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THE ACTION OF LOCAL ANAESTHETICS AND *d*-TUBOCURARINE ON THE ISOLATED INTESTINE OF THE RABBIT AND GUINEA-PIG

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This paper describes the action of local anaesthetics and of *d*-tubocurarine chloride on isolated intestinal preparations. In order to distinguish experimentally between the action of these substances on the muscle fibres and their action on the nervous structures present in the preparation the following procedure was adopted: an effect on the tone and spontaneous rhythm of the muscle and on the responses to acetylcholine or histamine was taken as evidence for action on the excitability of the muscle fibres; on the other hand the nicotine response and the local peristaltic reflex provided reactions with which the excitability of the nervous structures in the intestine could be tested.

METHODS

The experiments were carried out on isolated preparations of the rabbit's and guinea-pig's ileum suspended in aerated Tyrode's solution. A 16 c.c. bath was used when the contractions of the longitudinal muscle alone were recorded; this was done with a Lovatt Evans frontal writing lever. In the experiments on the peristaltic reflex the intestinal volume was recorded according to the method described by Trendelenburg (1917). A piston recorder of the Brodie type was used for this purpose, and the bath volume was 35 c.c. The method of suspending the intestinal preparation was identical with that described by Feldberg and Solandt (1942). The temperature of the bath was kept at 37° C. when the rabbit's, and at 35° C. when the guinea-pig's, ileum was used. The animals were killed by a blow on the head immediately before the intestine was removed.

The local anaesthetics used were cocaine hydrochloride, procaine hydrochloride, and nupercaine hydrochloride. The amounts stated in the text refer to the salts and not to the bases.

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RESULTS

I. Effect on the excitability of the muscle fibres

Movements and tone of the muscles of the small intestine of most animals are increased by small doses of cocaine and decreased by large ones (von Anrep, 1880; Bayliss and Starling, 1899; Langley and Magnus, 1905; Kuroda, 1915; and Trendelenburg, 1917). On the guinea-pig's intestine, however, the action of cocaine was found by Trendelenburg to be always depressant. In our experience this was not so.

Guinea-pig.—In some preparations small doses of cocaine had a stimulating effect. The contractions of the longitudinal muscle were usually found to be small and transient and obtainable only when cocaine was added to the bath for the first time. In a few preparations, however, cocaine produced strong and well-maintained contractions which could be obtained with each new administration of the drug and resembled the contractions produced by acetylcholine or histamine. Even when cocaine had no stimulating action of its own, small doses often rendered the preparation more sensitive to the stimulating action of histamine or acetylcholine.

In order to obtain the depressant action of cocaine it had to be given in concentrations stronger than 1 in 80,000 (see Fig. 1*b*). There might then be some relaxation of the tone of the longitudinal muscle and reduction of the spontaneous contractions if they were present. The most obvious effect, however, was the reduction in the response to acetylcholine, histamine, or potassium. The depression was greatest during the first few minutes of the cocaine action and decreased somewhat when the cocaine was kept in the bath for longer periods. After the cocaine had been

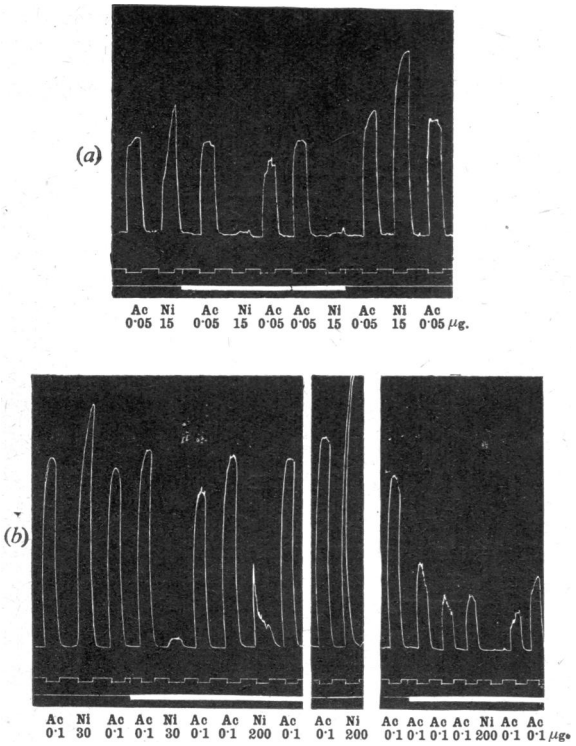


FIG. 1.—Contractions of guinea-pig's ileum in 16 c.c. Tyrode's solution. At (Ac) acetylcholine chloride, at (Ni) nicotine tartrate in μg., as indicated at the bottom of the figure, added to the bath. The broad white lines indicate the periods during which the bath contained cocaine, 1 in 80,000 (first two white lines) and 1 in 25,000 (last white line).

washed out the muscle quickly regained its original sensitivity, in fact for 10 to 15 min. it became even more sensitive to histamine or acetylcholine than it had been before the cocaine administration (see Fig. 1).

During a cocaine depression the muscle was unable to contract as strongly as the untreated muscle even if acetylcholine or histamine were given in hundredfold increased amounts. The extent of the reduction in contractibility depended on the concentration of cocaine present in the bath, becoming more pronounced with increasing concentration.

Other local anaesthetics examined depressed the muscle fibres in weaker concentrations than cocaine. A reduction of the acetylcholine response similar to that seen with 1 in 35,000 cocaine was seen with concentrations of 1 in 600,000 procaine and 1 in 800,000 nupercaine.

d-Tubocurarine chloride, even if given in strong concentrations (1 in 80,000 to 1 in 16,000), did not affect the muscle tone and had only a slight and irregular effect on the responses to acetylcholine and to histamine. The responses to histamine were sometimes slightly depressed, sometimes slightly augmented; those to acetylcholine, however, were usually slightly depressed; the depression did not become more pronounced when the concentration of tubocurarine was increased. After a strong concentration of tubocurarine had been washed out there was often a period of a few minutes in which the response to acetylcholine was augmented.

Rabbit.—In some preparations cocaine increased, in others it decreased, the amplitude of the rhythmic contractions and caused slight loss of tone of the longitudinal muscle. When different parts of the intestine were examined inhibition by cocaine was found to occur more regularly in the duodenum and jejunum and stimulation in the ileum, so that sometimes cocaine had opposite effects in different parts of the same intestine. In order to obtain inhibition of the acetylcholine response cocaine had to be given in concentrations higher than 1 in 30,000, except in preparations which had been kept suspended in the bath for several hours and had become more sensitive to the depressant action of cocaine. Nupercaine was about 20 times as active as cocaine. A similar inhibitory action of other local anaesthetics on the rabbit's duodenum has been described by Tripod (1940), and more recently by Dawes (1946) and de Elío (1948).

d-Tubocurarine chloride in a concentration of 1 in 30,000 or stronger usually caused slight and transient augmentation of the tone and amplitude of the rhythmic contractions. Since this effect was not prevented by the presence of a strong concentration of local anaesthetics in the bath fluid, it could not have been due to stimulation of the ganglion cells in the intestinal wall. In some preparations weak concentrations of tubocurarine (about 1 in 200,000) had a slight depressant effect on the response to acetylcholine, in others such an effect was obtained with very strong concentrations only (1 in 40,000 to 1 in 16,000).

II. Effect on the nervous structures in the intestinal preparation

(a) The nicotine response of the guinea-pig's ileum

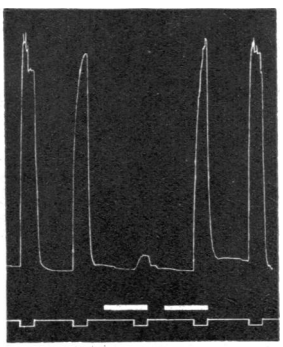
The addition of small doses of nicotine to the bath in which a guinea-pig's ileum is suspended produces contractions which differ from those produced by acetylcholine or histamine in that the

onset is delayed for a few seconds and the contraction itself proceeds more gradually. If a few minutes are allowed each time after the nicotine has been washed out and before its renewed administration, comparable results can be obtained without manifestations of a paralysing action of nicotine, but when such an experiment is continued for long periods the responses to nicotine often become gradually smaller.

The nicotine contractions are abolished by concentrations of local anaesthetics or tubocurarine chloride which do not yet affect or affect slightly only equally strong contractions produced by histamine or acetylcholine. In the experiment of Fig. 1, doses of 15 and 30 μg. of nicotine had stronger actions than 0.05 and 0.1 μg. of acetylcholine respectively. Cocaine (1 in 80,000) prevented the nicotine responses but left those to acetylcholine practically unaffected. The preparation was not completely insensitive to nicotine, which in a dose of 200 μg. caused a small contraction. In order to abolish this response the concentration of cocaine had to be increased to such an extent that it reduced the response to acetylcholine, i.e., the excitability of the muscle fibres (Fig. 1*b*). An experiment like that for cocaine in Fig. 1*a* is shown for tubocurarine in Fig. 2.

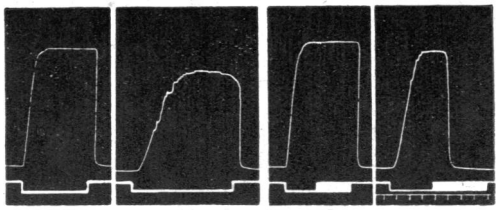
In the experiment of Fig. 3 the procedure was slightly modified. It illustrates that an acetylcholine, but not a nicotine, contraction is maintained in the presence of 1 in 80,000 cocaine; relaxation of the nicotine contraction started 13 sec. after adding the cocaine to the bath.

The inhibiting effect of cocaine and tubocurarine on the nicotine response is not so strong as that produced by paralysing doses of nicotine itself.



(*a*) (*b*) (*c*) (*d*) (*e*)

FIG. 2.—Contractions of guinea-pig's ileum in 16 c.c. Tyrode's solution. At (*a*), (*c*), and (*e*) 40 μg. nicotine tartrate, at (*b*) and (*d*) 0.4 μg. acetylcholine chloride for 1 min. The broad white lines indicate the presence of 200 μg. *d*-tubocurarine chloride in bath.



(*a*) (*b*) (*c*) (*d*)

FIG. 3.—Contractions of guinea-pig's ileum in 16 c.c. Tyrode s solution. At (*a*) and (*c*) 0.4 μg. acetylcholine chloride kept in the bath for 2½ min., but followed after the 1st min. at (*c*) by 200 μg. cocaine. At (*b*) and (*d*) 35 μg. nicotine tartrate kept in the bath for 4 min., but followed after the 2nd min. at (*d*) by 200 μg. cocaine. Period during which cocaine was present in bath indicated by broad white line. Time in 30 sec.

In the presence of 1 in 200,000 nicotine the preparation is paralysed to any dose of nicotine; but 1 in 40,000 tubocurarine chloride renders the preparation insensitive to small doses only of nicotine, the effect of 100 or 200 μg. of nicotine being greatly reduced but not abolished. Usually the sensitivity of the preparations to the inhibitory action of cocaine and tubocurarine increased after a few hours' suspension in the bath of Tyrode's solution.

(*b*) *The peristaltic reflex in the rabbit's ileum*

The reflex was initiated by raising the pressure in the lumen of the intestine by 2.5 to 4 centimetres of saline. In some preparations, in which the increased pressure in the lumen was maintained, peristaltic waves continued for hours at regular intervals of 15 to 25 sec., interrupted from time to time by short periods of inactivity of the circular muscle. In other preparations cessation of the reflex took place within a few minutes after the pressure in the lumen had been raised. The procedure usually adopted was to raise the pressure at every 4th min. for 1 min., and to keep it at zero or even below zero during the rest of the time. The reflex could then usually be initiated for hours without signs of fatigue, but in a few experiments the peristaltic reflex was not maintained even for the 1-min. period and in others fatigue occurred in the course of a prolonged experiment. Some improvement was obtained by adding 1 in 100,000,000 to 200,000,000 eserine to the Tyrode's solution and by keeping the pressure in the lumen of the intestine at zero for 60 to 90 min. before starting or continuing the experiment. This procedure was adopted as a routine in all experiments in which it was not possible to obtain the peristaltic reflex regularly. But even this procedure proved ineffective in a few preparations, which therefore had to be discarded.

As stated by Trendelenburg (1917) the onset of the contraction of the circular muscle layer, which starts at the stomach and spreads to the caecal end, is preceded by shortening of the preparation owing to contraction of the longitudinal muscle fibres. The degree of this shortening is dependent on the tone of the longitudinal muscle and varies greatly in different preparations. In addition we found that slight variations in the initial pressure in the lumen of the intestine greatly influenced the result. With a negative pressure in the lumen the longitudinal muscle is well relaxed and, when the pressure is raised, it contracts strongly before the first wave of contraction of the circular muscle spreads over the preparation. With an initial slight positive pressure in the lumen, the longitudinal muscle is already partly contracted and the additional rise in pressure leads to a relatively small further shortening of the preparation before it is stopped by the onset of the contraction of the circular muscle layer. In Fig. 4*a* and *c* the differences in the degrees of shortening in two preparations are illustrated; the initial pressure in the lumen at *a* was about zero, at *c* −1 cm. saline.

The fact that it is really the activity of the circular muscle which interupts the contraction of the longitudinal one can be seen from the results obtained when the pressure in the lumen was lowered at different phases of the peristaltic reflex. When this occurred during the end of a wave of contraction of the circular muscle there was no further shortening, but when it occurred in the interval between two peristaltic waves, when the longitudinal muscle had begun to contract, the shortening continued for some time after the pressure had been lowered. These differences are illustrated in the two controls shown in Fig. 7, before the administration of nupercaine.

With a negative pressure of 2 or 3 cm. saline in the lumen of the intestine its walls are in apposition, the longitudinal muscle is well relaxed and exhibits no or only small rhythmic contractions.

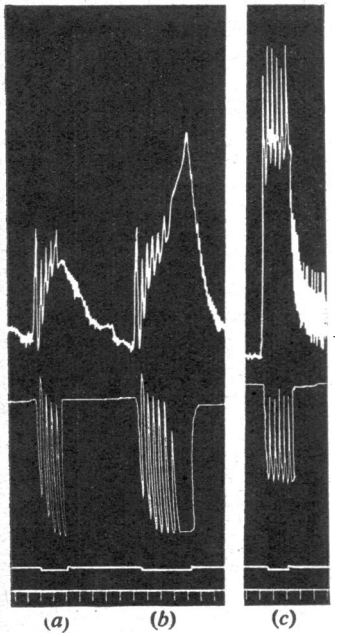


FIG. 4

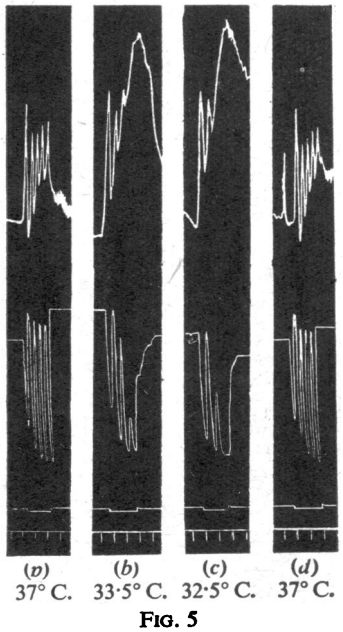


FIG. 5

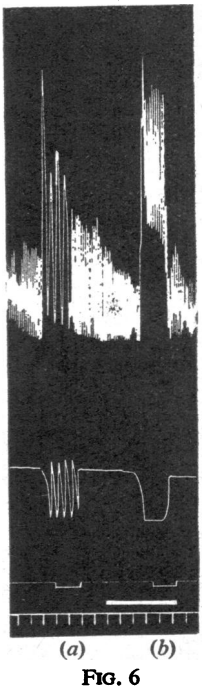


FIG. 6

FIG. 4.—Rabbit's ileum in 35 c.c. Tyrode's solution (6 μg./l. eserine). In this and the following figures the upper tracing records contractions of the longitudinal muscle, the lower tracing the intestinal volume. At (*a*), (*b*), and (*c*) pressure raised in intestinal lumen to about 3 cm. saline during the periods indicated. Time in 30 sec. Details in text.

FIG. 5.—Rabbit's ileum in 35 c.c. Tyrode's solution (6 μg./l. eserine). Effect of temperature on peristaltic reflex initiated by raising pressure in intestinal lumen to about 3 cm. saline for 1 min. Time in 30 sec.

FIG. 6.—Rabbit's ileum in 35 c.c. Tyrode's solution (6 μg./l. eserine). Effect of cocaine on peristaltic reflex. Pressure raised in intestinal lumen to about 3 cm. saline for 1 min. at (*a*) and (*b*). Broad white line indicates presence of 100 μg. cocaine in the bath. Time in 30 sec. Details in text.

In this condition the preparation is particularly suitable for testing quantitatively the effects of drugs like acetylcholine on the longitudinal muscle. The first effect of a rise of pressure in the lumen is the reappearance, or an increase in the amplitude, of the spontaneous rhythmic contractions; with a further rise in pressure the tone of the longitudinal muscle increases.

When fatigue of the peristaltic reflex takes place the response of the longitudinal muscle is not affected. The muscle either remains shortened or continues to do so after the contractions of the circular muscle have ceased (see Fig. 4*b*), and if the preparation had shown strong rhythmic contractions before the initiation of the reflex they reappear. The tracings then obtained resemble that illustrated for cocaine in Fig. 6.

The effect of lowering the bath temperature is seen in the experiment of Fig. 5. At 37° C. a 1-min. period of increased pressure in the intestinal lumen caused four peristaltic waves to spread over the whole length of the preparation (*a* and *d*); at 33.5° C. there were two such waves and a third incomplete one (*b*), and at 32.5° C. there occurred one full and one incomplete wave only (*c*). The contraction of the longitudinal muscle, however, was not inhibited when the bath temperature was lowered and the activity of the circular muscle had come to an end.

Local anaesthetics.—Von Anrep in 1880 and Bayliss and Starling in 1889 described inhibition of the peristaltic reflex in the dog when cocaine was applied in strong concentrations to the serosa of the small intestine *in situ*. According to Bayliss and Starling the spontaneous movements increased at the same time. On the isolated small intestine preparations of the rabbit and dog Trendelenburg (1917) obtained increased peristalsis with weak, but inhibition with strong, concentrations.

In our experiments the main effect of cocaine was inhibition of the peristaltic reflex, although there was some indication of a stimulating action. The inhibition by cocaine resembled the effect of fatigue or of lowering the bath temperature—i.e., the contractions of the circular but not of the longitudinal muscle were affected (see Figs. 6, 7, and 8). The concentrations of local anaesthetics necessary to inhibit the peristaltic reflex were of the same order as those which inhibit the response to small doses of nicotine.

In the experiment of Fig. 6 raising the pressure in the lumen of the intestine to 3 cm. saline for 1 min. produced four waves of contraction of the circular muscle layer (at *a*). At *b* 2 min. after the addition of 100 μg. of cocaine to the 35 c.c. bath these contractions no longer occurred when the pressure was raised, but the longitudinal muscle shortened and, in addition, exhibited its strong rhythmic contractions. The Figure illustrates well the difference in the mechanisms responsible for the contractions of the two muscle layers when the pressure in the lumen is raised. The waves of contraction of the circular muscle are inhibited because a local nerve reflex is involved in their initiation; the spontaneous rhythmic contractions, the so-called pendular movements, and the increase in tone of the longitudinal muscle persist because they are myogenic in origin. In the experiment of Fig. 7 raising the pressure in the lumen after the addition of 100 μg. cocaine to the bath still produced an initial small contraction of the circular muscle which affected the upper end of the preparation only; the longitudinal muscle, on the other hand, continued to contract during the whole period of increased pressure The quick reversi-

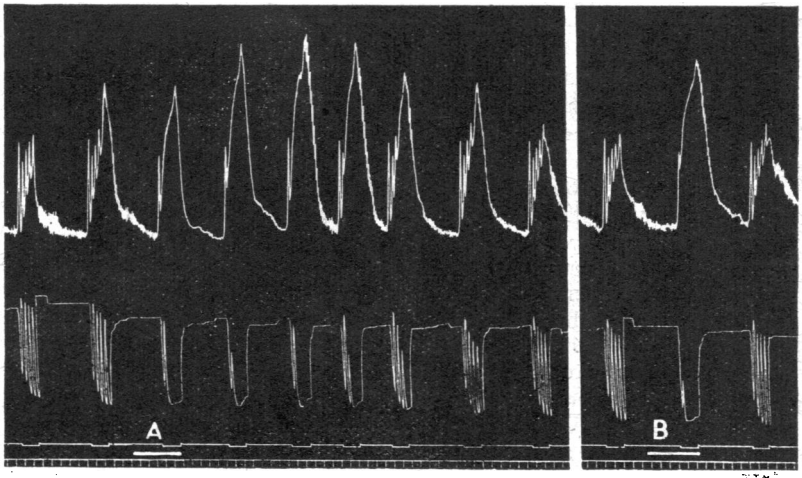


FIG. 7.—Rabbit's ileum in 35 c.c. Tyrode's solution (6 μg./l. eserin). Comparison of 8 μg. nupercaine (A) and 100 μg. cocaine (B) on peristaltic reflex. Pressure in intestinal lumen raised each time to about 3 cm. saline for 1 min. Broad white line indicates presence of local anaesthetics in bath. Time in 30 sec.

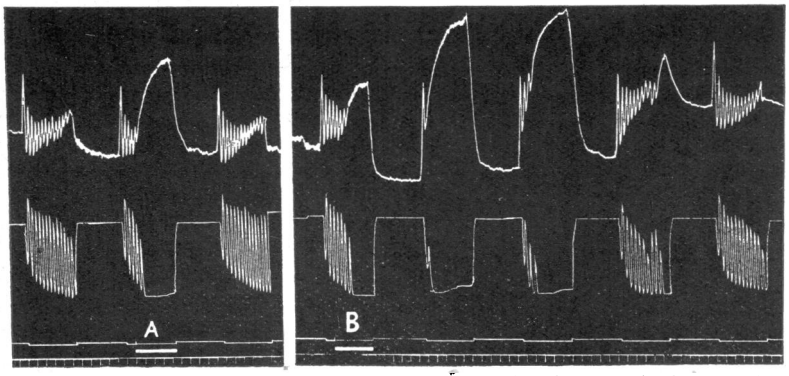


FIG. 8.—Continuation of Fig. 6. Pressure in intestinal lumen raised for 3 min. each time. Comparison of 100 μg. cocaine (A) and 10 μg. nupercaine (B) given 30 sec. after initiation of peristaltic reflex. Broad white lines indicate presence of local anaesthetics in bath. Time in 30 sec.

bility of the action of cocaine is seen from the fact that 3 min. after the cocaine had been washed out the peristaltic reflex was restored; indeed, it often became more regular and any signs of fatigue originally present disappeared. For instance, in the experiment of Fig. 8A the last two waves of contraction of the circular muscle occurring during the first 3-min. period of increased pressure in the lumen of the intestine were incomplete. After the cocaine had been washed out this "fatigue" was no longer seen. We do not know if this effect of cocaine is the result of increased excitability of the muscle fibres, or of the nervous elements, or of both.

The inhibitory effect of cocaine develops quickly. In the experiment of Fig. 8A the addition of cocaine to the bath 30 sec. after the peristaltic reflex had been initiated caused cessation of the peristaltic waves within 40 sec.

Partial inhibition of the peristaltic reflex which occurs with small doses of cocaine shows the following characteristics: (i) the contractions of the circular muscle layer come to an end after a short time; instead of the four or five waves usually passing over the preparation during a 1-min. period of increased pressure there remain the initial two or three waves or even a single contraction only; (ii) the waves of contraction become incomplete and confined to the upper end; or (iii) they become irregular and start at the middle or lower end or at different points simultaneously; dilated and constricted segments then alternate with each other. Such disorderly contractions are also frequently seen during the recovery period after a dose of cocaine greater than that necessary to produce complete inhibition of the peristaltic waves.

When cocaine was used in concentrations so strong as to depress the excitability of the muscle fibres the response of the longitudinal muscle to the stimulus of increased pressure in the lumen was also abolished.

The fact that the inhibiting action of cocaine on the peristaltic reflex is easily reversible, so that it is possible to obtain comparable effects on repeated administration of the same dose and graded responses with different doses, makes it possible to employ this reaction for the biological assay of cocaine and to compare its action with that of other local anaesthetics. When this was done it was found that procaine was slightly less active than cocaine in inhibiting the peristaltic reflex, whereas nupercaine was 12 to 12½ times as active as cocaine and, in addition, the effect of nupercaine was more prolonged and developed more gradually. In the experiment of Fig. 7 the nupercaine was added to the bath 2 min. before the pressure in the lumen was raised for 1 min. and washed out after 3 min. This was the procedure usually adopted when assaying the potency of local anaesthetics against cocaine. The greatest inhibition of the reflex did not occur during the presence of nupercaine in the bath but 3½ min. after it had been washed out, and about 20 min. elapsed before the reflex was restored. In the experiment of Fig. 8, 8 μg. nupercaine and 100 μg. cocaine were added to the bath after the reflex had begun. With cocaine it took about 40, with nupercaine about 70, sec. before the reflex was inhibited, and again the prolonged effect of nupercaine was evident. This delay in the action of nupercaine was taken into account when assaying its potency against cocaine. The maximal inhibition observed after the nupercaine had been washed out was used for comparison.

d-*Tubocurarine chloride*.—This drug has only an inhibitory and no stimulating action on the peristaltic reflex. As with the inhibitory effect of cocaine only the waves of contractions of the circular muscle layer are abolished, whereas the rhythmic contractions of the longitudinal muscle, if present, and its tonic contraction in response to the increase in pressure in the lumen, persist; this is illustrated in Fig. 9. In sensitive preparations

some inhibition of the reflex is seen with concentrations less than 1 in 10,000,000 (Fig. 10), but for complete inhibition higher concentrations are necessary. The effect is easily reversible; recovery is delayed for more than a few minutes only when concentrations are used which are stronger than those necessary for complete inhibition of the reflex. Graded responses are obtained with different doses and comparable results with the same dose given repeatedly.

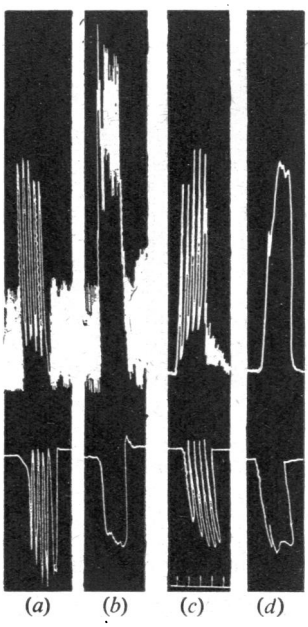


FIG. 9.—Rabbit's ileum in 35 c.c. Tyrode's solution (6 μg./l. eserine). The tracings (*a*) and (*b*) are from one, the tracings (*c*) and (*d*) from another, intestine. Inhibition of peristaltic reflex by 100 μg. *d*-tubocurarine chloride at (*b*) and (*d*), given 2 min. before the initiation of the reflex. Pressure in intestinal lumen raised each time for 1 min. to about 3 cm. saline. Time in 30 sec.

The inhibition of the peristaltic reflex can be used as a reaction for assaying unknown solutions of tubocurarine and other substances with curare-like action. Fig. 10 illustrates the inhibition produced by different doses of *d*-tubocurarine chloride. With 100 and 50 μg. inhibition was complete; with 25 μg. one wave, with 10 μg. one and a half, with 5 μg. practically two, and with 2 μg. two and a half waves remained. Each dose of tubocurarine was added to the bath 2 min. before the pressure was raised and washed out when the pressure was lowered again. The reflex was tested every 4th min. After the larger doses of tubocurarine recovery was delayed; the reflex had not fully returned when it was tested 3 and 7 min. after the tubocurarine had been washed out; it was retested only after full recovery had taken place, but with the exception of four controls, marked 0, all intermediate tests have been omitted in the Figure.

In the experiment of Fig. 10 slight quick volume changes of the intestine occurred during the tubocurarine paralysis; their frequency was the same as that of the rhythmic contractions of the longitudinal muscle and they might in part have been accounted for in this way. Partly, however, they resulted from the fact that the circular muscle layer itself exhibited rhythmic contractions at the lower end of the preparation; they were apparently myogenic in origin.

(c) The peristaltic reflex of the guinea-pig's ileum

The reflex could be obtained regularly, repeatedly, and without the necessity of adding eserine to the bath fluid. A rise of pressure of 2 cm. of saline in the lumen of the intestine was sufficient when the preparation was from a small, and of 2.5 cm. when from a large, guinea-pig. During a 1-min. period of increased pressure in the lumen, between 9 and 14 waves of contraction of the circular muscle layer passed over the preparation, and if the increased pressure was repeated every 4th min. the preparation remained active for many hours, sometimes up to 10 hours.

According to Trendelenburg cocaine had no stimulating effect on the peristaltic reflex of the guinea-pig's intestine: when kept in the bath for 1 min. cocaine had no effect at all, when kept for 5 min. the reflex was abolished. Our results were slightly different.

Usually no definite inhibitory effect on the peristaltic reflex could be obtained with cocaine, nupercaine, or tubocurarine during the first hour or so of an experiment, even if the substances were tested in relatively high concentrations and kept in the bath for several minutes. But nearly all preparations gradually became more sensitive to the inhibiting action of these substances, and then inhibition occurred whether the substances were kept in the bath for 5 min. or for 1 min. only.

During the first hour or so of an experiment the characteristic effect of cocaine, and to a lesser degree of nupercaine and tubocurarine, consisted in an increase in the frequency of the waves of contraction of the circular muscle layer. In some preparations in which 1 in 40,000 cocaine had this effect at the beginning of the experiment a 10 times weaker concentration caused complete inhibition

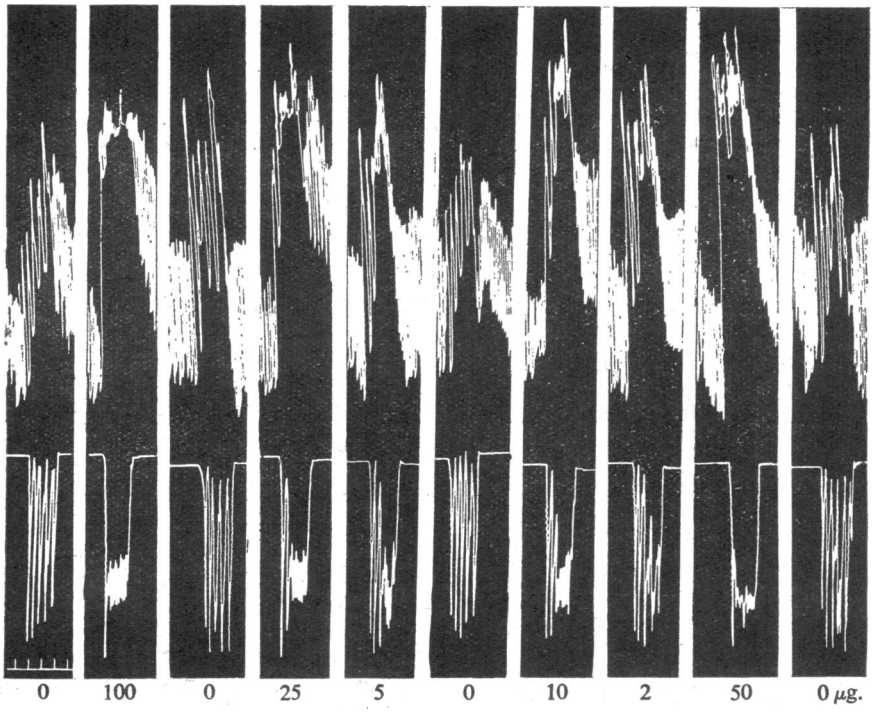


FIG. 10.—Rabbit's ileum in 35 c.c. Tyrode's solution (6 μg./l. eserine). Graded inhibition of peristaltic reflex by varying amounts of *d*-tubocurarine chloride. Amounts indicated in μg. at bottom of figure. Time in 30 sec. Details in text.

after the intestine had been in the bath for several hours. Although Trendelenburg does not mention this initial action of cocaine on the frequency of the peristaltic waves it is evident in an experiment which he illustrates in his paper.

In the experiments of Fig. 11 the different actions of cocaine on the peristaltic reflex are illustrated. Instead of the 12 peristaltic waves elicited by a 1-min. period of increased pressure in the lumen at *a*, there were 20 waves when the procedure was repeated at *b* 2 min. after the addition of 100 μg. of cocaine to the 35 c.c. bath. Sometimes up to 30 waves occurred. Each of these waves started at the upper end and extended over the whole preparation. This was not always so; often they were incomplete, irregular, and started at different parts of the preparation; at the same time the longitudinal muscle contracted. Such a result can be seen in the experiment of Fig. 11 *c* to *f*, in which another intestine was used. Instead of the 11 complete waves at *c* there were 18 incomplete waves along with shortening of the preparation at *d*. At a later stage of the experiment cocaine started to exert its inhibiting action and results like that seen at *e* were obtained: an initial period of incomplete waves at a high frequency was followed by cessation of the reflex. At an even later stage of the experiment there was complete inhibition of the peristaltic waves after the addition of the same dose of cocaine. When it was washed out and the peristaltic reflex retested at 4-min. intervals restoration of the reflex passed through a stage in which the frequency of the peristaltic waves was increased.

It was found difficult and sometimes impossible to obtain graded responses with increasing doses of cocaine and equal responses with the same dose, even at a later stage of an experiment, when cocaine exerted its strong inhibiting action on the peristaltic reflex. The isolated intestine of the guinea-pig, unlike that of the rabbit, therefore, is unsuitable for assaying the potency of an unknown solution of cocaine.

With nupercaine there were changes in the sensitivity of the preparation similar to those with cocaine, but it was usually possible to obtain some

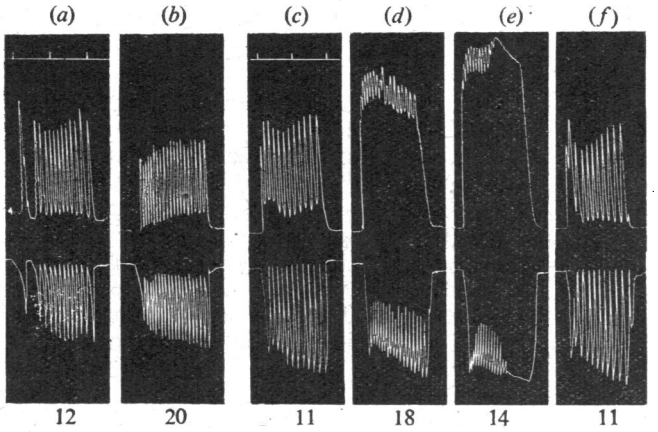


FIG. 11.—Guinea-pig's ileum in 35 c.c. Tyrode's solution. Effects of 100 μg. (at *b*) and 300 μg. (at *d* and *e*) of cocaine on the peristaltic reflex. The number of peristaltic waves during each 1-min. period is given at the bottom. Time in 30 sec. Details in text.

slight inhibition of the reflex even at the beginning of an experiment; therefore, when the potency of nupercaine was compared with that of cocaine no constant results could be obtained. In one preparation, for instance, 20 μg. nupercaine produced slight inhibition of the reflex at the beginning of the experiment whilst 800 μg. cocaine only increased the frequency of the peristaltic waves; 4 hours later 100 μg. cocaine as well as 20 μg. nupercaine abolished the reflex. In this experiment, therefore, nupercaine had an inhibiting action on the peristaltic reflex 40 times stronger than cocaine at the beginning of the experiment, but later on it was only five times as active.

The increase in the frequency of the peristaltic waves produced by nupercaine was not so pronounced as with cocaine. When nupercaine had inhibited the reflex, recovery passed through a stage in which the frequency of the peristaltic waves was increased. As in the experiments on the rabbit's intestine the action of nupercaine was delayed and prolonged: when it was kept in the bath for 3 min. the full effect only occurred a few minutes after it had been washed out.

At the beginning of an experiment tubocurarine, even in very strong concentrations, caused only slight acceleration of the frequency of the peristaltic waves; after several hours twentyfold weaker concentrations might abolish the reflex. The effects like those of cocaine were quickly reversible after washing out the drug. The difficulty of obtaining comparable responses on repeated administration of the same amount of tubocurarine, or graded responses on administration of varying amounts, made the guinea-pig's intestine preparation unsuitable for the quantitative assay of curare-like substances.

In Fig. 12 the changes in sensitivity to tubocurarine chloride are illustrated. The drug was added each time 2 min. before the pressure in the lumen was raised. At the beginning of the experiment 2,000 μg. increased the frequency of the peristaltic waves from 10 to 13 per min. (at *b*); 2½ hours later 600 μg. greatly inhibited the reflex (at *c*), and after a further 3½ hours 100 μg. practically abolished it (at *d*).

The paralysing action of local anaesthetics and of tubocurarine on the peristaltic reflex is much weaker than that of nicotine in paralysing doses,

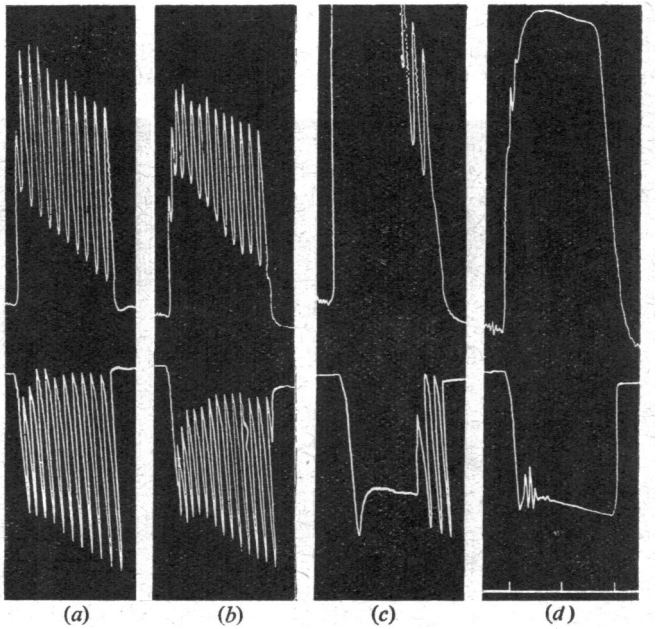


FIG. 12.—Guinea-pig's ileum in 35 c.c. Tyrode's solution. Gradual increase in the inhibition of the peristaltic reflex by *d*-tubocurarine chloride. At (*b*) 2,000 μg., at (*c*) 600 μg. two and a half hours later, at (*d*) 100 μg. six hours later. Time in 30 sec. Details in text.

particularly when the comparison is made on a fresh preparation. An analogous result has been obtained and referred to previously for the inhibition of the stimulating action of nicotine.

In the experiment of Fig. 13, 2,000 μg. of *d*-tubocurarine chloride had no inhibiting action; 600 μg. of nicotine, however, abolished the reflex. The nicotine was added to the bath between *a* and *b* whilst the pressure in the intestinal lumen was low; an immediate strong contraction of the longitudinal muscle occurred, but within 1 min. the muscle relaxed again although the nicotine was kept in the bath. At this stage raising the pressure in the lumen no longer initiated the peristaltic reflex (at *b*). In fact, both the longitudinal and the circular muscle layer were in an atonic condition. Instead of the usual strong contraction of the longitudinal muscle in response to the increased pressure in the lumen there was only a slight delayed shortening of the preparation, and the lack of tone of the circular muscle led to the great filling of the intestine with saline solution. This is seen on the volume record by the profound fall which is followed by a horizontal line because the maximal possible reduction in volume of the Brodie recorder has been reached. Between *b* and *c* the nicotine was washed out; before the reflex returned, the muscle tone was restored in both layers. With the return of the reflex the peristaltic contractions of the circular muscle were at first not sufficiently strong to overcome the contraction of the longitudinal muscle as will be seen from a comparison of *e* with *a* and *f*.

If the nicotine had remained in the bath the return of muscle tone seen at *c* and *d* in Fig. 13 would nevertheless have occurred but the abolition of the peristaltic waves would have remained. In the early stages of nicotine paralysis, but not later, the muscle layers are thus unable to develop tone in response to increased pressure in the lumen and in this condition they have also been found to be less excitable to drugs like acetylcholine or histamine (Cantoni and Eastman, 1946; Emmelin and Feldberg, 1947).

Discussion

On the isolated intestinal preparation it can easily be shown that the response of the intestinal wall to increased pressure in the lumen consists of two phases: a contraction of the longitudinal muscle and a wave of contraction of the circular muscle spreading aborally over the whole preparation. Trendelenburg has aptly termed the two responses the preparatory and the emptying phase of the peristaltic reflex. Only the peristaltic wave of contraction of the circular muscle is nervous in origin and therefore very susceptible to lowering of the bath temperature, to fatigue, and to drugs which affect nerve fibres or nerve cells in the intestinal wall. The contraction of the longitudinal muscle, on the other hand, is a response of the muscle fibres themselves to the stimulus of stretching and is therefore not abolished when the function of the nervous structures in the intestinal wall is interfered with by local anaesthetics or by tubocurarine. In the rabbit's intestine with its pronounced rhythmic contractions of the longitudinal muscle there occurs in addition, even before the pressure in the lumen is sufficiently raised to initiate the shortening of the preparation, an augmentation of the amplitude of the rhythmic contractions. This response also is myogenic in origin. We do not know whether similar myogenic responses to stretching occur in the circular muscle layer. This muscle certainly offers some resistance to the stimulus of filling the gut, and this resistance is not abolished when the nervous structures in the intestinal wall are inactivated by cocaine or tubo-

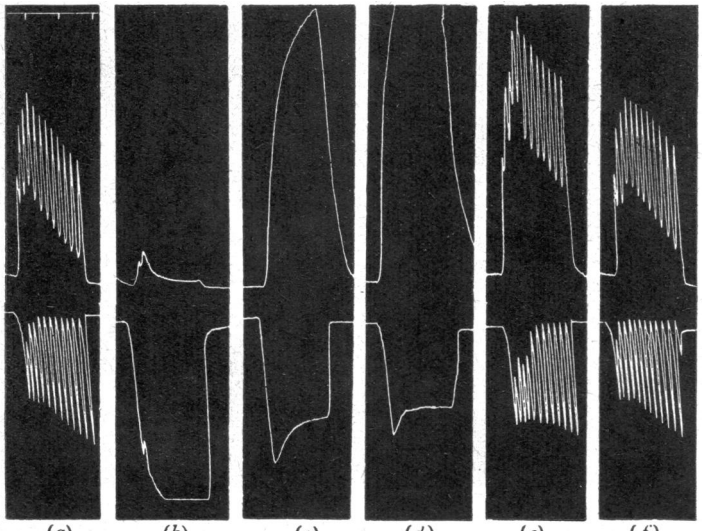


FIG. 13.—Guinea-pig's ileum in 35 c.c. Tyrode's solution. Nicotine paralysis of the peristaltic reflex. Between (*a*) and (*b*) 600 μg. nicotine tartrate were given and washed out at the end of (*b*). Time in 30 sec. Details in text.

curarine, but it is abolished when the muscle fibres are in an atonic condition as, for instance, in the early stages of nicotine paralysis. In addition we have found* that the whole layer of the circular muscle may be thrown into powerful rhythmic contractions at the same frequency as that of the rhythmic contractions of the longitudinal muscle, when eserine in a concentration of about 1 in 300,000 is added to the bath during a period of increased pressure in the lumen and whilst the peristaltic waves of contraction are abolished by cocaine or tubocurarine.

A study of the peristaltic reflex as well as of the simple contractions of the longitudinal muscle on the intestinal preparation reveals the fact that a careful analysis is always necessary before the effects of drugs can be attributed to a nervous or to a muscular site of action. For instance, the shortening of the intestinal preparation when the ganglion cells are stimulated by nicotine does not differ much from the contraction produced by muscle-stimulating drugs, such as histamine or acetylcholine. On the other hand, if the pressure exerted on the intestinal wall from the lumen is just insufficient to initiate the reflex, any drug which increases muscle tone may indirectly elicit the reflex. It is therefore not surprising that drugs which stimulate smooth muscle are known to augment, whereas drugs which relax them are known to inhibit, peristalsis. Straub and his co-workers applied Trendelenburg's technique to the intestine *in situ* and observed increased peristalsis with choline, acetylcholine, and physostigmine, and inhibition of the peristaltic reflex with adrenaline and ephedrine (Straub and Viaud, 1933; Straub and Leo, 1933; Leo, 1933; Straub and Stefánsson, 1937).

If drugs are known to have local anaesthetic or curare-like actions the abolition of the peristaltic reflex in the isolated rabbit's intestine provides a useful reaction for assaying their potency, provided that the concentrations used do not affect the muscle fibres. Nupercaine was found to be about 12 times as active as cocaine in abolishing the peristaltic reflex; this does not necessarily prove a similar relationship for the potency of the two drugs as local anaesthetics, since with different tests widely different results are obtained (see Goodman and Gilman, 1943).

When the peristaltic reflex is used as a reaction for assaying curare-like substances we compare their effectiveness in paralysing autonomic ganglia and not motor endplates. The two effects do not run parallel, as shown by Bovet, Depierre, and de Lestrange (1947) and by Depierre (1947). In previous experiments of this kind the paralysing effects on sympathetic ganglia were compared with those on motor endplates. The ganglia involved in the peristaltic reflex, however, probably belong to the parasympathetic nervous system, and their sensitivity to a curare-like acting substance may be different from that of sympathetic ganglia. The problem appears to be even more complicated since motor endplates of muscles from different species do not show the same sensitivity to different curare-like substances (Wien, 1948).

The greater frequency of the peristaltic waves after cocaine in the freshly suspended guinea-pig's intestine is difficult to explain. When at the same time the waves become incomplete, so that they affect parts of the preparation only, they resemble rhythmic contractions of myogenic origin. But they are not accounted for in this way, for the following reasons: (1) All grades of increased frequency may occur after cocaine. The frequency of the contractions may increase slightly or the rate may double or increase even more. If cocaine were to abolish the peristaltic waves and reveal myogenic contractions there would be only two frequencies—that of the peristaltic waves before, and that of the myogenic contractions after, cocaine. (2) When the contractions do not become incomplete after cocaine, they, like typical peristaltic waves, start at the oral end and spread over the whole preparation.

The frequency of the peristaltic contractions is to some extent dependent on the degree of pressure exerted in the intestinal lumen and increases with the pressure (Trendelenburg, 1917). Cocaine might be thought to produce a similar effect in the guinea-pig's intestine simply by increasing muscle tone. It is more likely, however, that cocaine, and to some extent other local anaesthetics, alter the excitability or the conductivity of either the muscle fibres or the nervous elements or of both structures in the intestinal wall of the guinea-pig in such a way as to speed up the rate of the rhythmic response which occurs when the pressure in the lumen is increased. This action could be grouped then with the well-known central stimulating actions of cocaine. To assume a similar action for tubocurarine would be strange; but its effect in increasing the frequency of the peristaltic waves is only very weak and it may have to be explained in a different way.

SUMMARY

1. Cocaine has stimulating and inhibiting effects on the excitability of the muscle fibres of the

*Unpublished experiments.

rabbit's and guinea-pig's intestine. *d*-Tubocurarine has but slight effects of this kind on these preparations.

2. It is possible to inactivate the nervous structures in the intestinal wall with cocaine or with *d*-tubocurarine. In concentrations which do not yet depress the excitability of the muscle fibres these substances inhibit the responses to small doses of nicotine as well as the peristaltic reflex.

3. The peristaltic reflex initiated by raising the pressure in the lumen of the isolated intestine (Trendelenburg's method) consists of two phases: (i) a contraction of the longitudinal muscle which is a response of the muscle fibres themselves to the stimulus of stretching, and (ii) waves of contractions of the circular muscle layer spreading aborally over the whole preparation and being nervous in origin. Only the latter are abolished when the nervous structures in the intestinal wall are paralysed by local anaesthetics or by tubocurarine. In the rabbit's intestine the familiar strong rhythmic contractions of the longitudinal muscle (pendular movements) also remain unaffected under these conditions.

4. In the guinea-pig's intestine local anaesthetics and *d*-tubocurarine exert their inhibiting effect on the peristaltic reflex only several hours after the preparation has been suspended in the bath. In the freshly suspended preparation the effect of these drugs is to increase the number of peristaltic waves per minute. This effect is strong with cocaine but weak with tubocurarine.

5. Inhibition of the peristaltic reflex in the isolated rabbit's intestine provides a useful reaction for assaying quantitatively the potency of local anaesthetics or curare-like substances, because the inhibition is easily reversible, and comparable effects are obtained on repeated administration of the same dose of these substances, and graded responses with different doses. By this method nupercaine was found to be about 12½ times as active as cocaine.

References

Anrep, B. von (1880). *Pflüg. Arch. ges. Physiol.*, **21**, 38.

Bayliss, W. M., and Starling, E. H. (1899). *J. Physiol.*, **24**, 99.

Bovet, D., Depierre, F., and de Lestrange, Y. (1947). *C. R. Acad. Sci., Paris*, **225**, 74.

Cantoni, G. L., and Eastman, G. (1946). *J. Pharmacol.*, **87**, 392.

Dawes, G. S. (1946). *Brit. J. Pharmacol.*, **1**, 90.

Depierre, F. (1947). *C. R. Acad. Sci., Paris*, **225**, 956.

Elfo, F. J. de (1948). *Brit. J. Pharmacol.*, **3**, 108.

Emmelin, N., and Feldberg, W. (1947). *J. Physiol.*, **106**, 482.

Feldberg, W., and Solandt, O. M. (1942). *J. Physiol.*, **101**, 137.

Goodman, L., and Gilman, A. (1943). *The Pharmacological Basis of Therapeutics*, p. 297, New York: The Macmillan Company.

Kuroda, M. (1915). *J. Pharmacol.*, **7**, 423.

Langley, J. N., and Magnus, R. (1905). *J. Physiol.*, **33**, 37.

Leo, E. (1933). *Arch. exp. Path. Pharmak.*, **169**, 25.

Straub, W., and Leo, E. (1933). *Arch. exp. Path. Pharmak.*, **169**, 18.

Straub, W., and Stefánsson, K. (1937). *Arch. exp. Path. Pharmak.*, **185**, 435.

Straub, W., and Viaud, P. (1933). *Arch. exp. Path. Pharmak.*, **169**, 1.

Trendelenburg, P. (1917). *Arch. exp. Path. Pharmak.*, **81**, 55.

Tripod, J. (1940). *J. Physiol.*, **97**, 289.

Wien, R. (1948). *J. Physiol.*, **107**, 44*P*.