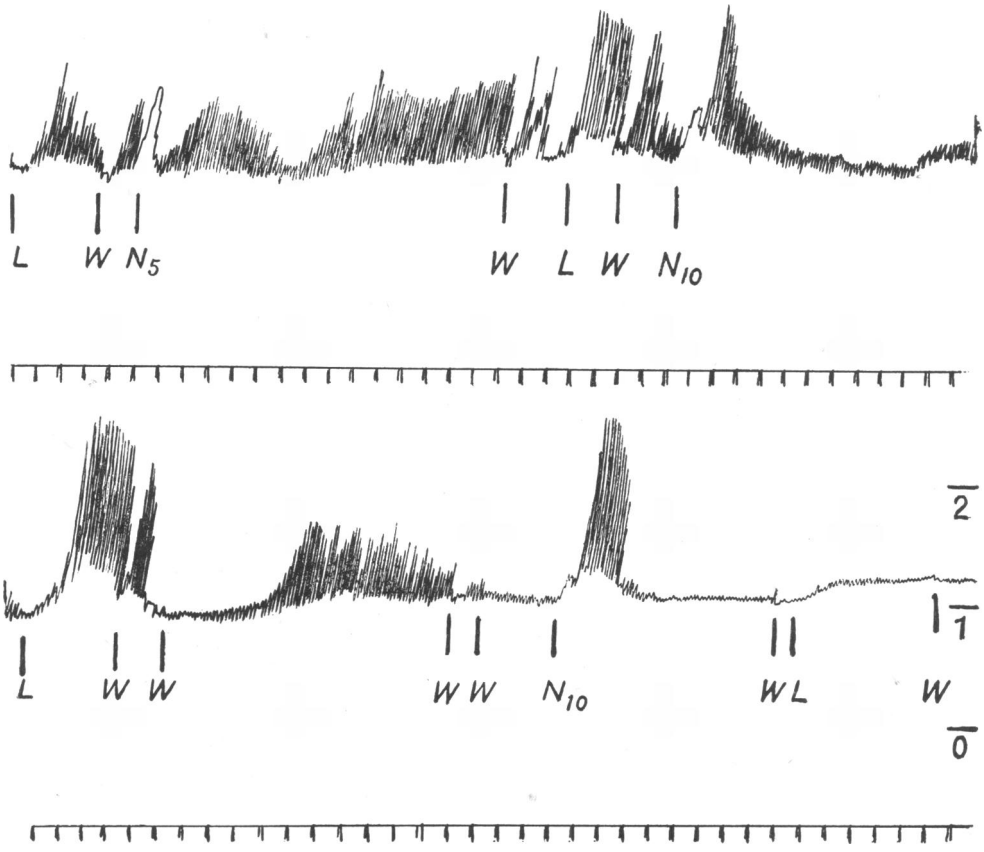

Fig. 5. 7. v. 42. Female rabbit, 2·0 kg. Strip of jejunum kept for 4 hr. in Tyrode at room temperature, then suspended in warm, oxygenated, glucose-free Tyrode. Tracing starts 40 min. later. Movements of circular coat registered with Krogh chamber. Calibration in ml. Time: 30 sec. Size of bath: 75 ml. *L*, 300 mg. sodium lactate in isotonic solution; *W*, strip washed; N_5, 5 mg., N_{10}, 10 mg. nicotine acid tartrate.

experiments to select strips of intestine which give a series of approximately equal responses to lactate. In some preparations the effect wears off rapidly and the influence of other drugs can, therefore, not be investigated. Moreover, the size of the initial response is very variable in different animals.

Discussion

The responses to hypertonic solutions and to sodium lactate have several features in common: they are rhythmic contractions, frequently followed by a short period of activity after the drug has been washed away; they are insensitive to doses of atropine which abolish the effects of parasympathomimetic drugs; their actions are frequently enhanced by nicotine in moderate doses and are always prevented by amounts of nicotine which paralyse the gut to the addition of further nicotine. In this context it is interesting to remember the observations by Bayliss & Starling [1899] on the small intestine of the dog. After splanchnotomy, stimulation of the vagi caused increased intestinal activity, which was not abolished by doses up to 30 mg. atropine, but disappeared after injection of 3 mg. nicotine. The circular coat was much more powerfully stimulated than the longitudinal muscle. The resemblance between vagal stimulation (in its preferential activity on the circular coat and in its sensitivity to drugs) and the effects of hypertonic solutions or sodium lactate is evident; the most likely conclusion is that the site of action of salt and lactate is Auerbach's plexus. If the action of these substances is on the nervous elements, the failure of some preparations to respond repeatedly to the same stimulus is not surprising, as fatigue or paralysis are readily elicited in nervous structures.

There is an interesting quantitative difference in the sensitivity to atropine of the vagus stimulation in the anaesthetized dog on the one hand, and application of the drugs in question to isolated pieces of intestine on the other: vagus stimulation remains effective even after excessive doses of atropine (30 mg.), whereas addition to the bath of two or three times the amount of atropine necessary to abolish the action of parasympathomimetic drugs will decrease or inhibit the action of salt or lactate. The failure of atropine to prevent the effect of vagal stimulation has been explained by Dale & Gaddum [1930] on the assumption of a close proximity of the site where the acetylcholine is liberated to the place where it causes stimulation. Reaction between the atropine and the region of the muscle excited by acetylcholine is thus prevented. It would appear that in the isolated preparation suspended in an unphysiological medium (Tyrode), this protecting structure is damaged and atropine, provided it is given in sufficient quantities, will get access to and react with the acetylcholine sensitive 'receptors' in the tissue.

The cooled preparation

In the dying tissue, nervous structures are known to succumb more rapidly than the musculature. The fact that pendulum movements still occur in rabbit's intestine kept for several days in the refrigerator has been used by Gunn & Underhill [1914] as an argument for their muscular origin. It seemed

of interest, in order to try and confirm the view that the effects of hypertonic NaCl or of lactate are due to stimulation of nervous elements, to compare the rate of disappearance of these responses with that of drugs of known site of action in the preparation which was slowly dying in the refrigerator at about 1° C. The effects were compared of

300–450 mg. sodium lactate,	0·3–2 mg. nicotine acid tartrate,
160 mg. sodium chloride,	0·5–1 ml. muscarine[1] 1:500,
10μg. eserine sulphate,	100 mg. potassium chloride.

The effect of lactate was the first to disappear; it was always absent if the intestine had been kept in the ice chest for 24–27 hr. Eserine and sodium chloride were the next to be abolished: they were usually present after 28, but always absent after 48 hr. At this latter period nicotine (0·3–1 mg.) had also become inactive, though a small effect on the tone of the muscle could still be elicited with 2 mg.; the rhythmic response had completely disappeared. Muscarine and potassium chloride had, at this stage, more effect on the gut than 2 mg. nicotine; the responses, however, were exclusively tonic in character.

The observation that the effects of lactate and hypertonic solutions are the first to be affected by cooling, supports the view that they are caused by stimulation of the nervous elements in the intestinal wall. The long persistence of the KCl effect, on the other hand, is in good agreement with the conclusion drawn at the beginning of this paper that its site of action is the muscle. Doses of nicotine which had an effect comparable in size to that of the other drugs employed, lost their efficacy a little later than NaCl or eserine, and earlier than muscarine or KCl. The persistence of a slight effect of 2 mg. nicotine after prolonged cooling is not at variance with the well-established fact that its site of action lies in the ganglion cells, as the dose of 2 mg. caused a much stronger reaction on the fresh preparation than any of the other substances used.

Summary

1. The response to certain drugs of the circular coat of the rabbit's intestine was investigated in a preparation, in which the activity of the longitudinal coat had been much reduced by suspension in a glucose-free Tyrode solution.

2. 25–100 mg. KCl in a 75 ml. bath strongly stimulate the circular muscle. The responses are mainly rhythmic with smaller doses and become more tonic with larger ones. The effect is not abolished by atropine or by paralysing doses of nicotine. Its site of action is the muscle.

3. Sodium chloride, given in a quantity which increases the tonicity of the bath by 20 %, causes an outburst of rhythmic activity in the circular coat. The action frequently has a latent period of about a minute and is of variable size in different animals. It is abolished by paralysing doses of nicotine and

[1] Natural muscarine, activity 1/450th of that of acetylcholine.

not affected by amounts of atropine which prevent the action of muscarine or eserine. Large doses of atropine, however, inhibit the effect. Similar responses of the circular coat are obtained, if hypertonicity of the bath is produced by the addition of lactose or of sucrose.

4. Sodium lactate, if applied to the intestine in such a way that it replaces part of the sodium chloride and therefore does not change the tonicity of the bath, stimulates the circular coat in a manner which resembles that of hypertonic solutions. Suitable doses are 300–450 mg. in 75 ml. The effect is abolished in the paralytic stage of nicotine poisoning but may be enhanced when paralysis has not yet been achieved. The effect is only abolished by atropine if the dose employed is several times that needed for the abolition of parasympathomimetic effects.

5. In the intestine undergoing slow disintegration at temperatures of 1° C., the response to a number of drugs was tested and seen to disappear in the following order:

Sodium lactate (300–450 mg.),
Sodium chloride (160 mg.) and eserine sulphate (10 μg.),
Nicotine acid tartrate (0·3–1·0 mg.),
Nicotine acid tartrate (2 mg.),
Muscarine (0·5–1 ml. solution 1:500) and potassium chloride (100 mg.).

6. The bearing of these results on the view that the mode of action of hypertonic solutions and of lactate is to stimulate Auerbach's plexus is discussed.

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