J. Physiol. (1951) 112, 84–94 577.174.5:612.337

THE EFFECT OF ADRENALINE ON THE GUINEA-PIG INTESTINE

By A. F. MUNRO

From the Department of Physiology, King's College, University of London

(*Received* 26 *January* 1950)

The inhibitory effect of adrenaline on the tone and rhythmic contractions of the mammalian small intestine is a well-known phenomenon. Observations have from time to time been reported, however, which suggest that adrenaline may also have a stimulating action on the muscle of the intestinal wall. Bunch (1898) observed augmentation of intestinal contraction in some instances after injection of adrenaline or stimulation of the splanchnics. Magnus (1903) recorded augmentation of contraction in a single case after adrenaline. Hoskins (1912) found that the pendular movements of isolated segments of rabbit intestine were inhibited by dilute adrenaline, but with great dilution ($1:400 \times 10^6$) they were increased. Bernheim & Blocksom (1932) found that adrenaline stimulated the muscle of the small intestine of the guinea-pig, subsequent to the induction of tone by pilocarpine, but in no case when adrenaline was added alone. When studying the reaction of the guinea-pig's intestine, for another purpose, it was found in isolated segments from the terminal region of the ileum that adrenaline alone consistently produced longitudinal muscle contraction and sometimes onset of rhythmicity. This paper describes the response and certain factors which modify it.

METHODS

The animals were killed by bleeding after a blow on the head. Segments of the ileum about 3 cm. in length were suspended in a bath of capacity 60 c.c., containing Krebs's physiological salt solution with 0·18% glucose. Tyrode's solution with a similar glucose content was employed for some experiments and gave comparable results. The Trendelenburg (1917) method of suspending the tissue was used and the bath temperature was maintained at 36° C. Adrenaline tablets (Parke Davis and Co.) were generally used in the experiments, but the results were checked by using crystalline adrenaline ('Ciba'). The adrenaline was made up to a working dilution of $1:20 \times 10^3$ in 0·9% NaCl, the usual concentration employed in the bath being $1:1 \times 10^6$. The lever length was such as to produce a fivefold magnification of the actual contraction.

RESULTS

Adrenaline had no apparent effect in any concentration on isolated segments of the guinea-pig's jejunum, or the upper part of the ileum. It caused immediate relaxation of tone in segments taken from the duodenum and the large intestine (Fig. 1). Segments from the terminal ileum, however, consistently showed contraction of the longitudinal muscle layer on addition of adrenaline to the bath fluid. The circular muscle of the same segments appeared to be relatively unaffected by adrenaline.

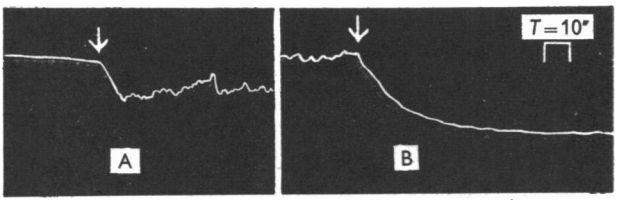

Fig. 1. Addition of adrenaline at the points indicated by (↓), to produce a concentration of $1:1 \times 10^6$ in Krebs's solution, causes relaxation of longitudinal muscle in isolated segments from (A) the duodenum, (B) the large intestine of the guinea-pig.

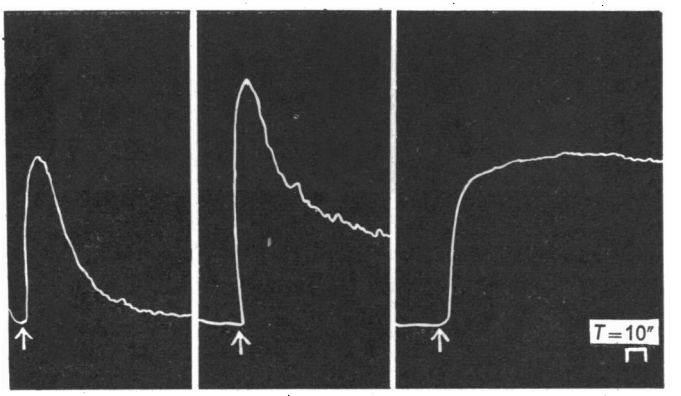

Fig. 2. Variations in the responses of the longitudinal muscle in isolated segments from three guinea-pigs on adding adrenaline at (↑), to give a concentration of $1:1 \times 10^6$ in the bath solution. These and subsequent tracings are from segments of the terminal ileum of the guinea-pig suspended in Krebs's solution.

The form of the response varied somewhat in different animals and sometimes even in the same segment. In some the contraction was maintained, in some it was short-lived and in others the recovery from the contraction was incomplete (Fig. 2).

In some animals contractile responses to adrenaline were obtained throughout the terminal 9–15 cm. of the ileum, the most vigorous responses occurring in the 3–5 cm. immediately adjoining the caecum (Fig. 3). A concentration of $1:1\times10^6$ adrenaline in the bath gave contractions of similar magnitude to those obtained after histamine $1:5\times10^6$ (Fig. 8B, C). In some animals, the adrenaline response was weaker and extended less far along the ileum, whilst again in others the response was virtually absent on the first addition of adrenaline, but could be obtained with subsequent additions as described in the next section.

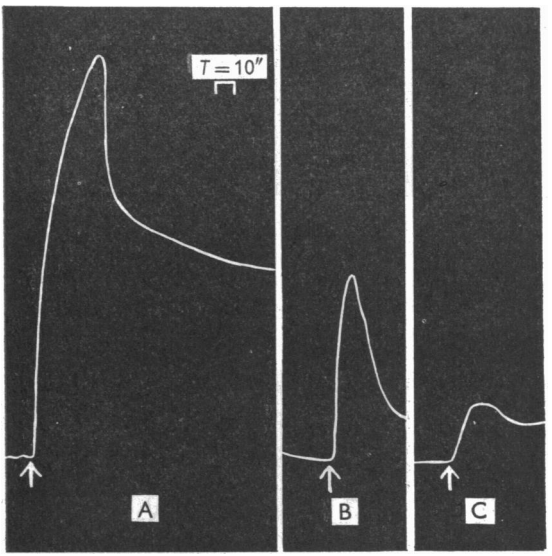

Fig. 3. Variations of responses in segments according to their distance from the ileo-caecal sphincter, on addition of adrenaline ($1:1\times10^6$) at (↑). (A), segment of ileum (4 cm. in length), adjoining caecum; (B), segment (4 cm. in length), next to (A); (C), segment (4 cm. in length), next to (B).

Factors influencing the contractile response to adrenaline

Relation to dose. With those segments which were observed to be most sensitive to adrenaline, increasing the strength of adrenaline in the bath from $1:40\times10^6$ to $1:6{\cdot}3\times10^5$ led to a progressively increased contraction of the longitudinal muscle, the latter dilution apparently producing a maximal contraction (Fig. 4). When the first addition of adrenaline produced a maximal contraction, further additions without washing even after partial relaxation, were for a time much less effective.

On the other hand, if the additional adrenaline was added about 15 min. after the addition of a maximal dose, the resultant contraction was often as great as that preceding it.

Repetition of dose and washing. The usual procedure in evoking successive contractile responses to adrenaline was to renew the Krebs's solution in the bath each time before adding the drug. When the initial contractile response

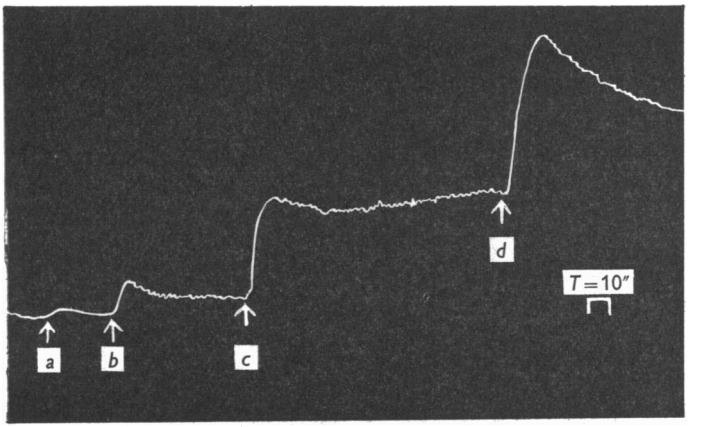

Fig. 4. Increasing the concentration of adrenaline in the bath, causes increased longitudinal muscle contraction of a segment from the terminal ileum. The adrenaline concentration was at (*a*) $1:40 \times 10^6$, at (*b*) increased to $1:13 \times 10^6$, at (*c*) to $1:1{\cdot}7 \times 10^6$, and at (*d*) to $1:6{\cdot}3 \times 10^5$.

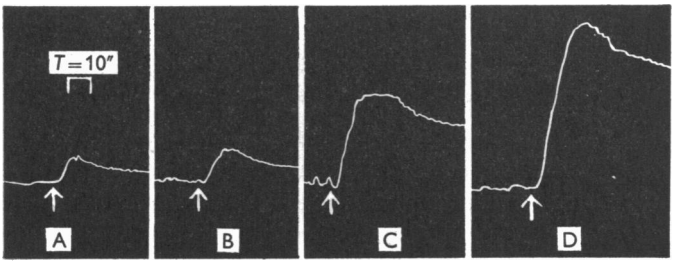

Fig. 5. Successive washing with Krebs's solution of an isolated segment from the terminal ileum, results in a progressive increase in the longitudinal muscle contractions, in response to the same concentration of adrenaline ($1:1 \times 10^6$). Additions of adrenaline were made at (↑). The tracings show the contractions obtained at (A), 1st washing; (B), 4th washing; (C) 7th washing; and (D), 10th washing.

was strong, subsequent contractions also remained uniformly strong. When, however, the initial response was weak or apparently absent, washing with fresh solution increased the sensitivity of the preparation to the dose (Fig. 5). The speed at which the response increased with successive washings varied, but usually attained a steady value after the 6th–10th change. Only in two or three segments from the fifty animals examined was this 'staircase' response to adrenaline absent. The effect did not seem to be associated with recovery of

tissue function after excision and handling, since a preparation could be left for an hour or longer suspended in the bath and then show little response to a first addition of adrenaline, whereas another preparation subjected to a number of washings with saline within the same period, showed a progressive increase in the response to adrenaline.

Relation to acetylcholine. The effect of acetylcholine on the terminal ileum was to produce strong contraction of the longitudinal muscle. There was no gradient in response along the ileum corresponding to that obtained with adrenaline. The consistency of the contractile response to acetylcholine in different animals compared strikingly with the variability in adrenaline response.

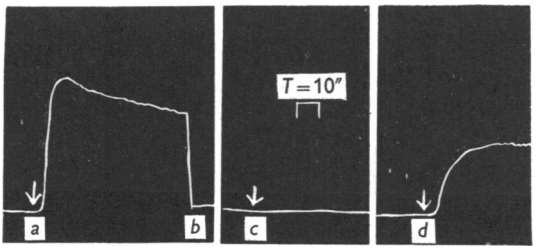

Fig. 6. The longitudinal muscle response to adrenaline ($1:1 \times 10^6$) of an isolated segment from the terminal ileum, in Krebs's solution containing ephedrine ($1:2 \times 10^5$) is less than when adrenaline alone is present. At (*a*) adrenaline ($1:1 \times 10^6$); at (*b*), the tissue was washed. Ephedrine to concentration ($1:2 \times 10^5$) at (*c*). Without again washing the tissue adrenaline to concentration $1:1 \times 10^6$ added at (*d*).

Response in the presence of other drugs

Ephedrine. Ephedrine in concentrations up to $1:2 \times 10^5$ did not produce any contractile response from a segment which was already sensitive to adrenaline. In contrast with the sensitizing action of ephedrine on the adrenaline response of such tissues as the nictitating membrane (Gaddum & Kwiatkowski, 1938), it was found that the presence of ephedrine in the bath in a concentration of ($1:1 \times 10^6$) had no effect on the subsequent response to adrenaline, while higher concentrations of ephedrine ($1:2 \times 10^5$) actually diminished it (Fig. 6). With subsequent washings, the response to adrenaline attained its original level.

Cocaine. The presence in the bath of cocaine hydrochloride in concentrations varying from $1:1 \times 10^6$ to $1:5 \times 10^4$ had little apparent effect on the adrenaline response except, possibly, for a very slight potentiation in the lower concentration of cocaine. There was no depression of the response as with ephedrine.

Ergotoxine. When ergotoxine ($1:5 \times 10^6$) alone was added to the bath it usually had either no effect or produced a feeble evanescent contraction. Occasionally, after repeated changes of the bath fluid, which resulted in an enhanced response to adrenaline, the sensitivity to ergotoxine was also increased.

The addition of ergotoxine to produce a concentration of $1:1\times10^6$ at the height of an adrenaline contraction resulted in a superimposed contraction. When the same dilution of ergotoxine was left in contact with the tissue for 2–3 min., the addition of adrenaline had no effect, although a strong contraction was still obtained with acetylcholine ($1:40\times10^6$).

Atropine. The contractile response to adrenaline was obtained after addition of atropine in sufficient concentration to abolish the effect of added acetylcholine (Fig. 9C).

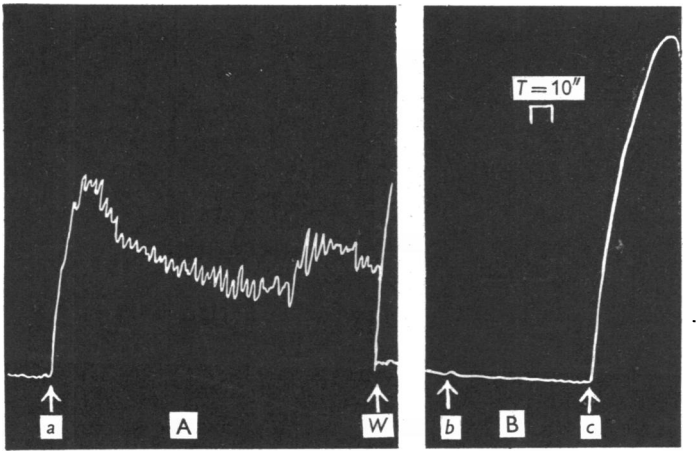

Fig. 7. An isolated segment from the terminal ileum gave no response to adrenaline ($1:1\times10^6$) after eserine ($1:5\times10^6$) had been added. (A), Adrenaline to concentration $1:1\times10^6$ added at (*a*). At (*W*) the tissue was washed and eserine ($1:5\times10^6$) added. (B), the same tissue 5 min. later; at (*b*) adrenaline ($1:1\times10^6$); at (*c*) acetylcholine ($1:40\times10^6$).

Eserine. Addition of eserine ($1:5\times10^6$) to the bath fluid resulted in very strong intermittent contractions of the longitudinal muscle and usually some increase in longitudinal muscle tone. A simultaneously maintained contraction of the circular muscle was also observed. With this concentration of eserine, adrenaline ($1:1\times10^6$) was ineffective in evoking a contraction from a segment which previously gave strong contractile responses to adrenaline (Fig. 7). Eserine in a concentration of $1:10\times10^6$, which was insufficient to produce intestinal contractions, markedly reduced the contractile response to adrenaline.

Histamine. Addition of histamine ($1:5\times10^6$) gave the usual rapid contraction of the longitudinal muscle, followed by a rapid partial relaxation and maintenance of tone at an intermediate level. When adrenaline ($1:1\times10^6$) was then added, it caused initially a superimposed contraction followed by a rapid relaxation of the muscle towards the resting level (Fig. 8A). When no adrenaline was added, it was always observed that the histamine contraction was main-

tained for a considerably longer period as indicated by the dotted line (Fig. 8A). Contractile responses to adrenaline would thus appear to predominate in this part of the ileum, but secondary inhibitory responses may be revealed when the initial tone is increased.

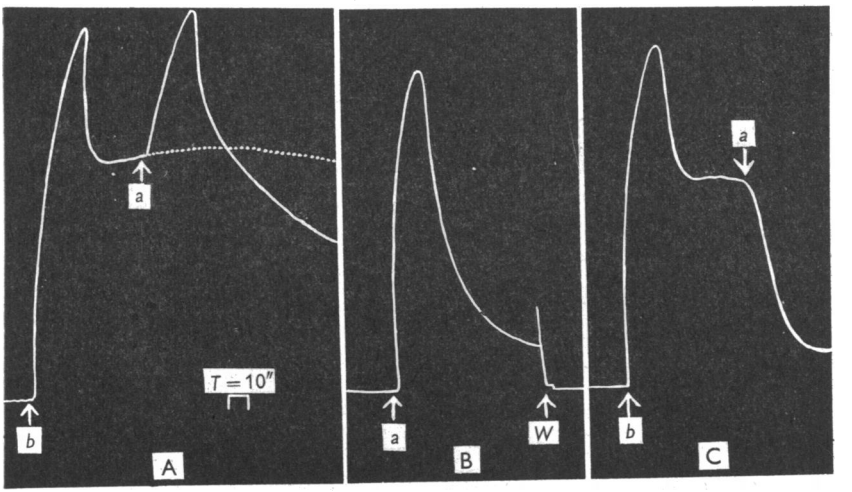

Fig. 8. Shows the reversal of the longitudinal muscle response to adrenaline of an isolated terminal segment after addition of ergotoxine. (A), histamine ($1:5 \times 10^6$) at (*b*); adrenaline ($1:1 \times 10^6$) at (*a*). (B), adrenaline ($1:1 \times 10^6$) at (*a*). The drum was stopped and the tissue was then washed (*W*) and ergotoxine ($1:1 \times 10^6$) added to the Krebs's solution in the bath. (C), the same tissue 10 min. later; at (*b*) histamine ($1:5 \times 10^6$); at (*a*) adrenaline ($1:1 \times 10^6$). The dotted line (Fig. 8A) shows how a contraction by histamine is normally maintained when adrenaline is not added.

The addition of ergotoxine did not prevent contraction by histamine but abolished the motor response on the addition of adrenaline, leaving the inhibitory response unaffected (Fig. 8C). Similar motor and inhibitory effects of adrenaline before and after addition of ergotoxine could be obtained when terminal segments were initially contracted by acetylcholine.

Nicotine. After nicotine ($1:1 \times 10^3$), which itself caused initially a strong contraction followed by relaxation, there was with adrenaline either an absence of any contraction (Fig. 9A) or, where nicotine produced an increase in tone, relaxation (Fig. 9B). Fig. 9A also shows that the effect of nicotine was not generally depressant on the tissue, since the acetylcholine response remained strong. When nicotine was added at the height of an adrenaline contraction the result was immediate relaxation of the longitudinal muscle (Fig. 9C). The previous atropinization of the segment was not responsible for the nicotine relaxation of the adrenaline contraction, since the effect was also obtained in

a fresh segment. Nicotine, however, similarly inhibited an acetylcholine contraction (Fig. 9D).

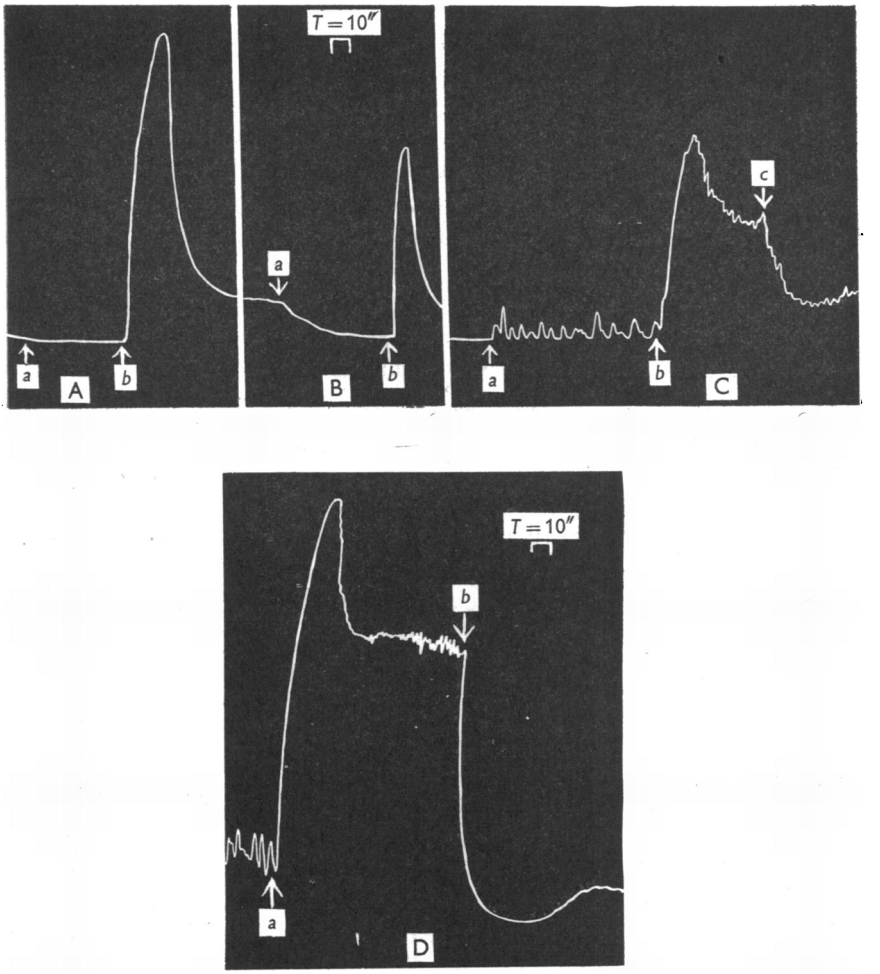

Fig. 9. (A), nicotine (1:1000) had been present for 5 min. in the Krebs's solution containing the isolated terminal ileum. At (*a*) adrenaline ($1:1 \times 10^6$); at (*b*) acetylcholine ($1:40 \times 10^6$). (B), another terminal segment nicotinized as in (A); at (*a*) adrenaline ($1:1 \times 10^6$) causes relaxation of the longitudinal muscle; at (*b*) acetylcholine ($1:40 \times 10^6$). (C), another terminal segment suspended in Krebs's solution containing atropine ($1:8 \times 10^6$), added 10 min. previous to addition at (*a*) of acetylcholine ($1:40 \times 10^6$); at (*b*) adrenaline ($1:1 \times 10^6$); at (*c*) nicotine (1:1000) in the bath. (D), another terminal segment suspended in Krebs's solution. Acetylcholine ($1:40 \times 10^6$) at (*a*); nicotine 1:1000 at (*b*).

Individual variations in response to adrenaline. The reason for the great variation in the strength of the initial adrenaline response in isolated segments

from different animals is still obscure. When no contraction could be obtained with adrenaline, the segment was usually, but not invariably, from an animal which had not been fed that day. The sex of the animal did not appear to modify the response. Stronger initial responses, however, tended to be found with segments from younger animals.

DISCUSSION

The foregoing experiments show that the effect of adrenaline on the intestine of the guinea-pig varies along its length from one of inhibition of tone and rhythm in the duodenum to increase of tone and rhythmic response in the lower part of the ileum, whilst the effect on the large intestine is again one of inhibition.

The experiments also indicate the existence of a dual action of adrenaline on the terminal ileum of the guinea-pig, but the mechanism responsible is by no means clear. Heinekamp (1925) found that in the dog's intestine, adrenaline stimulation subsequent to tonic contraction of the muscle by eserine was abolished by atropine. This would relate the effect to the liberation of acetylcholine at the nerve endings in the muscle. Chakrabarty (1947) also found that a stimulating effect of adrenaline after eserine in the rabbit ileum was abolished by atropine as well as by cooling the gut for more than 48 hr., and by nicotinization. The present observations differ from the above in the absence of any inhibitory effect of atropine on the adrenaline response; but the experiments with nicotine support the view that contraction with adrenaline is in some way dependent upon the functioning of ganglionic elements in the muscular wall.

The question arises whether contraction is mediated directly by the action of adrenaline or whether the latter enhances the production of another mediator, e.g. acetylcholine. Bülbring & Burn (1942), for instance, have shown that the action of acetylcholine on sympathetic ganglia is potentiated by adrenaline. If the increased activity in the intestine is due to the liberation of acetylcholine by post-ganglionic fibres, the absence of an increased response after eserine and the continuance of effect after atropine is puzzling. The action might, however, occur by way of central cholinergic fibres, in which case atropine would be unlikely to prevent it.

The possibility that nervous elements are implicated in the reaction of the terminal ileum to adrenaline rests on the supposedly specific inhibition of ganglionic function by moderate concentrations of nicotine. When it is found that a contraction induced by acetylcholine is relaxed by nicotine, doubts arise whether the paralysing effects of this substance are not extending to other elements of the tissue. On the other hand, nicotine did not prevent the response of the relaxed tissue to acetylcholine. It may be that smooth muscle in the contracted state shows different reactions to a drug than when relaxed.

The dual response obtained (Fig. 8A) when adrenaline is added during tonic contraction of a segment by histamine is possibly an example; another may be Bernheim & Blocksom's observation (1932) that guinea-pig ileum responds to adrenaline by contraction only after the induction of tone by pilocarpine. However, the fact that ergotoxine can reverse or abolish the response to adrenaline suggests a direct effect of the latter on the receptors in the muscle.

That the terminal region of the ileum in different species responds somewhat differently from higher levels has been commented on by various workers. Alvarez (1915), for instance, observed a gradient of rhythmicity in the last 25 cm. of the rabbit ileum, extending upwards to the ileo-caecal sphincter, and he suggested that the reversal of the general gradient in this region prevents material from packing too strongly against the sphincter. Barclay (1936) observed that the last few inches of the human ileum seem to be different in function from the part above, and that the region is apparently in a state of persistent tone, contrasting sharply with the restlessness of the duodenum and remainder of the ileum.

These observations would appear to associate closely this region of the ileum with the ileo-caecal sphincter, in controlling the transfer of the ileal contents to the large intestine. Observations on the human subject indeed suggest that the sphincteric responses are identical with those of the terminal ileum (White, Rainey, Monaghan & Harris, 1934). Elliott's experiments (1904) on the cat, however, indicate opposite and sharply differentiated responses of the ileo-caecal sphincter and the adjacent gut wall to adrenaline, except for a band about 1 cm. broad on either side of the sphincter. It would seem that there are species variations in the response of this region and indeed of other levels of the bowel to adrenaline. Brunaud & Labouche (1947), for instance, found that isolated duodenal segments from the horse were contracted by adrenaline.

King & Arnold (1922) found that splanchnic stimulation relaxed the outer muscular coat of the dog's intestine but contracted the muscularis mucosae. If the latter were sufficiently developed in the terminal region of the guinea-pig ileum, it might account for the adrenaline contractile response. A histological examination of the ileum at different levels is being made to examine the point. Further observations are also being made to establish how far the responses to adrenaline of isolated segments correspond to those of the same region when stimulated *in vivo*.

SUMMARY

1. Adrenaline causes contraction of isolated segments from the lower end of the guinea-pig intestine and relaxation of those from the upper end.

2. The response to adrenaline remains in the presence of atropine, but is antagonized by eserine, nicotine or ergotoxine.

3. The possible relation of the response to that of the ileo-caecal sphincter, in controlling the transfer of food to the colon, is considered.

I am very grateful to Prof. R. J. S. McDowall for advice during the course of the experiments. Thanks are also due to Messrs Parke Davis and Co. for providing the adrenaline chloride used in the experiments.

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