THE ANTAGONISM OF SYMPATHOMIMETIC DRUGS BY ERGOTAMINE.

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(II). Paper showing fuller evidence for (I).

(III). Paper in Journ. of Pharmacol. and Exp. Therap. "Action of Ergotamine on the Response of the Rabbit's Gut to Adrenaline. 1931, vol. 42, p.9.

Contents.

THE ANTAGONISM OF SYMPATHOMIMETIC DRUGS BY ERGOTAMINE.

A. LITERATURE.

(1) Introduction.

Barger and Dale (1910) showed that the pharmacological action of adrenaline was shared by a large number of primary and secondary amines, and they termed this group of drugs the sympathomimetic group. Tyramine (para-hydroxyphenyl-ethyl-amine) which was isolated by Barger in 1909 was found to be one of this group which closely resembled adrenaline in its action. Ephedrine, an alkaloid obtained from the ancient Chinese remedy Ma Haung, was isolated in 1887, by Nagai, but its pharmacological action was only established in 1917 by Amatsu and Kubata. The fact that it is a relatively stable substance with a strong sympathomimetic action has led to its extensive clinical use.

Recent work on these important drugs has shown that their action differs in many important respects from/

from those of adrenaline and their right in the title of sympathomimetic has been questioned.

The author has examined the mode of action of these drugs on isolated plain muscle and the manner in which these actions are modified or antagonised by ergotamine and atropine. It was found necessary to determine many elementary actions, because, although these had been determined by many previous workers, yet the results were so contradictory that no certain conclusions could be drawn from the evidence.

(2) Theories of Drug Antagonism.

1. The site of action of autonomic drugs.

Until recently the specific autonomic drugs were believed to act on nerve endings and a distinction was drawn between neurotropic and musculotropic drugs. For example adrenaline and acetyl choline were believed to stimulate the nerve endings of the sympathetic and para-sympathetic systems and ergotamine and atropine were believed to paralyse these endings. Loewi (1921) and his school have, however, provided firm evidence that the autonomic nerves act by liberating appropriate drugs and he has actually shown that atropine does not prevent the release of acetyl choline by the vagus/

vagus in the heart but that it renders the cells insusceptible to the liberated drug.

The old distinction between musculotropic and neurotropic drugs must therefore be abandoned and the term sympathomimetic means not a drug that acts on sympathetic nerve endings but one which produces the same effect as does the stimulation of sympathetic nerves.

2. The mode of action of autonomic drugs.

Most observers who have made quantitative measurements of the action of drugs have assumed that the action of most drugs is proportionate to their concentration in the fluids around the cell. Straub (1907) put forward the potential theory of the action of drugs, according to which the action of a drug depended on the difference in the concentration of the drug without and within the cell. Jendrassik (1924-29) in recent years has endeavoured to show that this theory is of wide and even general application. According to this view the action of most drugs would vary continuously on prolonged application and quantitative study of actions and antagonisms would be almost impossible.

The author has examined the evidence for the potential action of the autonomic drugs on plain muscle, and has found that that most of the evidence/

evidence can be explained as experimental error. This work is being published in the Quarterly Journal of Physiology as a separate paper (Appendix I and II).

In this paper it will be assumed that the action produced by the drugs under discussion is dependent on the concentration of drug in the fluid around the muscle cells and that the action is maintained without important alterations so long as the drug remains at unaltered concentration around the cell. This general rule is subject to certain minor exceptions which are probably due to slow changes in the viscous elastic properties of plain muscle when this either contracts or relaxes.

3. The nature of drug antagonism.

Straub (1907) held that atropine opposed muscarine by retarding its penetration into the cell and thus produced the same result as if the concentration was lessened. Langley (1914) studied the antagonism of nicotine by curara on the rectus abdominis of frog and concluded that curara and nicotine acted on the neural region of the muscle and not on the nerve endings and formed chemical compounds with the tissues. Langley put forward the view that (i) these alkaloids entered into chemical/

chemical combination with the same constituent in the tissues, (ii) that the compounds formed were reversible, and (iii) that the amount of the compound formed by each drug depended on their relative mass, i.e. concentrations. However he qualified his above conclusions by the statement that whilst advocating the chemical theory as being at present the one most in accordance with the facts, he did not consider it to be more than a tentative theory.

The abolition by curara of an immediate tonic contraction produced by nicotine was strictly dependent on the ratio of the concentration of curara and nicotine. Langley found, however, that this was not true in the case of the fibrillar twitchings produced by nicotine, for if sufficient curara was given to abolish these, then the antagonism could not be overcome without a great increase in the concentration of nicotine.

Langley and Kato (1915) found that the antagonism of physostigmine by curara as regards twitchings and nerve irritability resembled the antagonism of nicotine by curara in the case of muscular twitchings and not the type observed with the tonic contractions.

Cushny/

Cushny (1914) in his quantitative observations on antagonism, noted that in the case of neutralisation of toxins by antitoxins, the law of multiple proportions held good, but did not do so in the case of alkaloidal antagonism (e.g. atropine and pilocarpine). These appeared to be governed by mass action, i.e. the degree of antagonism exercised by atropine and pilocarpine depended on the ratio in which they were presented to the organ on which they acted (the relative concentration of the drug being the determining factor and not the amount. He also held that the time factor was very important, e.g. the atropine given ten minutes before pilocarpine had already proceeded to a certain length of concentration before pilocarpine started its action to displace it.

Cushny/

Cushny observed further that if Straub's view of antagonism of muscarine by atropine be extended to that of pilocarpine by atropine, the large dose of atropine reduced the permeability of cell membrane to such an extent that the entry of pilocarpine in massive doses was greatly delayed while a smaller amount of atropine was less efficient against a correspondingly reduced dose of pilocarpine.

Gaddum (1926) determined the antagonism of adrenaline by ergotamine on rabbit's uterus and found that after exerting its action on the uterine strip ergotamine did not disappear in any measurable quantity from the bath and that the amount of the drug (ergotamine) passing into the muscle depended on (i) its concentration in the bath, and (ii) the time the muscle was in contact with it, and (iii) on the temperature of the bath.

About (ii) or time factor, he observed that the degree of paralysis of the uterine strip to the adrenaline after ergotamine increased with time, which he plotted in curves. He noted that in some experiments with smaller concentrations of ergotamine there were indications of paralysis having reached a maximal value in 3-20 minutes, but he found it impossible to get precise information as to the shape of the curves.

He/

He also noted that whenever ergotamine was left in bath for a long time the strip developed additional tone which obscured all further curves. His curves showing the ratio of the action of ergotamine to its concentrations, however, corresponded with those of Sollmann, Mendenhall and Stingel (1915). Gaddum also confirmed Cushny's findings that the combination of adrenaline and ergotamine showing their mutual antagonism was not of the nature of chemical neutralization. For this purpose he incubated overnight, a mixture of both these drugs and then, on trying this mixture on the uterine strips,found it to act as effectively as if each of the drugs was applied separately, i.e. the action of adrenaline began to wane as that of ergotamine developed, in paralysing the muscle to the action of the former.

Gasser and Dale (1926) in trying the antagonism of acetyl choline and nicotine by atropine and curara on denervated mammalian muscle mentioned that large amounts of atropine were stated by Reisser (1921-22) to cause a slow relaxation of the contraction produced by acetyl choline or by nicotine in normal frog muscle and that on the other hand Frank, Nathmann and Hirsch-Kaufmann (1922-23) could not detect any antagonism of atropine to the action of these drugs on/

on denervated mammalian muscle. Both the findings of these authors in normal frog muscle as well as in denervated mammalian muscle were confirmed by Gasser and Dale. Hence they concluded that the augmentor action of acetyl choline and other bases of nicoline choline group on denervated mammalian muscle was not definitely affected by curara in doses sufficient to abolish the indirect excitability of normal muscle, or by atropine in concentrations sufficient to abolish the parasympathetic effect of acetyl choline. However they found that adrenaline completely abolished the action of these bases producing maximal contraction and that ergotamine only partially antagonised this action of adrenaline affecting the action of these bases.

Clark (1926) tried the antagonism of acetyl choline by atropine in the frog's heart and rectus abdominis. He came to the conclusion that the antagonism between the two drugs depended on the atropine being fixed in some manner by the tissues as he noted that atropine continued to exert its antagonistic action on that of acetyl choline a long time after thorough and repeated washing out of the former around the heart muscle. He held also that this/

this excluded the possibility of antagonism to be due to any reaction between the drugs outside the heart cells as, he observed, two drugs did not appear to react when one of them, i.e. atropine, was fixed on heart cells because the application of concentrated solutions of acetyl choline did not hasten the rate at which the atropine was washed out.

Referring to Straub's views on atropine-muscarine antagonism, Clark pointed out that whilst Straub believed in atropine rendering tissues impermeable to drugs like acetyl choline and held that in the case of aplysia heart muscarine showed its action only on entering or leaving the tissues, he(Straub)admitted that it did not happen so with the frog's heart. According to Clark both atropine and acetyl choline therefore appear to act on different receptors on the heart cells and the antagonism between them appears to be an antagonism of "effects" rather than of combinations.

Cook (1926) dealt with the antagonism of acetyl choline by methylene blue in the frog's heart. He found that heart cells absorbed methylene blue slowly and this action of the drug was practically irreversible, but on the contrary the antagonism of the two drugs was quite reversible. He noted that/

that the action of methylene blue in antagonising acetyl choline was produced almost as rapidly as it was removed on washing out the former. Cook therefore concluded that the entrance of the dye into the nerve and muscle cells did not materially affect the antagonism and that the dye produced its antagonism to the action of acetyl choline by a freely reversible action on the surface of the cells.

The general conception of drug antagonism adopted by the author is that the antagonistic drugs combine with receptors on the cell surfaces. These combinations are in some cases freely reversible (e.g. acetyl choline and adrenaline) and in other cases very slowly reversible (e.g. atropine and ergotamine). The presence of one drug interferes with the action of the other, although it is uncertain whether they both combine with the same receptor. The amount of antagonism produced depends on the ratio of the concentrations of the two drugs.

(3) /

(3) Antagonism of Sympathomimetic Drugs.

The antagonism of adrenaline by ergotamine -

Quantitative determinations of the antagonism of adrenaline by ergotamine on the rabbit's uterus were made by Gaddum (1926) and by Mendez (1928). The evidence was conflicting concerning the antagonism by ergotamine of the inhibitor action of adrenaline on the gut. The author therefore studied this question and has published a paper (1931) on the subject (Appendix III).

He found that ergotamine antagonised both inhibitor and motor actions of adrenaline but that the intensity of the antagonism varied very greatly, the augmentor action of adrenaline on the rabbit's uterus was abolished by far lower concentrations of ergotamine than were required to abolish the inhibitor action of adrenaline on the ileum, and the action of adrenaline on the colon was still less affected.

The antagonism of adrenaline by ergotamine is therefore a graded effect which is produced more readily/

readily in some tissues than in others, and in any single tissue may affect one action more than another. Incidentally these results show that it is necessary to modify the usually accepted statement regarding the action of ergotamine, namely that it abolishes the motor action but does not interfere with the inhibitor actions produced by adrenaline. The truth appears to be that ergotamine antagonises practically all the actions produced by adrenaline but that the intensity of antagonism varies very greatly and in most, if not all, cases motor actions are affected far more powerfully than are inhibitor actions.

The mode of action of ephedrine and its antagonism by ergotamine and other drugs.

The clinical value of ephedrine depends chiefly on its power to relax the bronchial muscles and hence it might be expected to produce a powerful inhibitor action on the isolated gut or uterus. The evidence regarding this latter action is, however, somewhat confused.

Amatsu and Kubata (1913 and 1917) found that ephedrine depressed the isolated gut of cat and rabbit. To (1921) and Chen and Schmidt (1924) found/

found that ephedrine depressed the isolated rabbit's gut, the latter also observed that its depressant action could be readily overcome by barium or by pilocarpine but not by nicotine, and they concluded that the drug was sympathomimetic and was not musculotropic, nor parasympathomimetic, nor did it act on the ganglia.

Fujii (1925) also noted the depressant action of the drug on the isolated gut. Kreitmair (1927) found that the surviving gut was relaxed by concentrations between 1 in 1,000,000 and 1 in 100,000, but that it was stimulated by concentrations above 1 in 6,000. Renitz (1928) concluded that the surviving rabbit's gut was sometimes inhibited, often stimulated and sometimes first inhibited and then stimulated, by the same concentrations of ephedrine. Nagel (1925) found the drug to be purely stimulant to the surviving rabbit's and cat's gut. Lim and Chen (1925) noted stimulation of cat's intestine, isolated but with intact circulation caused by ephedrine. De Eds, Rosenthal and Voegtlin (1929) reported pure stimulation of rabbit's gut with 1 in 5,000 concentration of ephedrine. Halsey (1928-29) always noted stimulation of isolated rabbit's gut, caused by ephedrine. Mehes and Kokas (1929) found

isolated/

isolated rabbit's gut stimulated by low concentrations between 1 in 150,000 to 1 in 50,000 and relaxed by high concentrations 1 in 10,000 and over. Rudolf and Graham (1927) noted ephedrine causing only slight and transitory inhibition of the surviving rabbit's gut. In the case of the large intestine Kreitmair found the same results as with the small intestine, i.e. inhibition by low and stimulation by high concentrations.

The evidence regarding the action of ephedrine on the isolated gut is therefore conflicting, but the majority of workers agree that low concentrations inhibit and high concentrations stimulate this tissue.

Fortunately there is a general agreement regarding the action of ephedrine on the isolated rabbit's uterus. Chen and Schmidt (1924) found that ephedrine stimulated the isolated uterus of the rabbit except in the case of uteri from recently delivered animals. The following observers have observed a purely stimulant action of ephedrine on isolated uteri:-

Nagel (1925), guinea pig; Fujii (1925), rabbit; Kreitmair (1927), rabbit; Reinitz (1928), rabbit/

rabbit; De Eds and Butt (1927), De Eds, Rosenthal and Voegtlin (1928), guinea pig and rabbit; Thienes (1929), cat, rabbit, guinea pig, rat and dog. In a few cases inhibitory effects have been observed:- To (1921) found that dilute solutions (1 in 100,000 to 1 in 10,000) inhibited the isolated uteri of the rat and rabbit, whilst strong solutions (1 in 10,000 to 1 in 2,000) caused stimulation. Burn and Tainter (1930) found that ephedrine inhibited the cat's uterus. De Eds and Butt (1927) also noted that rabbits' uteri which were inhibited by adrenaline (an effect which occurs in a small minority of cases) were stimulated by ephedrine.

The evidence regarding the action of ephedrine on the uterus is also conflicting. Curtis (1929) found that ephedrine stimulated cat's and guinea pig's uteri; he also noted that the uteri in different animals showed a much lower sensitiveness to ephedrine than to adrenaline and observed that whereas in many cases the uterus responded by contraction to 1 in 5 million concentration of adrenaline, it remained quite insensitive to 1 in 10,000 and even stronger concentrations of ephedrine, but these high concentrations of the latter drug did not injure the preparation. He found/

found that it was rare for ephedrine to produce a single rapid contraction of the uterus like that produced by adrenaline and that the commonest effect seen was a gradual increase of tone and of rate and amplitude of rhythmic movements.

The effect of ephedrine on the response of uterus to adrenaline appears to be doubtful. Renitz (1928) noted that very small quantities of ephedrine augmented the stimulant effect of adrenaline on isolated rabbit's uterus, but larger doses reduced it. Thienes (1929) found that ephedrine prevented the inhibitory effect of adrenaline seen in the case of various uteri and similarly it antagonised the inhibition produced by adrenaline on the isolated large bowels of cat, rabbit, dog, rat and guinea pig. Roth (1930) found ephedrine and adrenaline when applied alternately acted independently after each other on giving their sub-maximal stimulant doses in the case of dog's ureter, e.g. aquiscent segment receiving 20 mgm. of ephedrine (1 in 5,000) at 10.15 a.m. showing no response after 13 minutes, gave a characteristic adrenaline response when it was treated with 0.5 mgm. of the latter drug (i.e. 1 in 200,000) in the presence of ephedrine given/

given before. Similarly if adrenaline, which was applied first, proved ineffective, a comparatively small dose of ephedrine added caused stimulation, e.g. 0.2 mgm. of adrenaline (1 in 500,000) showed no effect, but on adding ephedrine (1 in 10,000) stimulation occurred. He, however, observed further on that the results obtained with sub-maximal doses could not be had constantly with high concentrations.

The action of atropine on the ephedrine response is doubtful and also the action of ergotamine on the ephedrine response is doubtful. Kreitmair (1927) found that the action of ephedrine on the uterus was not reversed or even abolished by ergotamine in concentrations which antagonised the stimulant action of adrenaline on that organ. Curtis (1929) found that the action of ephedrine (1 in 10,000) on uterus was antagonised by ergotamine (1 in 33,000). Roth (1930) found that ephedrine stimulated the dog's ureter and that this action was not antagonised by ergotamine. He also observed that small doses of ergotamine failed to antagonise even the action of adrenaline on this preparation.

Summary/

Summary of Existing Evidence:-

The evidence regarding the action of ephedrine on isolated plain muscle is conflicting.

The majority of authors describe it as inhibiting the isolated rabbit's gut and stimulating the uterus. The effect of ephedrine on the adrenaline response is doubtful since some authors describe synergism and others antagonism.

Whether ergotamine can antagonise ephedrine is also doubtful.

The author's experiments were designed to determine these doubtful points and to try to discover whether ephedrine and tyramine resembled adrenaline in the manner in which they were antagonised by drugs.

B. EXPERIMENTAL EVIDENCE.

(1) The action of ephedrine on plain muscle.

Pieces of rabbit's gut and uterus were suspended according to the well known method of Magnus. The bath used contained 25 c.c. and the Locke's solution had the following percentage composition: NaCl 0.9, KCl 0.042, $CaCl_2$ (anhydrous) 0.024, $NaHCO_3$,0.05 and glucose 0.05. The drugs used were obtained from Messrs British Drug Houses.

1. The response of the duodenum.

Concentrations of ephedrine sulphate varying from 1 in 1 million to 1 in 2,000 were studied. A concentration of 1 in 100,000 usually produced a definite but transient inhibitory effect. Increase of dosage did not greatly increase the effect produced, but the inhibition produced persisted when concentrations of 1 in 2,000 and over were applied, and with these higher concentrations there was a fall of tonus as well as an inhibition of pendulum movements. These effects are shown in Fig. 1. a. and b.

Fig. 1 /

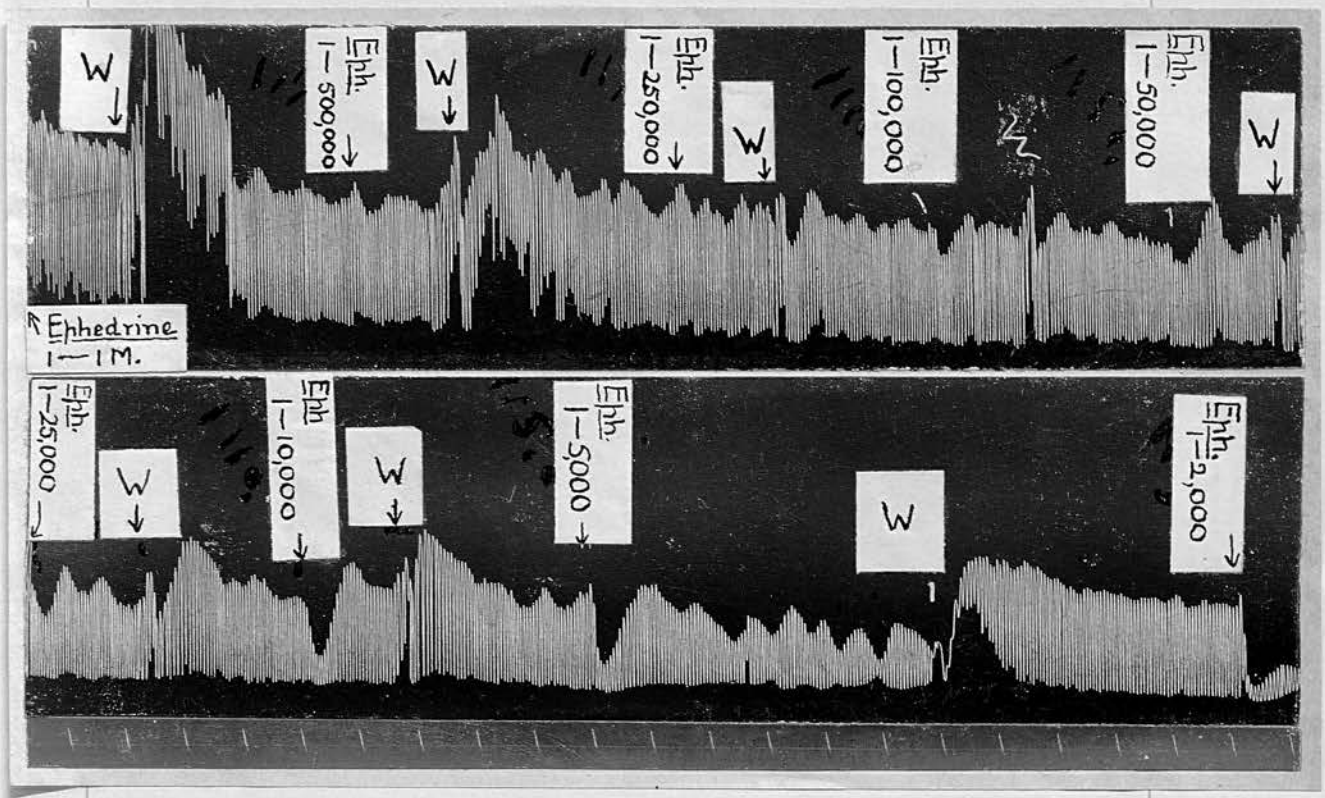


Fig. 1 (a). Preparations from rabbit's duodenum showing the action of ephedrine applied in concentrations 1 in million to 1 in 2,000, (a) before ergotamine.

Fig. 1 (b) /

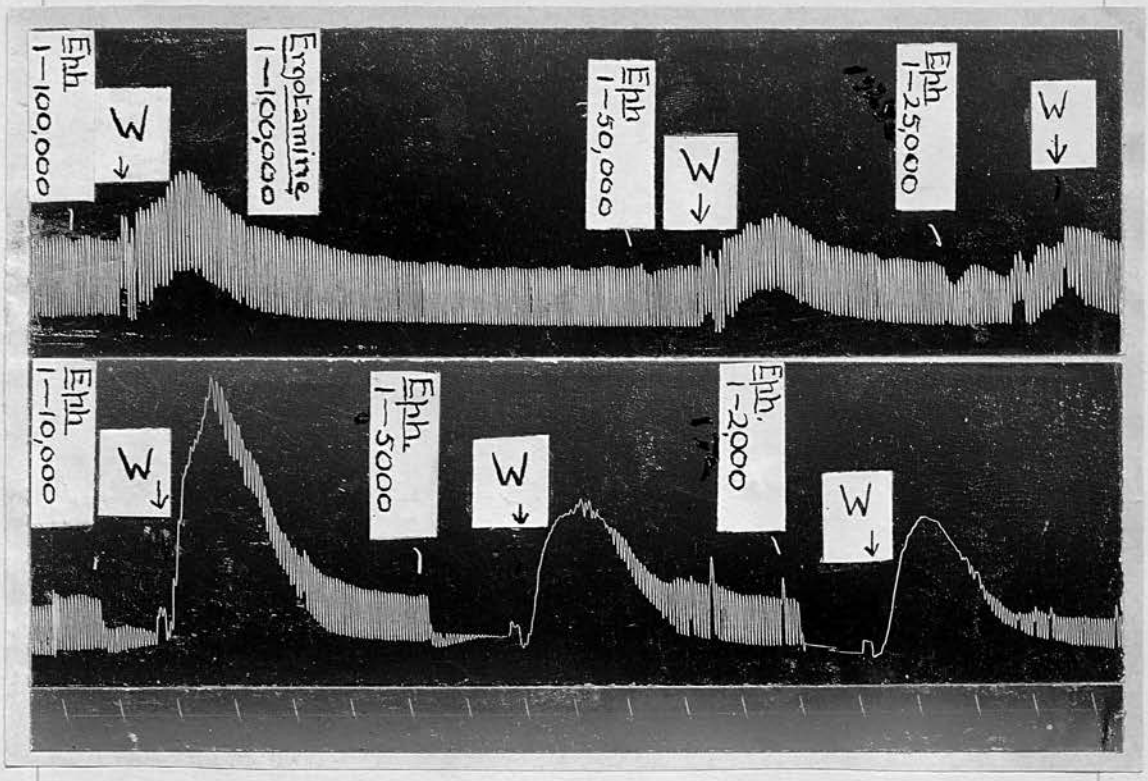


Fig. 1 (b). Preparations from rabbit's duodenum showing the action of ephedrine applied in concentrations 1 in million to 1 in 2,000, (b) after ergotamine.

2. The response of the ileum.

This was in general the same as that of the duodenum except that little effect on the tonus was observed even when concentrations of 1 in 2,000 were applied.

The action of ephedrine on the ileum is shown in Fig. 2.

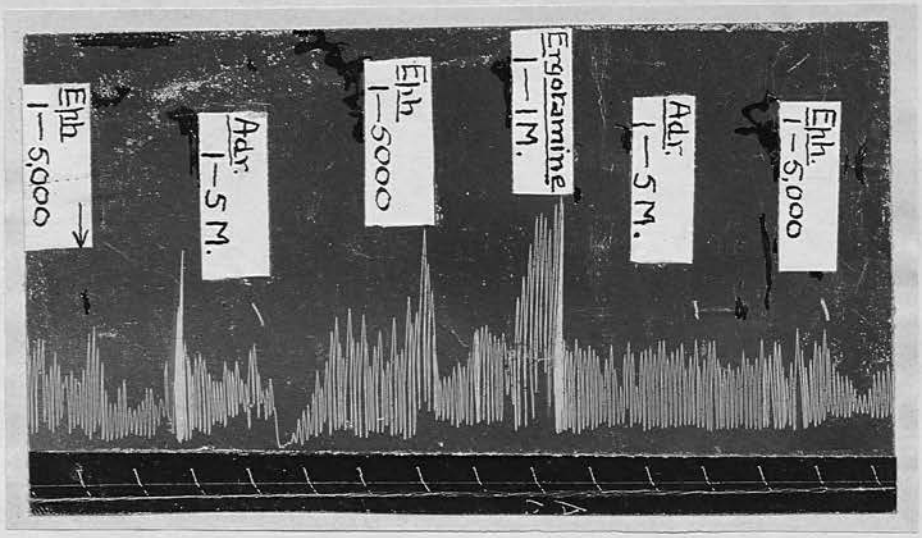


Fig. 2. Preparation from rabbit's ileum showing the action of ephedrine (1 in 5,000) and adrenaline (1 in 5 million) applied before and after ergotamine (1 in 1 million). It will be seen that whereas the inhibitor action of adrenaline on pendulum movements has been paralysed by ergotamine, that of ephedrine remains unaffected.

3. /

3. The response of the colon.

In most cases ephedrine produced a double action on this portion of the gut, for concentrations ranging from 1 in 250,000 to 1 in 10,000 produced a fall of tonus, whilst concentrations greater than 1 in 10,000 produced a rise of tonus. These effects are shown in Figs. 3 and 4.(a) and (b).

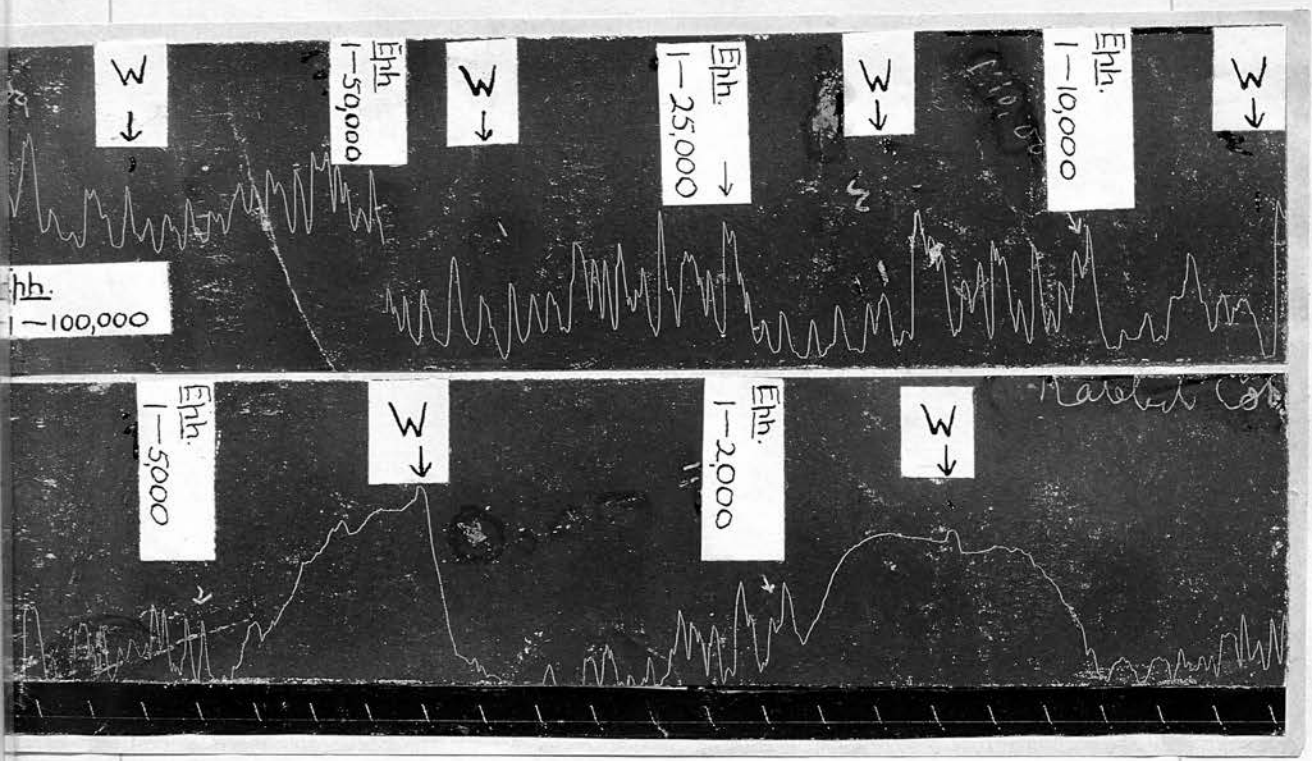


Fig. 3. Preparation from rabbit's colon showing the action of ephedrine. Concentrations of the drug varying from 1 in 100,000 to 1 in 10,000 are seen producing fall of tonus maintained till the wash-out of the drug, whilst the concentrations 1 in 5,000 and 1 in 2,000 have produced augmentor effect by causing rise of tonus also maintained till wash-out.

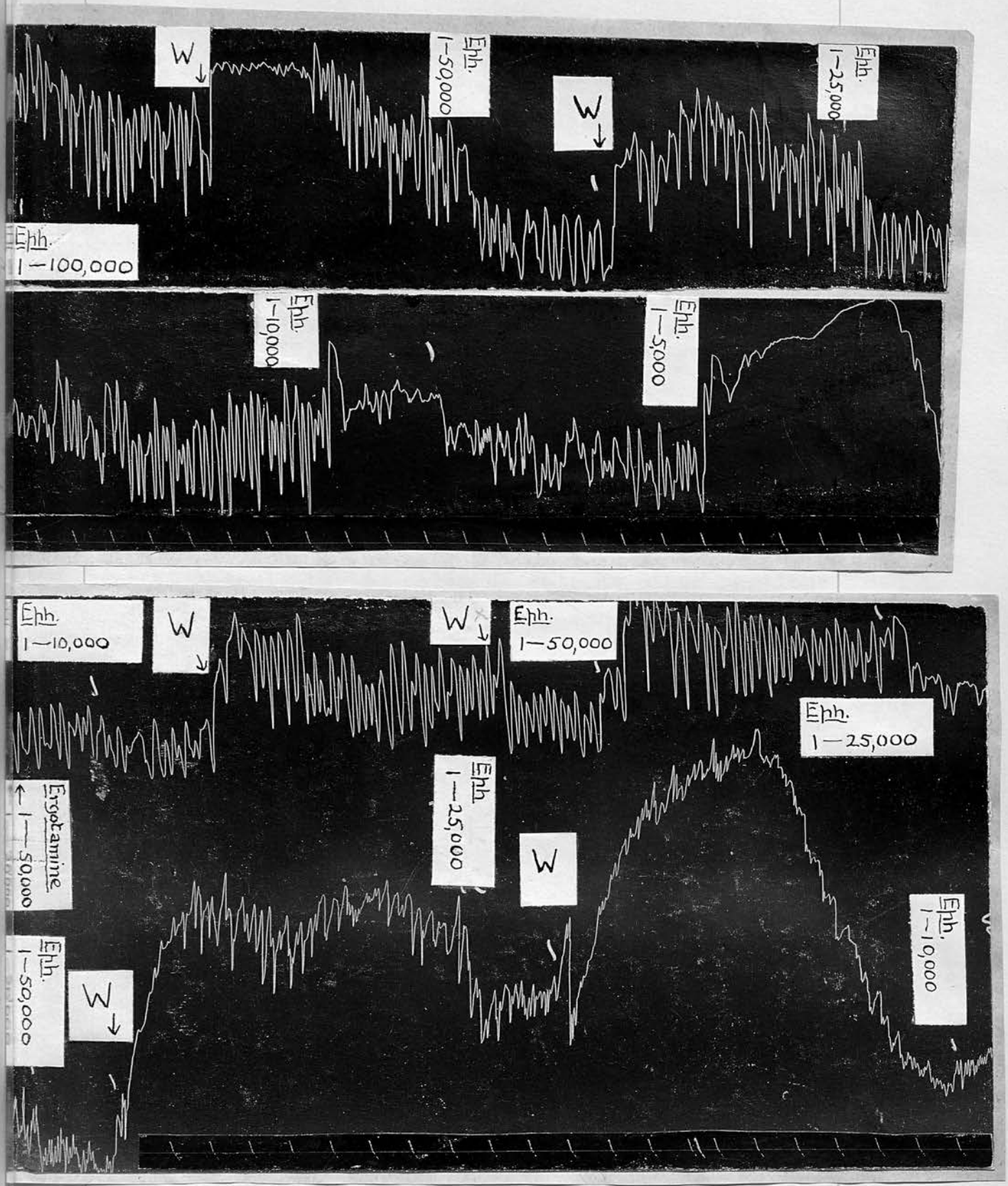


Fig. 4. (a) and (b). Preparations from rabbit's colon showing the action of ephedrine applied in concentrations (1 in 100,000 to 1 in 10,000) before and after ergotamine (1 in 50,000). Augmentor effect of ephedrine 1 in 10,000 seen in (a) appears to have been partially paralysed by ergotamine (1 in 50,000 as seen in (b).

In some cases low concentrations produced no effect, whilst high concentrations produced stimulation, whilst occasionally both high and low concentrations produced stimulation.

These experiments confirm, therefore, the results obtained by the majority of investigators for they show that the chief action of ephedrine is to inhibit the isolated gut, but that it fairly regularly causes a rise of tonus in the colon. This latter effect distinguishes it from adrenaline, for this inhibits the colon as well as other portions of the gut.

4. The response of the uterus.

The lowest concentrations of ephedrine that produced a demonstrable action was about 1 in 50,000 and concentrations of this strength and over always produced a purely stimulant action. This effect is shown in Figs. 5 and 6.

Fig. 5/

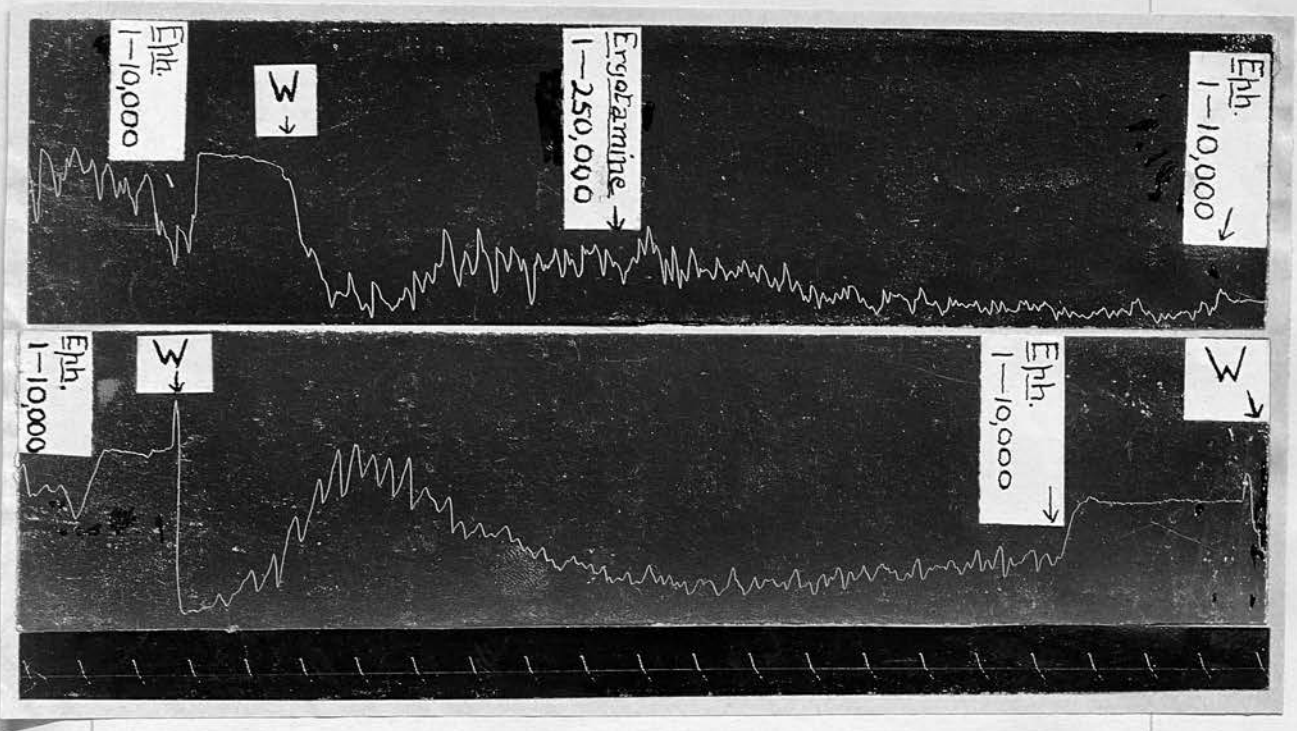


Fig. 5 (a) and (b). Preparations of rabbit's uterus showing the action of ephedrine (1 in 10,000) before and after ergotamine (1 in 250,000), the lower preparation (b) serving as control. In the upper, augmentor action of ephedrine on tonus seen before application of ergotamine has been seen mostly paralysed after ergotamine, which is not shown in the lower.

Fig. 6/

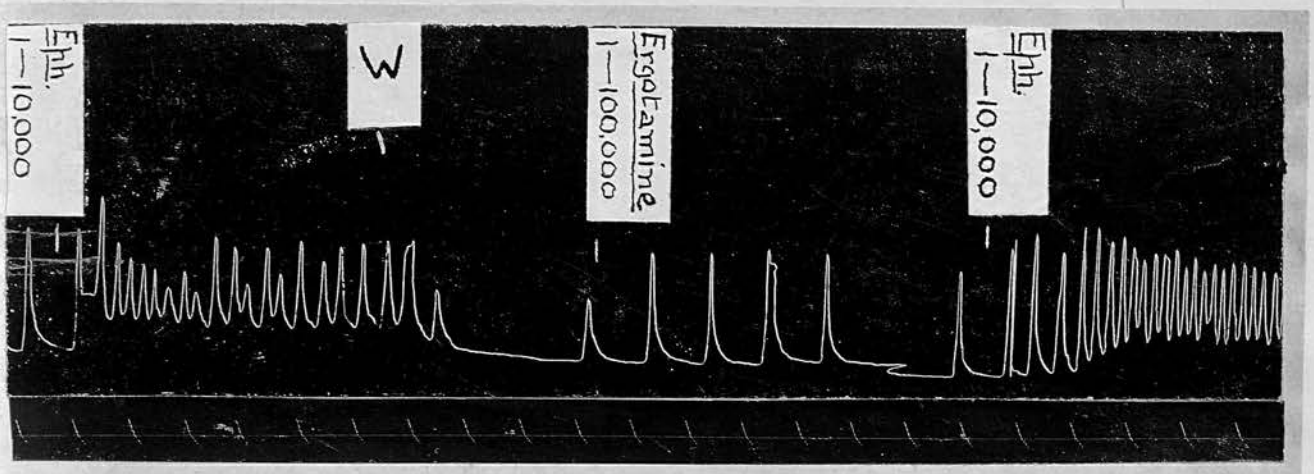


Fig. 6. Preparation of rabbit's uterus showing the effect of ephedrine mostly marked on pendulum movements. This effect of the drug remains unaffected by the application of ergotamine (1 in 100,000).

The author confirmed the conclusion of Curtis that the isolated uterus was much less sensitive to ephedrine than to adrenaline. In most cases 1 in 5 million adrenaline produced as great a stimulant action on the isolated rabbit's uterus as did 1 in 10,000 ephedrine.

The typical effect of ephedrine was to produce both a rise of tonus and an increase in the amplitude of the rhythmic movements. The rise of tonus produced by/

by ephedrine was much slower than that produced by adrenaline.

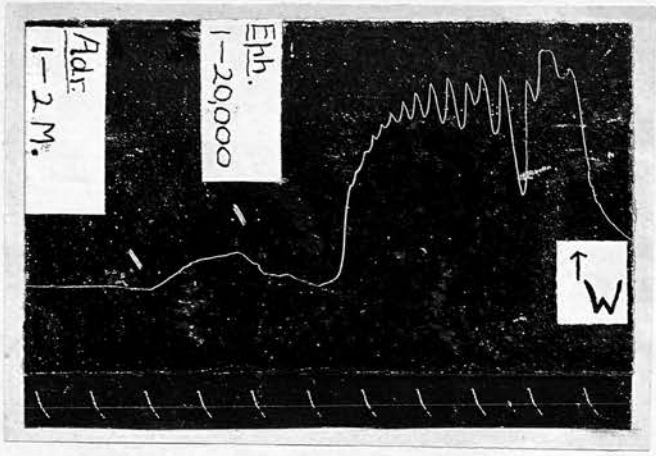
The action of ephedrine on the tissues tested can be summarised as follows:-

Duodenum and Ileum ...	purely inhibitor action
Colon ...	inhibition with low concentrations and rise of tonus with high concentrations.
Uterus...	purely augmentor action.

The influence of ephedrine on the adrenaline response.

Adrenaline and ephedrine both produce an augmentation on the uterus and I found that if the two drugs were added successively their action was additive. This effect is shown in Fig. 7. (a) and (b).

Fig. 7. /



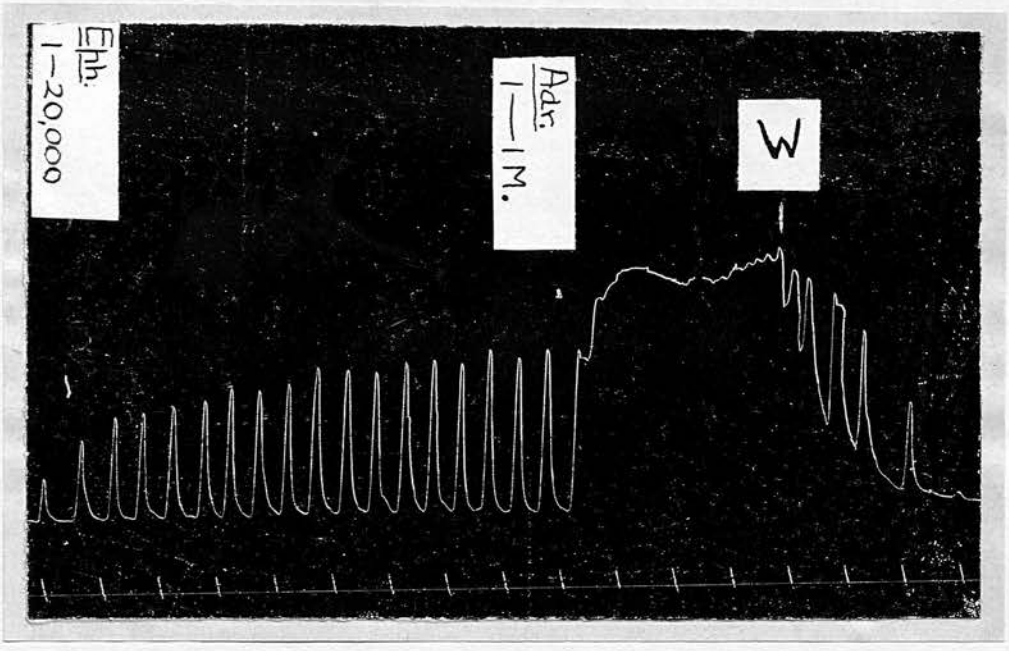


Fig. 7. (a) and (b). Preparations from rabbit's uterus showing the effect of ephedrine on the response of uterus to adrenaline. Both drugs act as augmentors and thus augmentor action of both was additive.

Ephedrine/

Ephedrine causes rise of tonus in the colon whereas adrenaline causes fall of tonus, and on this preparation they acted as antagonists. This effect is shown in Fig. 8. (a) and (b).

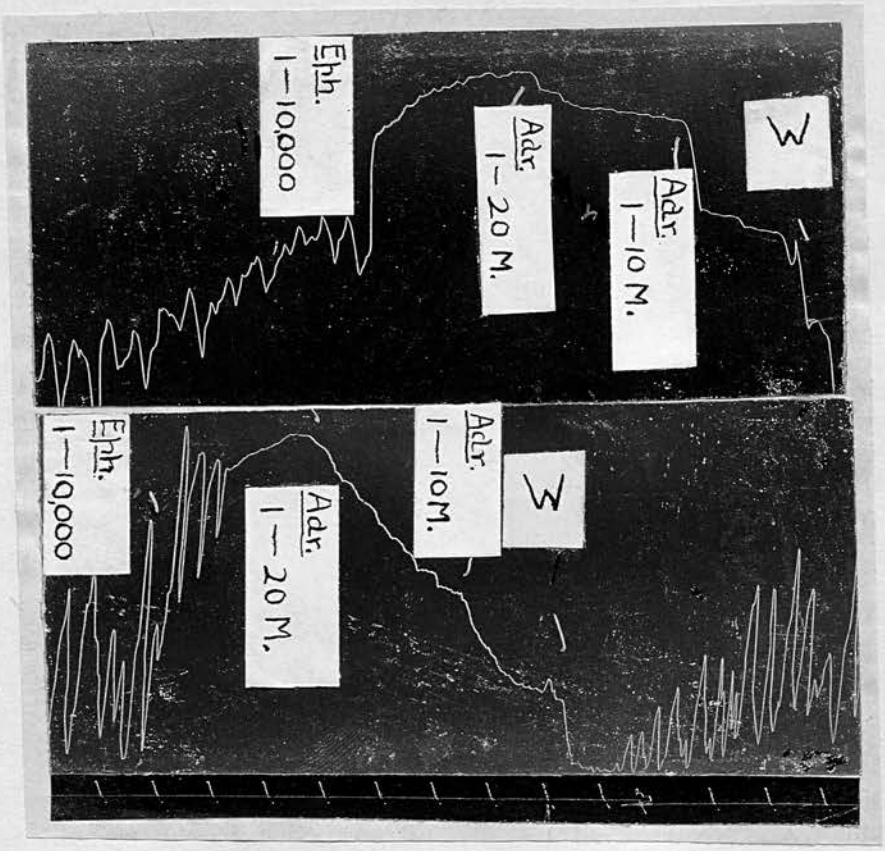


Fig. 8. (a) and (b). Preparations from rabbit's colon showing the effect of adrenaline on the augmentor response of colon to ephedrine. This augmentor action of ephedrine is antagonised by adrenaline. (Ephedrine 1 in 10,000; adrenaline 1 in 20 million and 1 in 10 million).

Adrenaline/

Adrenaline and ephedrine can therefore act either as synergists or antagonists according to the tissue on which they are tested.

I tested also the actions of various other pairs of drugs that produced a rise of tonus in the isolated rabbit's uterus. Pilocarpine, adrenaline, ephedrine and tyramine all produce this effect and in all cases these drugs when given successively produced a summation of effect and no evidence of antagonism was obtained. Fig. 9 shows the action of adrenaline (1 in 50,000), ephedrine (1 in 10,000) and pilocarpine (1 in 10,000) respectively added one after the other.

Fig. 9./

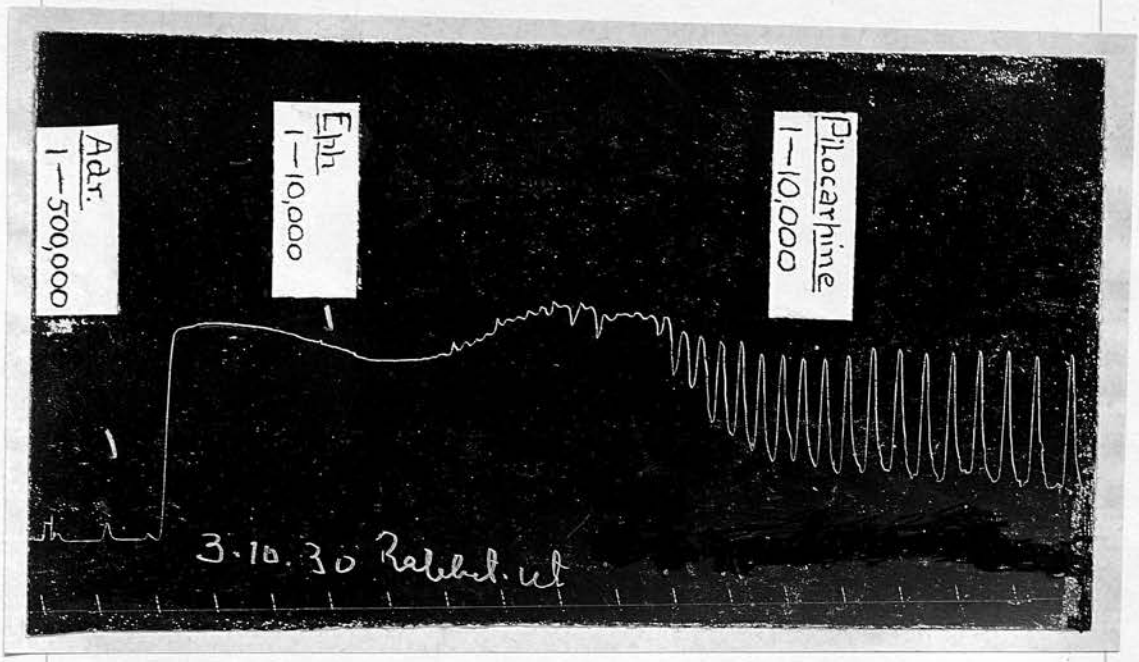


Fig. 9. Preparation from rabbit's uterus showing the additive action of adrenaline (1 in 500,000), ephedrine (1 in 10,000) and pilocarpine (1 in 10,000). First two drugs are seen affecting the tonus whilst the third one, pilocarpine, has produced increase of rhythmic movements.

(2) /

(2) The Antagonism between Ergotamine and Adrenaline.

(a) In the rabbit's uterus:

Broom and Clark (1923) showed that the antagonism between these two drugs as shown in this preparation could be used for estimating the biological activity of ergotamine. Other authors have confirmed this conclusion: Braun (1925); Gaddum (1926); Burn and Ellis (1927); Mendez (1928); Pattee and Nelson (1929).

Mendez (1928) showed that the antagonism between the two drugs could be expressed by the following formula:-

$$\frac{C_{A_2} - C_{A_1}}{C_E} = \text{constant} = 40$$

where C_{A_1} = concentration of adrenaline required to produce a certain rise of tonus in the absence of ergotamine and C_{A_2} = concentration of adrenaline required to produce the same effect in the presence of a concentration of ergotamine, C_E.

I made a series of observations and obtained the following figures.

Table I /

Table I.

The antagonism between ergotamine and adrenaline in the isolated rabbit's uterus.

The figures show the contraction recorded after adrenaline; measured in mm. The figures are averages from several experiments.

Concentration of ergotamine. Parts per 100 million.	Concentration of adrenaline. Parts per 100 million.							
	5	10	20	50	100	200	400	1000
0	30	33	35	38	40	43	45	45
1	-	-	-	34	36	39	41	43
1.25	-	-	-	33	35	38	40	43
2.0	-	-	-	-	34	36	39	41
2.5	-	-	-	-	33	35	38	40
4.0	-	-	-	-	-	34	36	39
5.0	-	-	-	-	-	33	35	38
8.0	-	-	-	-	-	-	34	36
10.0	-	-	-	-	-	-	33	35

These figures are plotted in Figs. 10 and 11.

Figs./

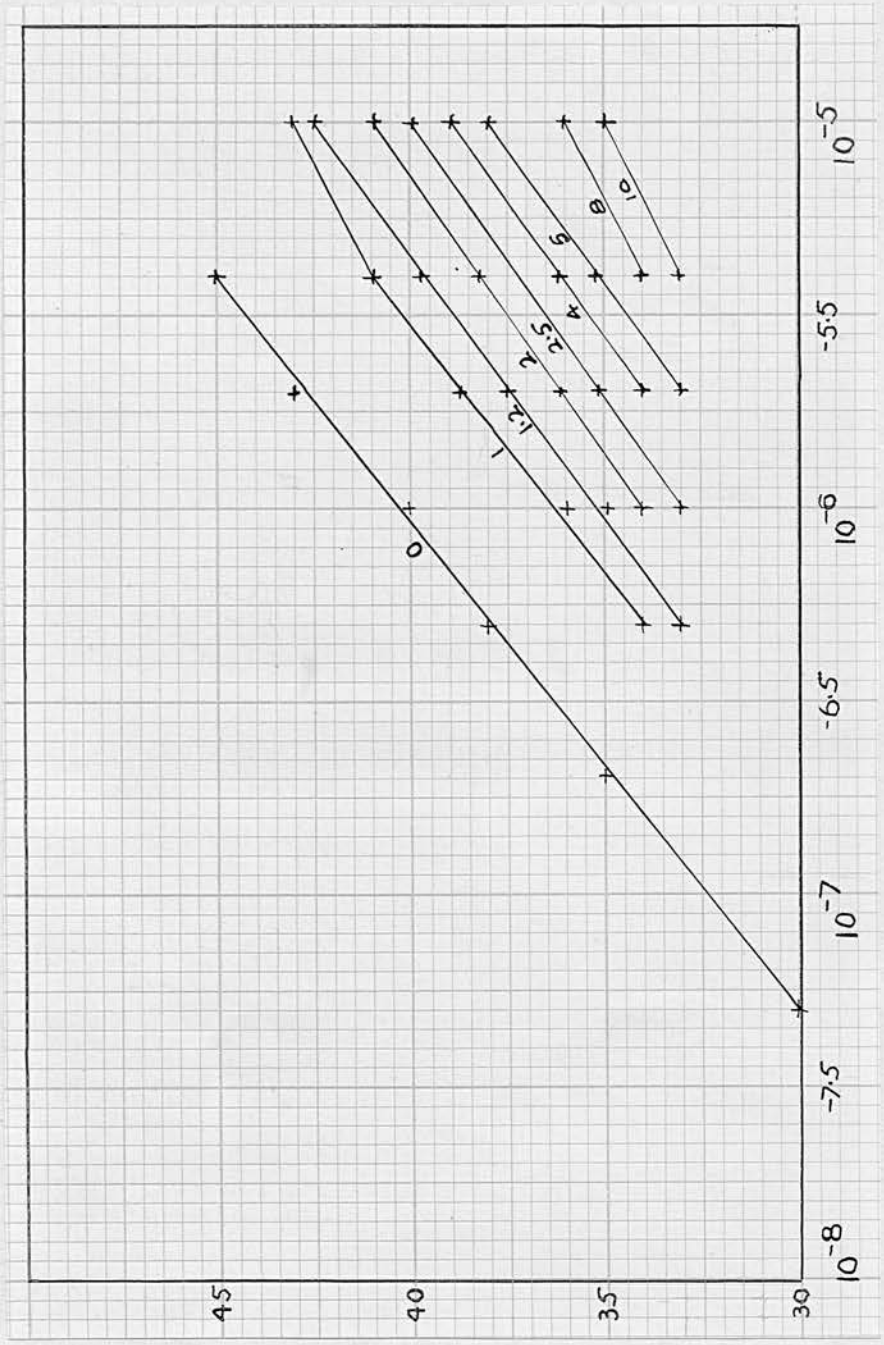


Fig. 10. Antagonism of adrenaline by ergotamine in the isolated rabbit's uterus.
Ordinate: Height of contraction ; Abscissa: Log. concentration of adrenaline. Curves numbered with conc. of ergotamine in parts per 100 million. Curve 0 = nil.

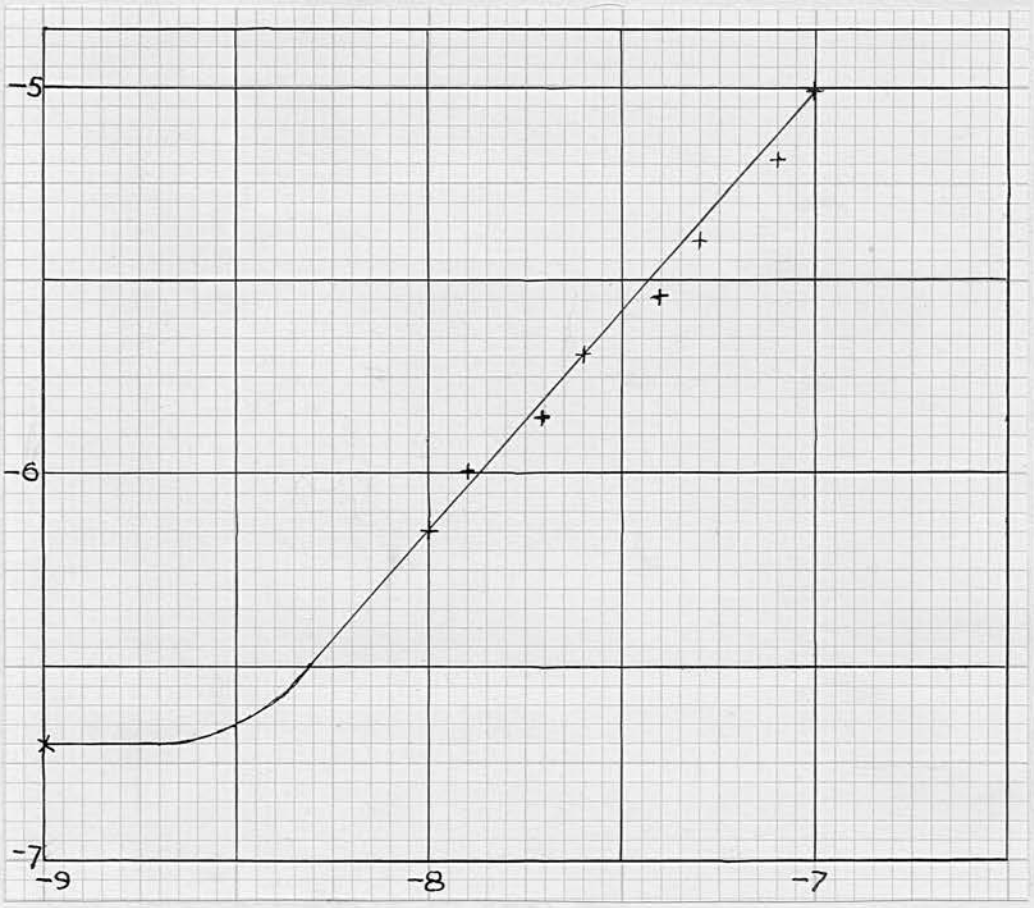


Fig. 11. Antagonism of adrenaline by ergotamine in the isolated rabbit's uterus.
Ordinate: Log.conc. adrenaline; abscissa: Log. conc. ergotamine. The curve shows the concentrations at which an equal response occurred.

Table I and Fig. 10 show that the following concentrations of adrenaline and ergotamine result in an equal response, namely a rise of 35 mm.

Concentrations of ergotamine per 100 million	0	1	1.25	2	2.5	4	5	8	10
Concentrations of adrenaline per 100 million	20	71	100	140	200	280	400	640	1000
$\frac{C_{A_2} - C_{A_1}}{C_E} =$	-	51	64	60	73	65	76	77	98

These figures plotted on logarithmic scale give the linear relation shown in Fig. 11.

Application of the formula gives a fairly constant figure except in the case of the two extreme results. My results give a value for the constant about 70, which is nearly twice the value obtained by Mendez.

(b) /

(b) The antagonism between ergotamine and adrenaline in the isolated rabbit's gut.

My work on this subject has been published in a separate paper (Appendix III). I found that ergotamine antagonised the action of adrenaline on the gut, but that the degree of antagonism depended on the portion of the gut selected.

The chief action of adrenaline on the isolated ileum is to diminish or abolish the pendulum movements and this effect was very clearly antagonised by ergotamine, although this action was considerably less powerful than the antagonism observed on the uterus.

Adrenaline when it acts on the duodenum, produces a fall in tonus as well as a diminution in pendulum movements and I found that the former effect was antagonised much more feebly by ergotamine than was the latter.

In the case of colon adrenaline produces a well marked fall in tonus and ergotamine had an extremely feeble antagonistic action on this effect.

(3) /

(3) The Antagonism Between Ergotamine and Ephedrine.

I found that ergotamine produced no certain effect on the response of the duodenum, ileum or colon to ephedrine. These effects are shown in Fig. 1 (a) and 1 (b), Fig. 2 and Fig. 4 (a) and (b) respectively. The antagonism of adrenaline by ergotamine is shown well in the ileum and quite clearly in the duodenum, and therefore there is a clear difference between the effect produced by ergotamine on the actions of adrenaline and ephedrine on the small intestine.

Ergotamine sometimes has no effect on the action of ephedrine on uterus (Fig. 6) and sometimes exerts a weak antagonistic action (Fig. 5). The antagonistic effect shown in Fig. 5 is far weaker than the antagonism between ergotamine and adrenaline in the uterus. This difference is shown in Fig. 12 (a) and (b).

Fig. /

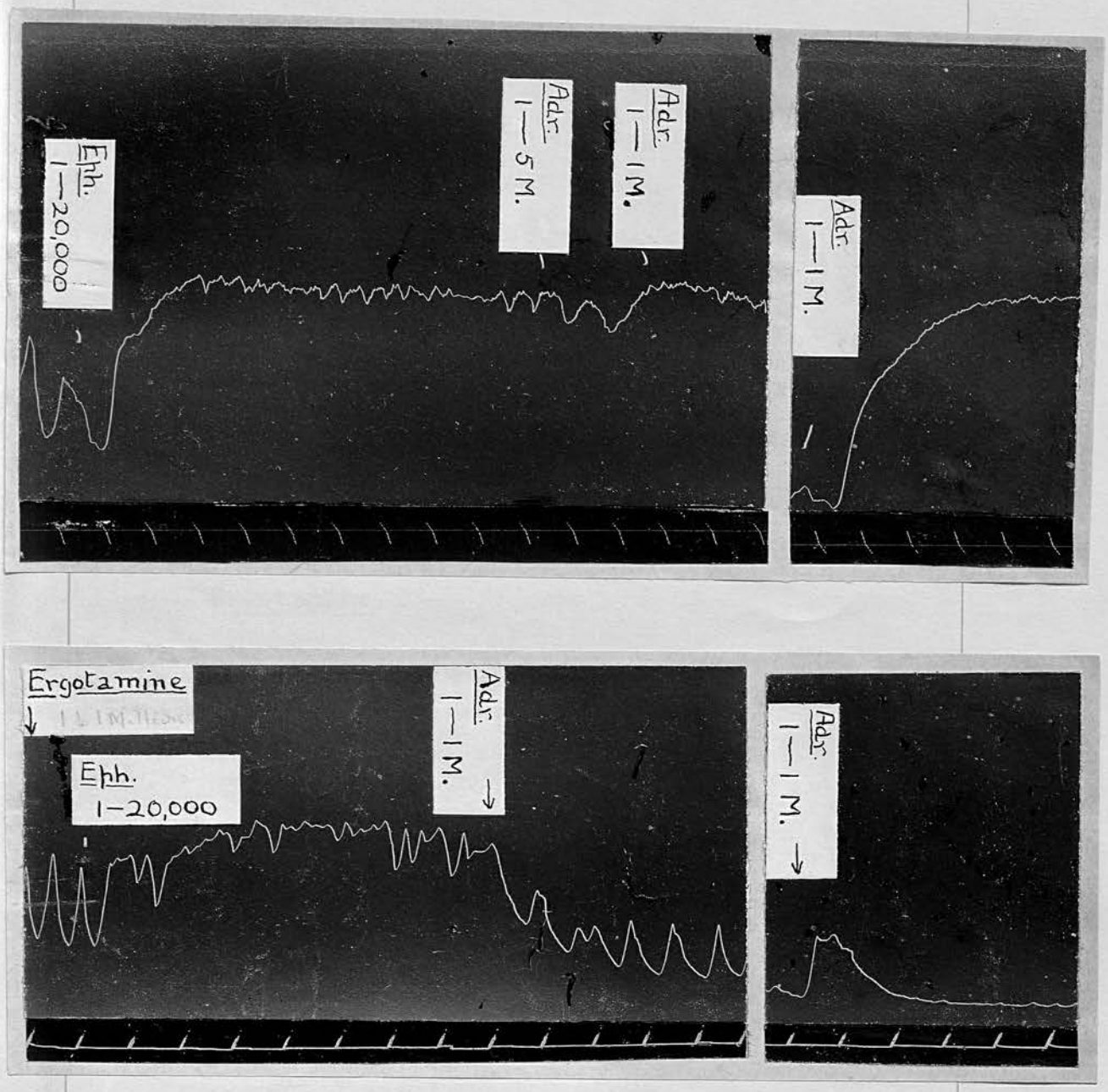


Fig. 12 (a) and (b). Preparations from rabbit's uterus showing the effect of ergotamine on the response of uterus to ephedrine (1 in 20,000) and adrenaline (1 in million). It will be seen that whereas the augmentor action of adrenaline on tonus has been reversed into inhibition by ergotamine 1 in million, ephedrine action on tonus is unaffected.

My results indicate therefore that ergotamine exerts a certain feeble antagonistic action to ephedrine. This can sometimes be demonstrated in the most favourable preparation, namely the rabbit's uterus, but cannot be demonstrated at all in the rabbit's gut, which is a less favourable preparation on which to show this antagonism.

(4) The Action of Tyramine and its Angatonism by Ergotamine.

The action of tyramine on plain muscle is only partly sympathomimetic. De Eds (1927) stated that the drug was purely musculotropic. Burn and Tainter (1930) concluded that tyramine in its action was intermediate between adrenaline and histamine, although nearer the former than the latter.

I found that tyramine in all concentrations (1 in 100,000 to 1 in 2,000) caused a rise in tonus of the isolated rabbit's gut (duodenum, ileum and colon) as seen in Figs. 13 (a) and (b), 14 (a) and (b), 15 (a) and (b), and 16.

Figs./

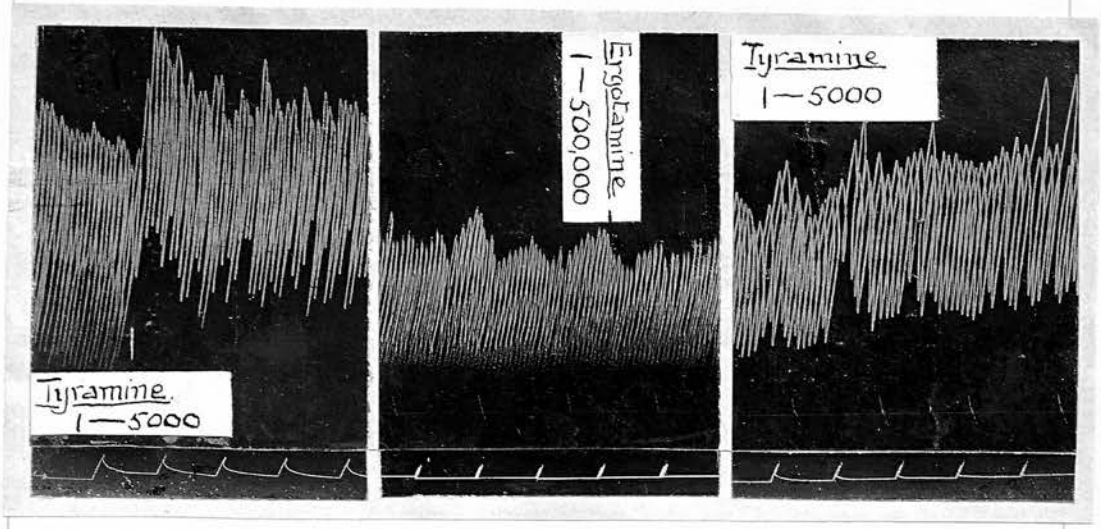


Fig. 13 (a). Preparation from duodenum showing the effect of tyramine (1 in 5,000) on tonus before and after ergotamine (1 in 500,000).

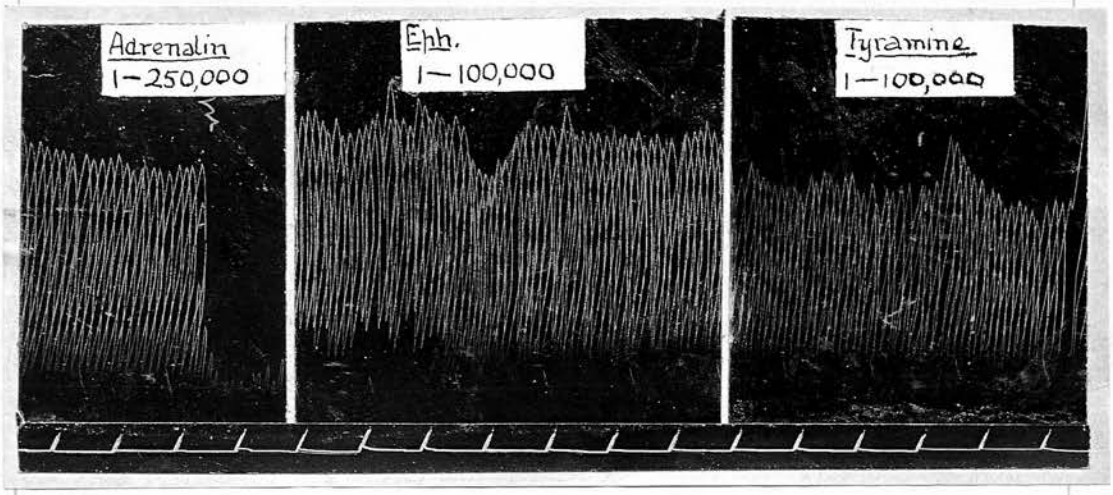


Fig. 13 (b). Preparation from duodenum showing the effect of adrenaline (1 in 250,000), ephedrine (1 in 100,000) and tyramine (1 in 100,000) respectively. Both adrenaline and ephedrine are seen inhibiting the pendulum movements, whilst tyramine has produced temporary rise of tonus (a twitch).

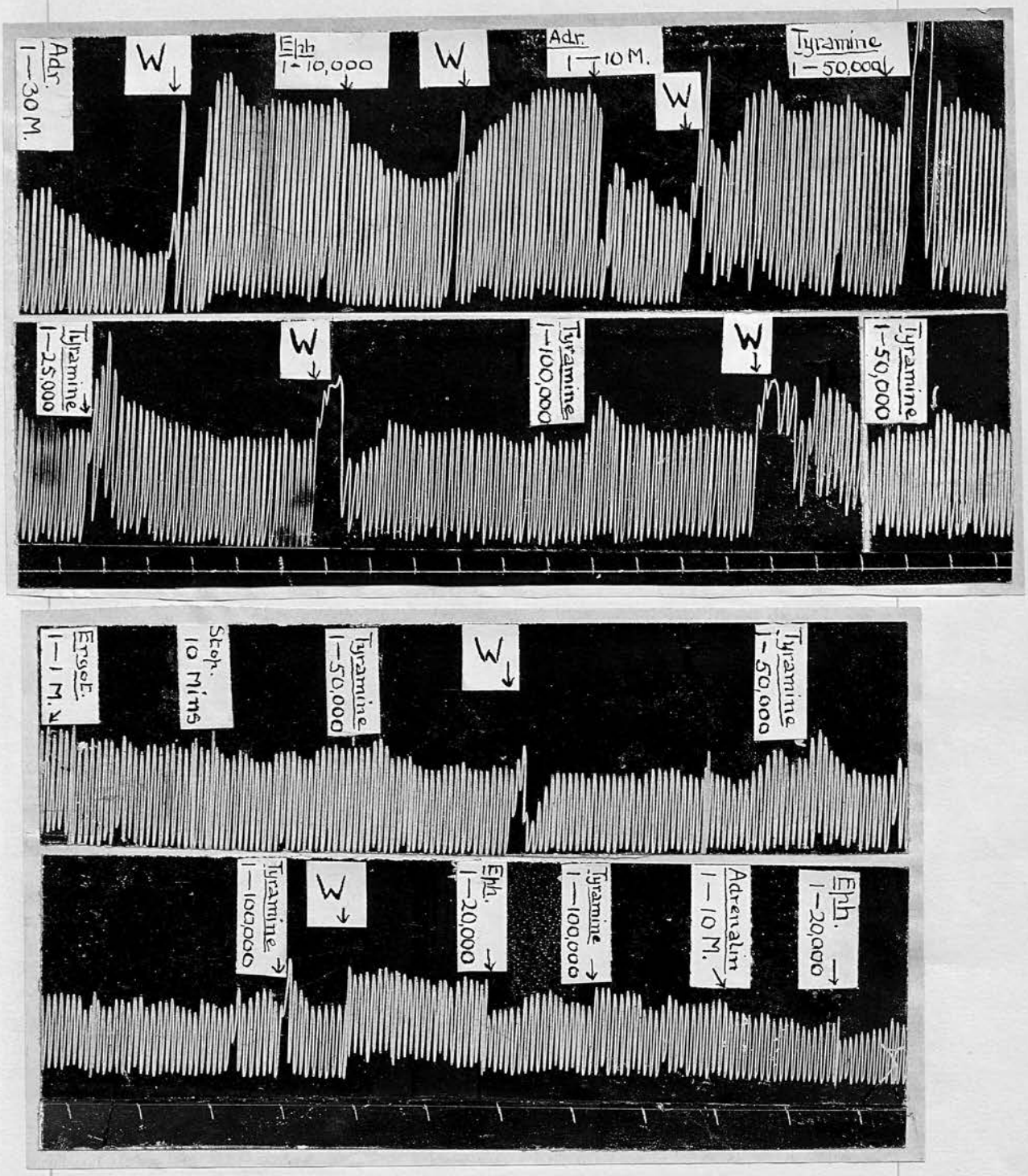
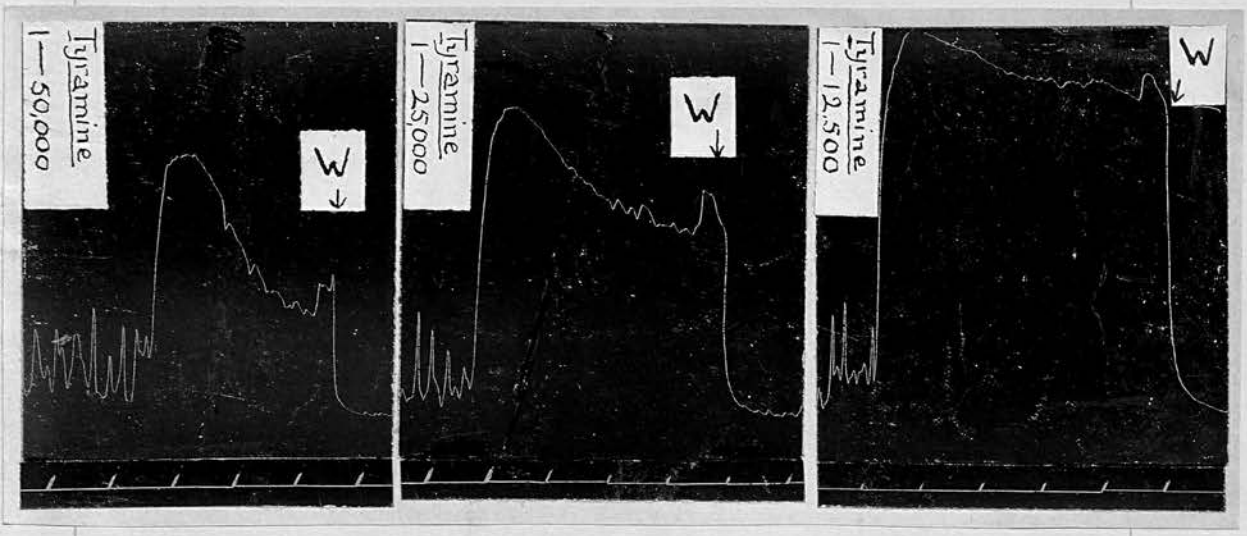


Fig. 14 (a) and (b). Preparation from ileum showing the effect of adrenaline (1 in 30 million and 1 in 10 million), ephedrine (1 in 10,000 and 1 in 20,000) and tyramine (1 in 100,000 and 1 in 50,000) before and after ergotamine (1 in 1 million). Whilst the inhibitory action of adrenaline has been wiped out after ergotamine, both ephedrine and tyramine actions are left unaffected.



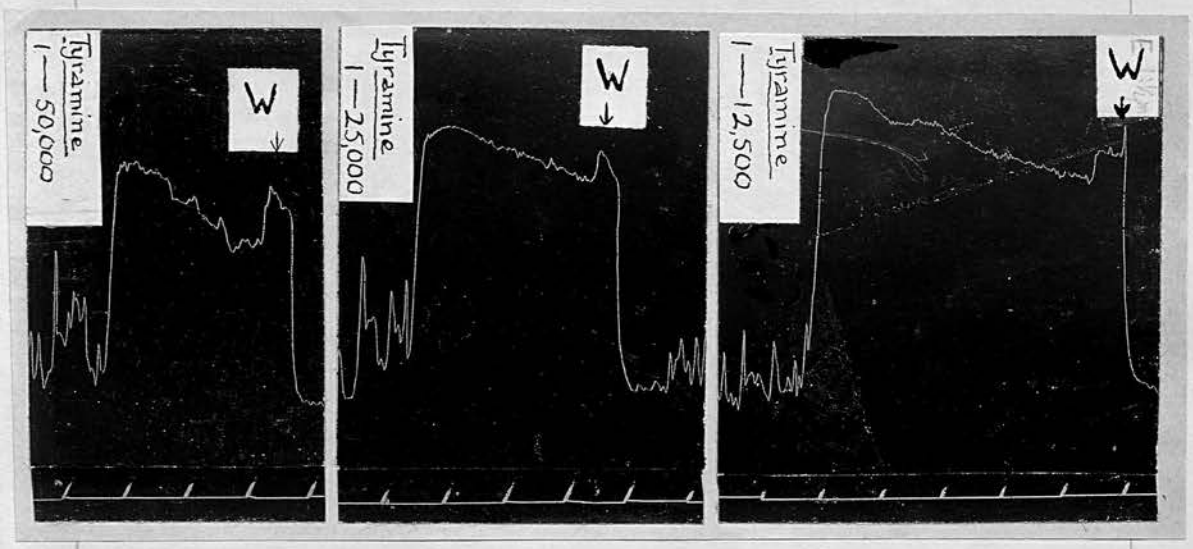


Fig. 15 (a) and (b). Preparation from rabbit's colon showing the action of tyramine (1 in 5,000) most marked on tonus and not affected by ergotamine (1 in 50,000).

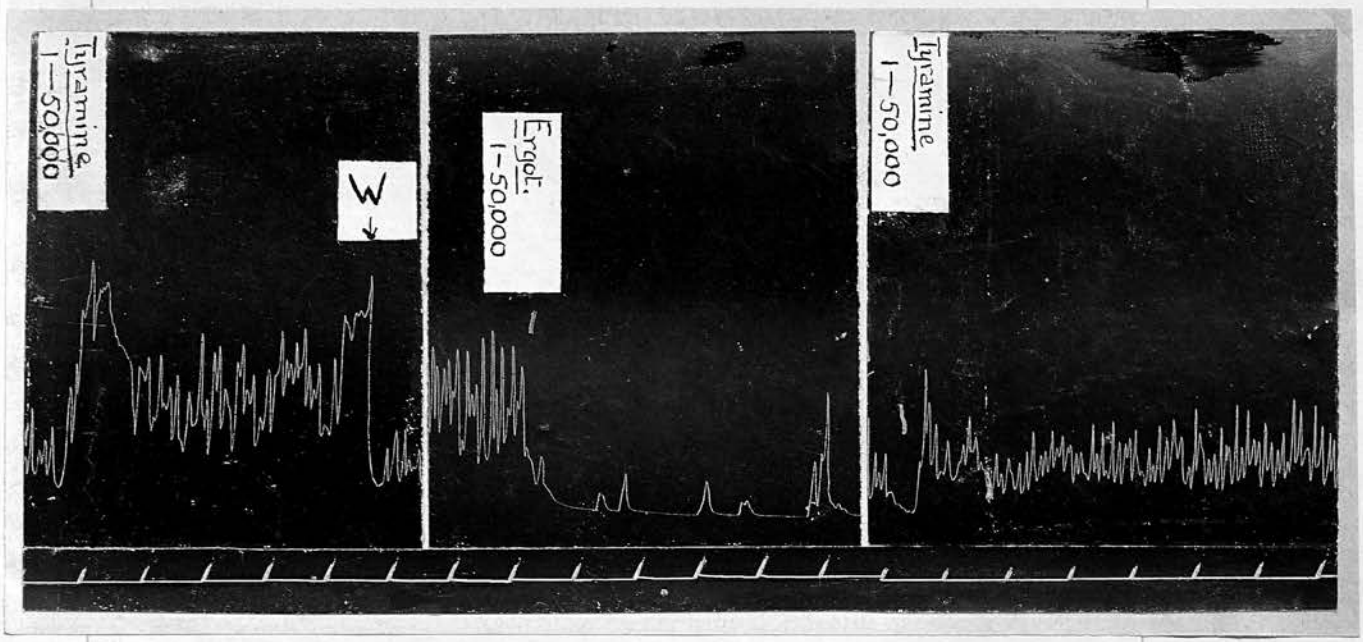


Fig. 16. Preparation from rabbit's colon showing the action of tyramine (1 in 5,000) most marked on pendulum movements and not paralysed by ergotamine (1 in 50,000).

Low concentrations produced a transient rise of tonus and larger concentrations produced a permanent action. These effects are shown in Fig.

In the uterus tyramine also produced a rise of tonus and an increase in rhythmic movements as seen in Figs. 17 and 18.

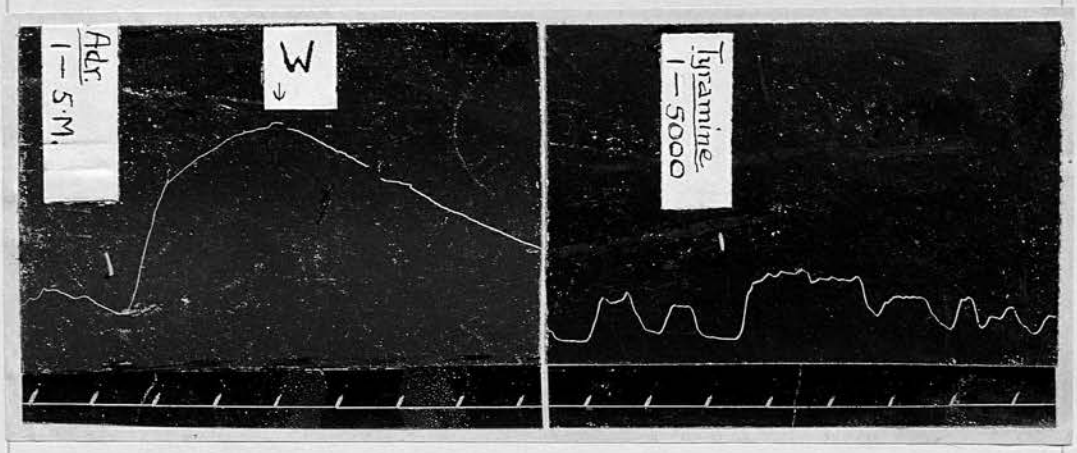


Fig. 17. Preparation from rabbit's uterus showing the augmentor action of adrenaline (1 in 5 million) and tyramine (1 in 5,000) most marked on tonus.

Fig. /

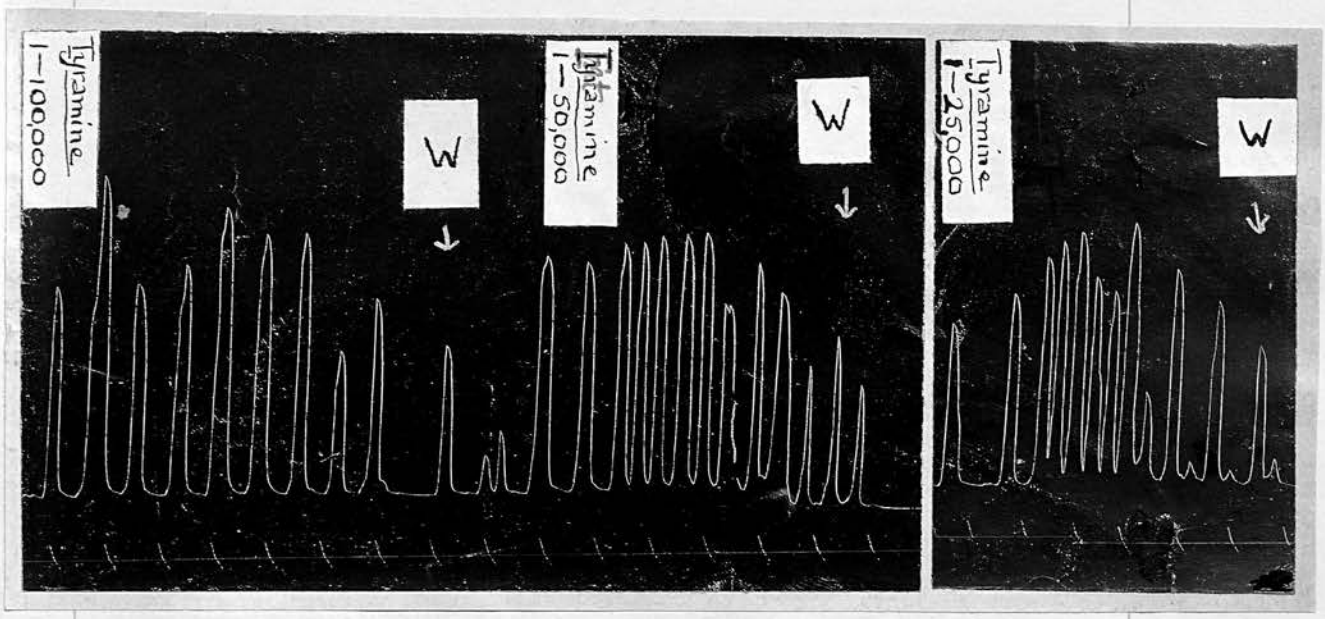


Fig. 18. Preparation from rabbit's uterus showing the effect of tyramine (1 in 100,000 to 1 in 25,000) most marked on rhythmic movements and not paralysed by ergotamine (1 in 50,000).

The influence of ergotamine on the response of the gut and the uterus to tyramine was studied by the same methods as those employed to study the ergotamine-adrenaline antagonism. I was unable, however, to detect any antagonistic action in this case.

(5) /

(5) Action of Atropine on Response to Sympathomimetic Drugs.

(a) Atropine and Adrenaline:

Atropine has been shown to inhibit many actions produced by adrenaline. Hildebrandt (1920) showed that atropine inhibited the vaso-constriction produced both by adrenaline and by stimulation of the sympathetic in the frog. Luckhardt and Carlson (1921) showed that atropine abolished the constriction produced by adrenaline in the pulmonary arteries of the frog and the turtle. Sugimoto (1913) showed that atropine abolished the augmentor action produced by adrenaline on the isolated rabbit's uterus and Ogata (1921) confirmed this. Cushny, however, found that atropine did not inhibit the action of adrenaline on the uterus of the pregnant rabbit in situ.

The writer measured the antagonism of adrenaline by atropine,using the same method as that employed to determine the antagonism of adrenaline by ergotamine, as seen in Figs. 19 and 20.

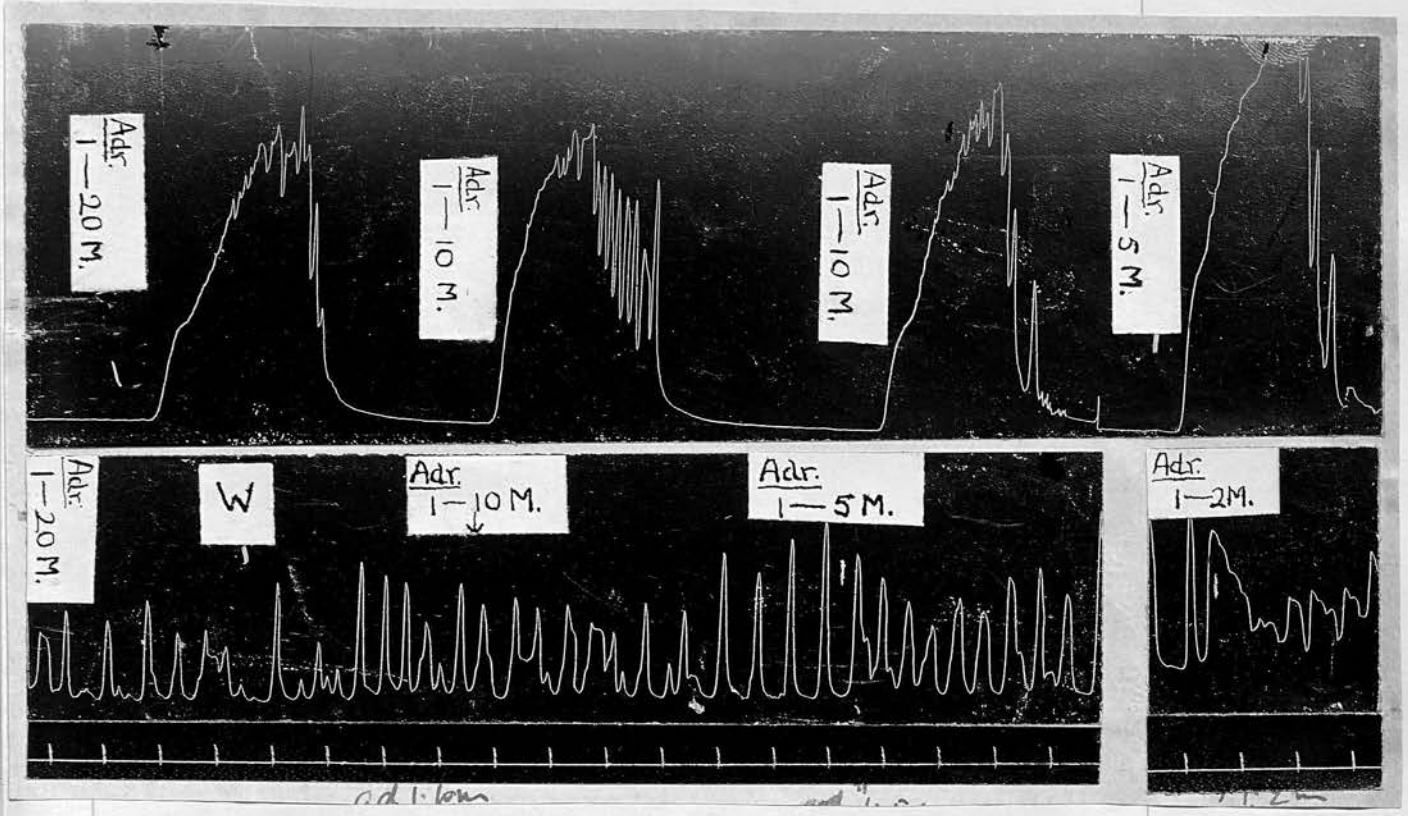


Fig. 19 (a) and (b). Preparation of rabbit's uterus showing antagonism of adrenaline by atropine.

Fig. /

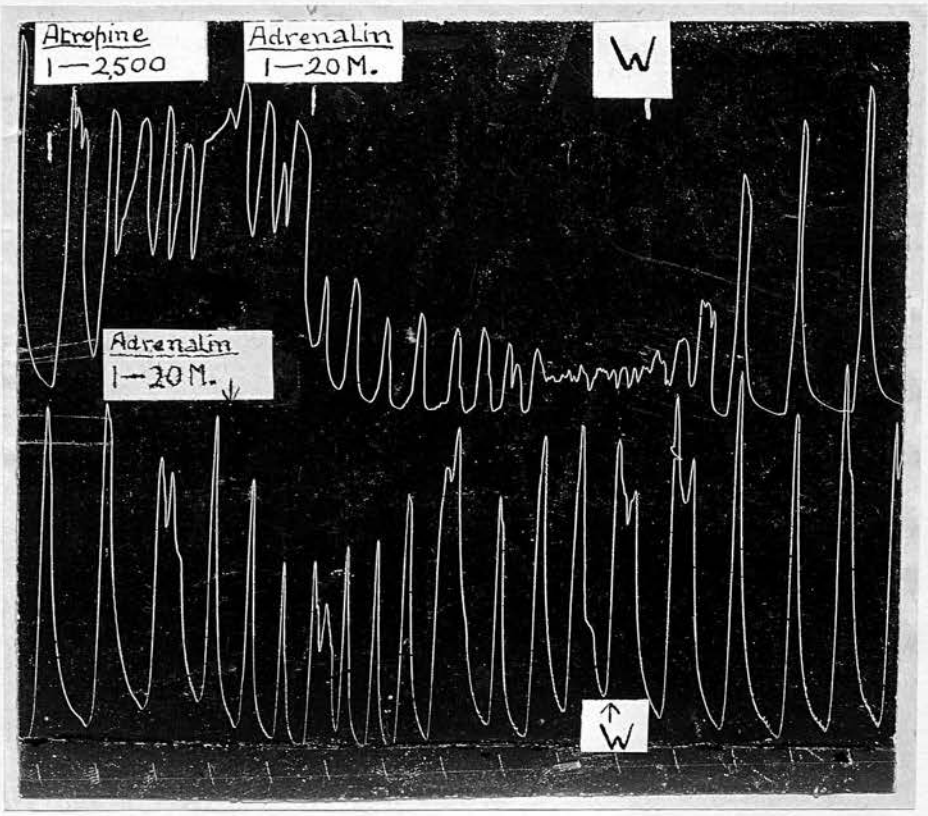


Fig. 20. Preparation from rabbit's uterus showing marked inhibition of automatic movement produced by adrenaline (1 in 20 million) after atropine (1 in 2,500).

The results are shown in Table II and are plotted in Figs. 21 and 22.

Table II.

The antagonism of adrenaline by atropine in the isolated rabbit's uterus.

The figures show the average height (in mm.) of the response to adrenaline.

Concentrations of atropine in parts per 100 thousand.	Concentrations of adrenaline in parts per 100 million.						
	5	10	20	50	100	200	400
0	31	33	35	38	40	43	45
1	-	-	-	34	38	40	43
1.6	-	-	-	32	36	39	41
2	-	-	-	31	34	37	40
2.5	-	-	-	30	33	35	39
4	-	-	-	-	31	34	37
5	-	-	-	-	-	33	35
8	-	-	-	-	-	-	34
10	-	-	-	-	-	-	33

Figs./

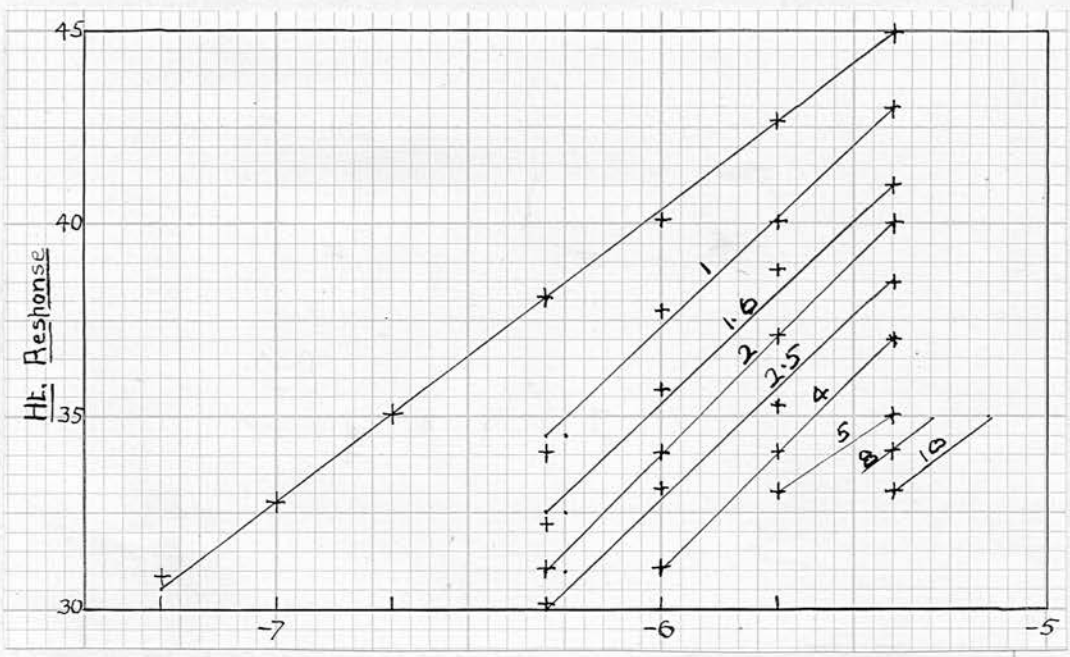


Fig. 21. Antagonism of adrenaline by atropine in the isolated rabbit's uterus.
Ordinate: Height of contraction (in mm.);
Abscissa: Log. conc. of adrenaline. Curves marked: Conc. atropine parts per 100 thousand.

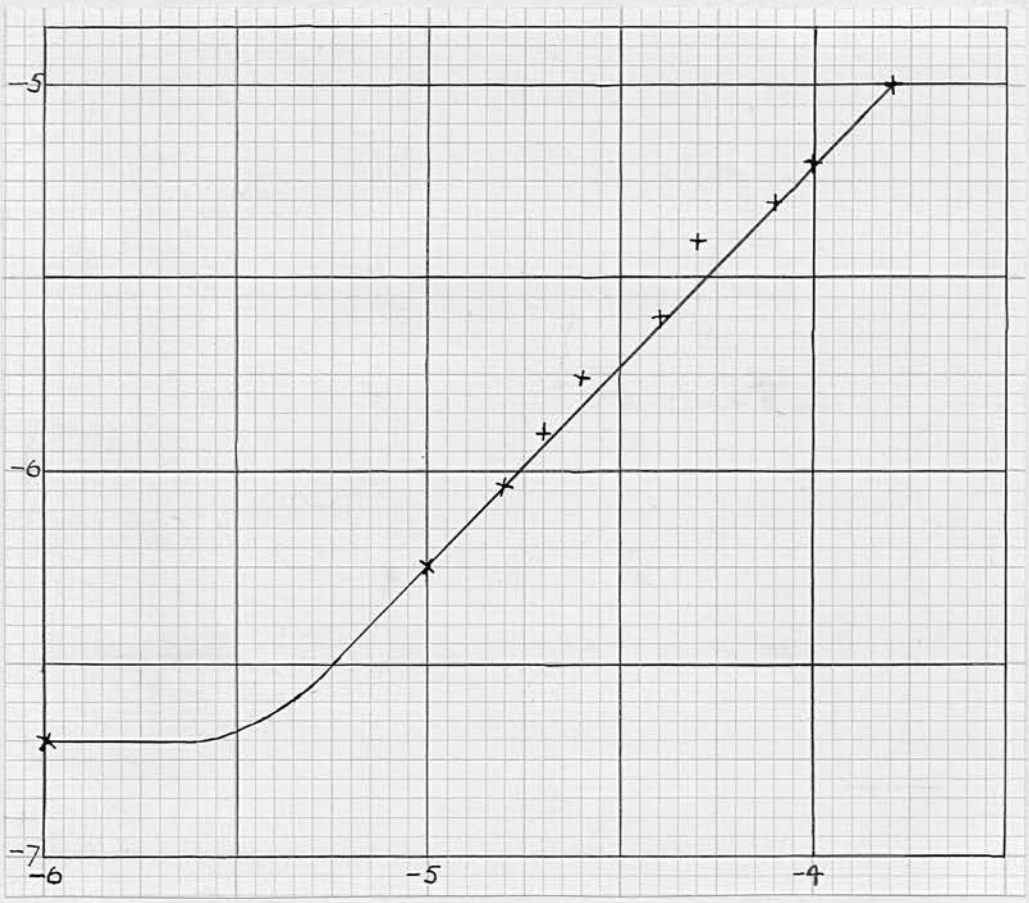


Fig. 22. Antagonism of adrenaline by atropine in the isolated rabbit's uterus.
Ordinate: Log. conc. adrenaline; Abscissa: Log. conc. atropine at which equal action on uterus is produced.

The following concentrations produce a response of 33 mm.

Concentrations of atropine, parts per 100 thousand	0	1	1.6	2	2.5	4	5	8	10
Concentrations of adrenaline in parts per 100 million	10	40	60	80	100	150	200	300	400
$\frac{C_{Ad_2} - C_{Ad_1}}{C_{Atr}} \times 1000$	30	30	35	36	35	38	36	39	

These figures when plotted on the logarithmic scale give the linear relation shown in Fig. 21 and it will be seen that the formula gives an approximately constant result, namely 0.035.

The antagonism of adrenaline by atropine appears therefore to be of the same general nature as the antagonism of adrenaline by ergotamine. This resemblance is the more remarkable because the intensity of the antagonism varies so enormously in/

in the two cases. A marked reduction in adrenaline response is produced when the concentration of ergotoxine is one fortieth the concentration of adrenaline, but to produce a similar effect with atropine, the concentration of atropine must be about thirty times that of adrenaline. Ergotamine is therefore about 1,000 times more efficient than atropine as an antagonist to adrenaline.

Atropine only antagonises the augmentor action of adrenaline on the uterus and does not affect the inhibitor action; and hence the application of high concentrations of atropine will usually cause a reversal of the adrenaline response from augmentor to inhibitor. Atropine does not antagonism the action of adrenaline on the gut. The antagonism by atropine of the motor action of adrenaline on the uterus is remarkable because atropine has apparently no effect on the nerves supplying the uterus.

Cushny (1906) found that the stimulation of the hypogastric nerves produced exactly the same effects before and after the administration of atropine (i.e. inhibitor/

inhibitor or augmentor according to the species of animal and its condition, whether virgin, parous or pregnant). He concluded that atropine had no effect on these nerves. Rohrig, Langley and Anderson (1879, 1895) had previously arrived at similar conclusions.

(b) The Antagonism of Tyramine by Atropine.

I tried the effect of atropine (1 in 100,000 to 1 in 10,000) on the response of gut to tyramine (1 in 100,000 to 1 in 10,000). No antagonism could be detected. (Vide Fig. 23).

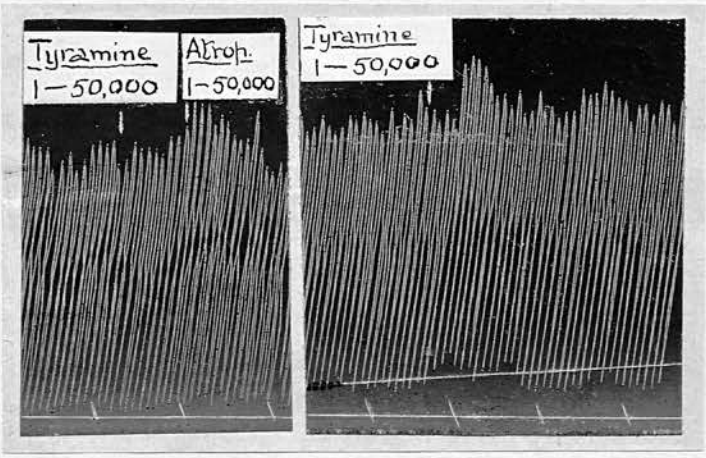


Fig. 23. Preparation from rabbit's ileum showing the augmentor action of tyramine (1:50,000) before and after atropine (1:50,000) - no antagonism seen.

In/

In the case of rabbit's uterus a feeble antagonism was observed in most cases but not in all cases. (Vide Fig. 24).

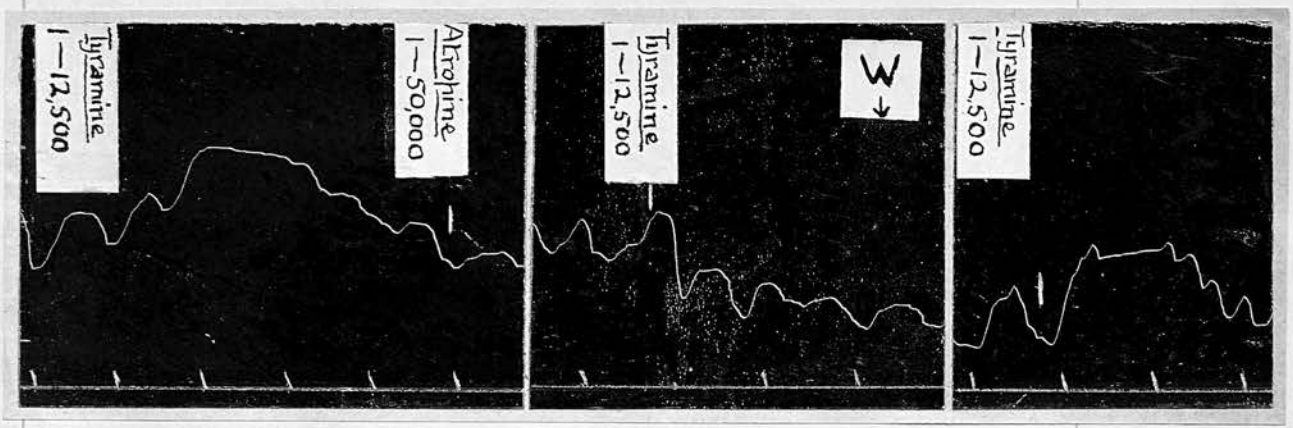


Fig. 24. Preparation from rabbit's uterus showing the augmentor action of tyramine (1:12,500) reversed into inhibitor action after atropine (1:50,000) - antagonism seen.

(c) The Antagonism of Ephedrine by Atropine.

Kreitmeir (1927) found that 1 in 6,000 ephedrine caused a slight stimulation of the isolated intestine of the cat and that this effect was abolished by atropine.

Renitz (1928) found that ephedrine produced stimulation of the isolated gut of the rabbit, but that atropine produced no effect on this action.

I found that ephedrine produced pure inhibition in the duodenum and ileum of the rabbit but rise of tonus/

tonus of the colon. Ephedrine also produced an augmentor action on the uterus. None of these effects, either inhibitor or augmentor, was antagonised by atropine. (Fig. 25 (a) and (b).

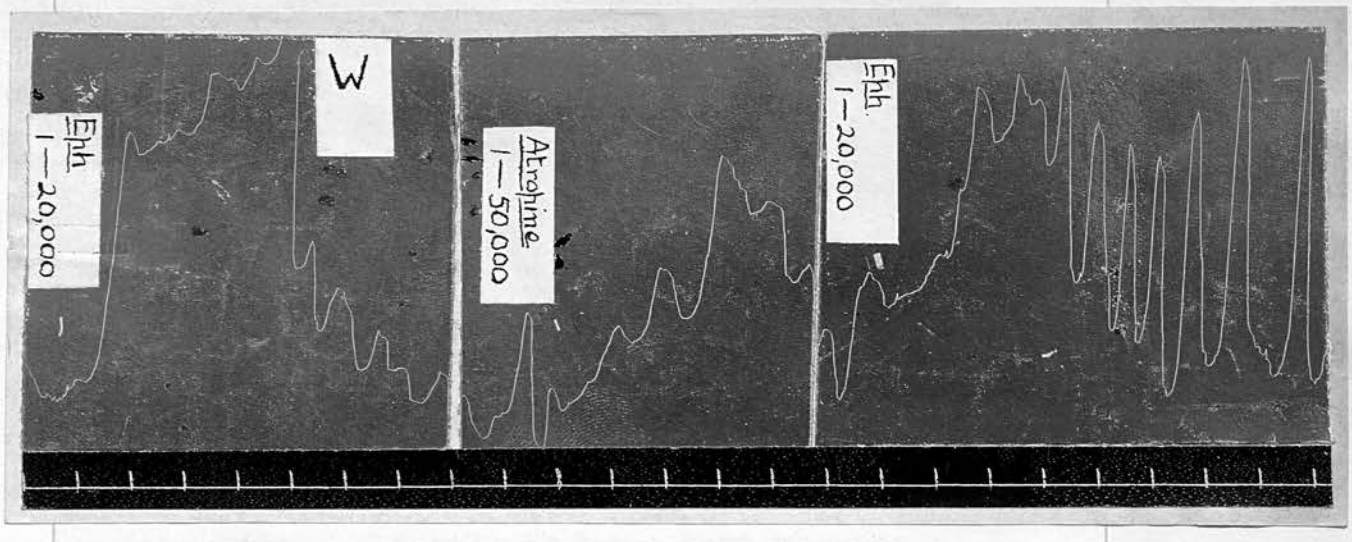


Fig. 25 (a). Preparation from rabbit's uterus showing the augmentor action of ephedrine (1:20,000) before and after atropine (1:50,000) - no antagonism seen.

Fig. 25 (b) /

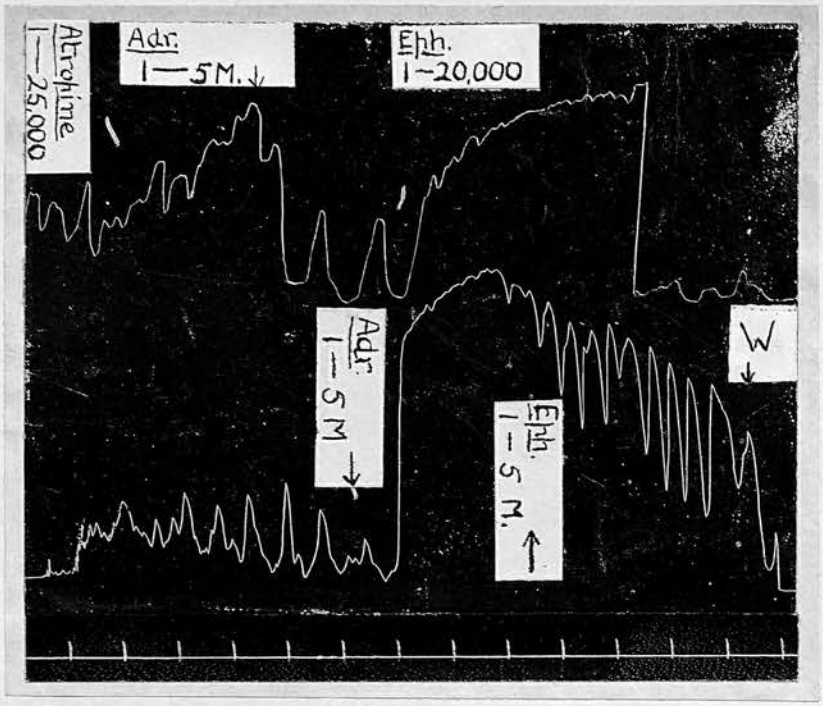


Fig. 25 (b). Preparation from rabbit's uterus showing the contrast of adrenaline and of ephedrine action in the presence of atropine or otherwise. It will be seen that whereas adrenaline (1:5 m.) causes inhibition of tonus after atropine, ephedrine causes augmentation. (Atropine 1:25,000; ephedrine 1:20,000).

(6) /

(6) Action of Ergotamine on Response to Para-Sympathomimetic Drugs.

Adrenaline produces on the uterus of the rabbit an action similar to that produced by the para-sympathomimetic drugs and since the adrenaline action is paralysed both by ergotamine and atropine, it would seem likely that the parasympathomimetic drugs would be paralysed by both drugs.

Cushny (1910) found that the pressor effect of pilocarpine on the uterus of intact cats was to some extent antagonised by ergotoxine, although not so completely as by atropine.

Dale and Laidlaw (1912) found, however, that ergotoxine did not antagonise the action of pilocarpine on the isolated uterus. They showed that the intravenous injections of pilocarpine caused an increased secretion of adrenaline by the supra-renal glands. Hence the action of pilocarpine on the uterus of an intact animal was due partly to its direct action and partly to the action of the adrenaline which it caused to be liberated. Ergotoxine abolished the second action but not the first one.

The/

The writer found that the action of pilocarpine, physostigmine and acetyl choline on the isolated uterus and gut of the rabbit was not affected by ergotamine.

(7) Response of Uterus to Parasympathomimetic Drugs and Effect of Atropine.

Pilocarpine and physostigmine have a relatively feeble action on the isolated uterus of the rabbit. They cause an increase in rhythmic movements rather than a definite rise of tonus. However their augmentor action on tonus is antagonised by atropine but not that on rhythmic movements as is seen in Figs. 26 and 27.

Fig. 26/

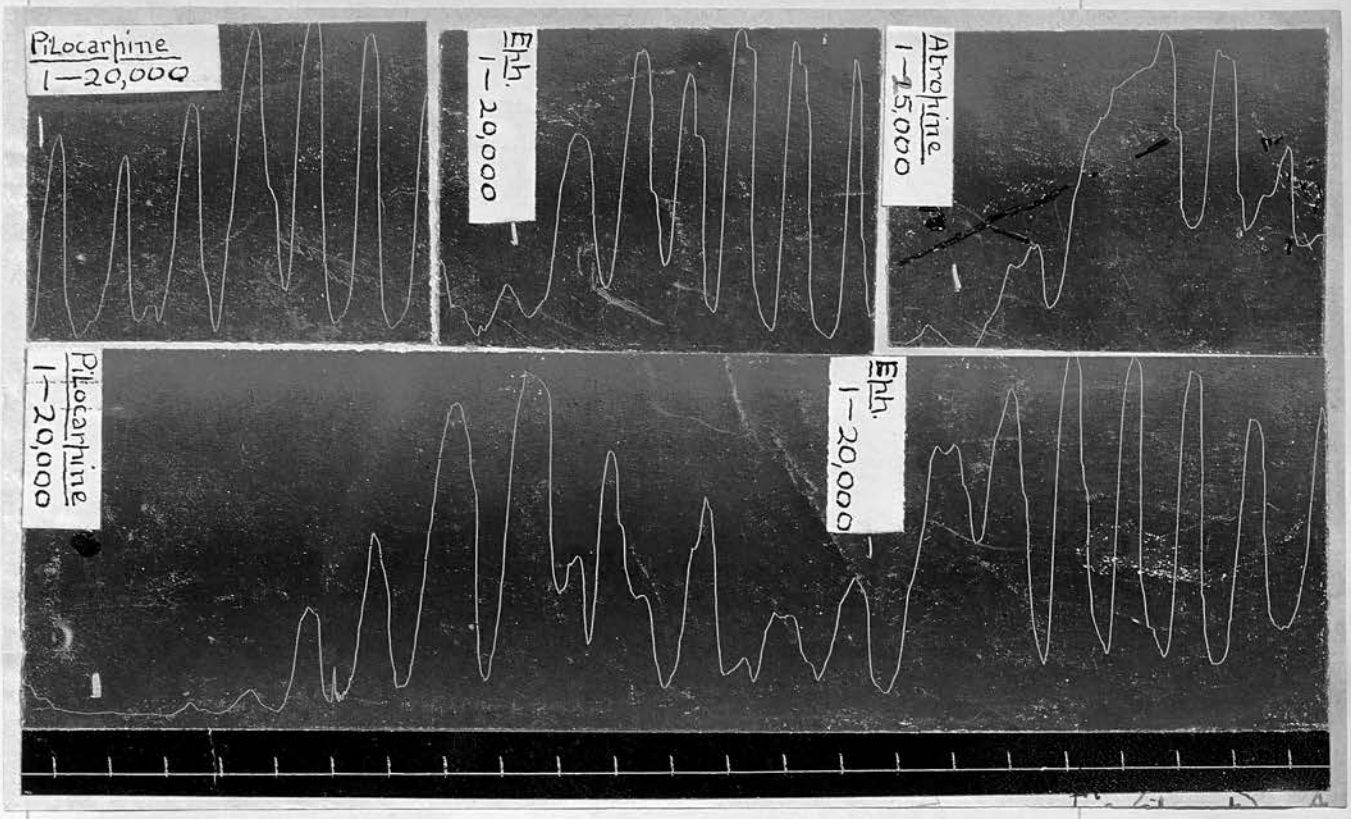


Fig. 26. Preparation from rabbit's uterus showing the action of pilocarpine and ephedrine before and after atropine (1:25,000). It will be seen that whereas the augmentor action of pilocarpine (1:20,000) on rhythmic movements has been antagonised by atropine, that of ephedrine (1:20,000) remains unaffected.

Fig. 27/

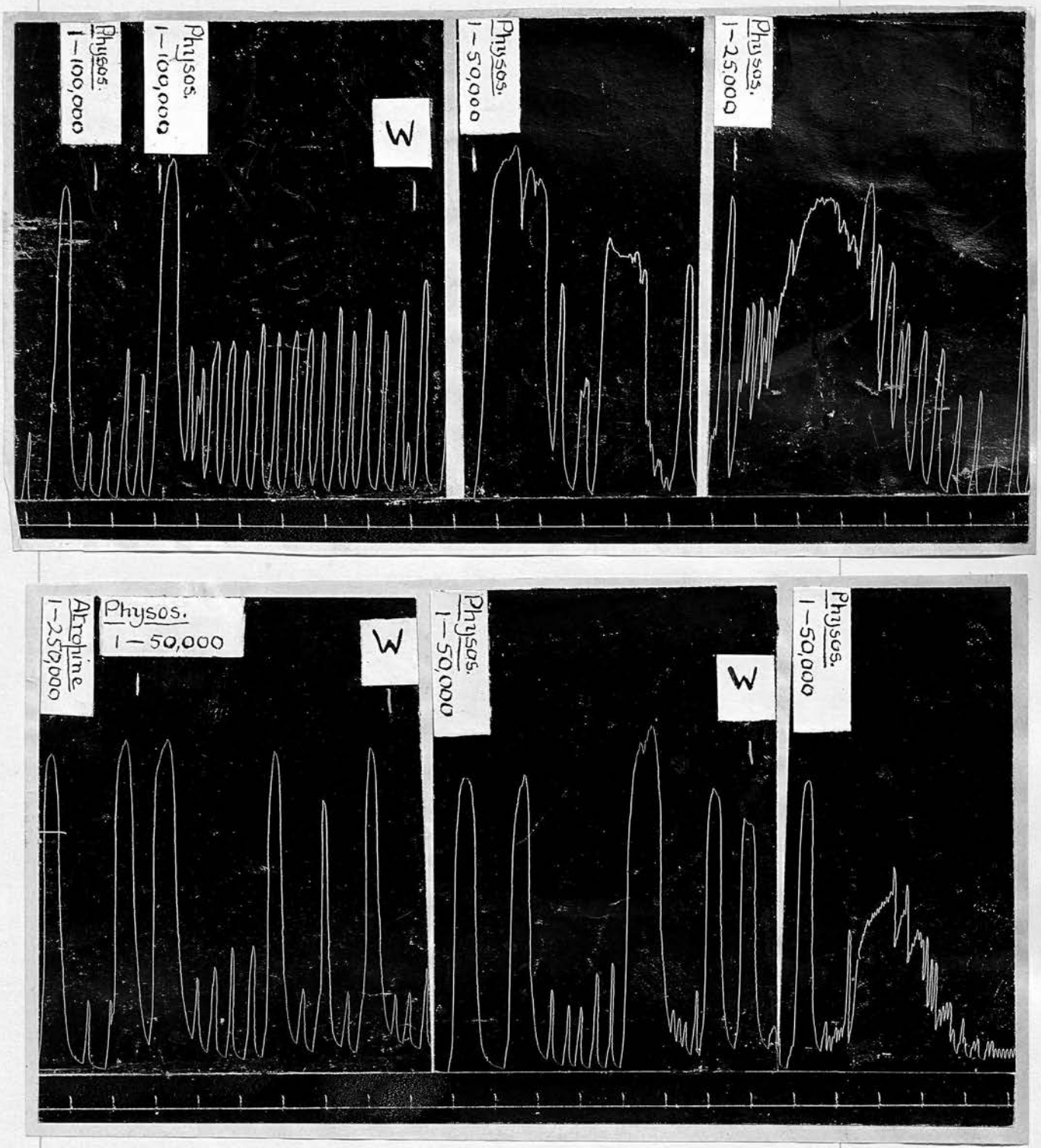


Fig. 27 (a) and (b). Preparations from rabbit's uterus showing the response of the organ to physostigmine before and after atropine (1:250,000). It will be seen that augmentor action of physostigmine in concentrations varying from 1:100,000 to 1:25,000 on rhythmic movements before atropine is paralysed after its application.

Rohrig (1879), Langley and Anderson (1895) and Cushny (1906) all found that atropine did not act by paralysing the hypogastric nerves in the uterus. Cushny (1910) observed that pilocarpine produced its augmentor action on uterus through hypogastric nerves and this action of pilocarpine was antagonised by atropine which in the absence of pilocarpine had no effect on the above nerves of uterus.

Dixon (1925) held that physostigmine acted on skeletal muscle through its nerve endings which, if paralysed by curare, prevented this action and that the action was also prevented by atropine and calcium which themselves had no action on motor nerves. He also held that in mammals also, like frog, slowing of heart could be affected with physostigmine after paralysing vagus with atropine, pointing to the fact that physostigmine either acted on heart muscle or depressed sympathetics.

Langley and Kato (1915) held that the theory of the action of physostigmine on nerve endings in the light of the fact that the nerve endings showed degeneration within 2-3 days after section whilst physostigmine continued to produce its augmentor action as long as 18 days, was rendered impossible.

Anderson (1905) however found that whereas pilocarpine still showed its myotic effect on pupil after section of post ganglionic fibres, physostigmine failed to do so.

C. DISCUSSION.

The reactions observed of the isolated gut and uterus of the rabbit to the drugs under investigation are summarised in Table III. This table shows that ephedrine differs from adrenaline in that it produces an augmentor action on the colon, whilst tyramine produces an augmentor action on all portions of the gut. There is the further important difference that the concentrations of ephedrine and tyramine needed to produce an action on isolated muscle are from 1000 to 10,000 times greater than the active concentrations of adrenaline.

Ergotamine Antagonism.

The four tissues, uterus, ileum, duodenum and colon are all extremely sensitive to the action of adrenaline. The concentrations of ergotamine needed to antagonise the action of adrenaline vary very greatly in these different tissues. If the concentration of adrenaline producing an action be taken as unity in all cases, then the concentration of ergotamine required to produce equal antagonism in the four cases is as follows (Appendix III)

Uterus/

Table III.

Antagonism of autonomic drugs by ergotamine and atropine in isolated rabbit's gut and uterus.

Extent of inhibition of action = +, ++, +++, ++++.
Reversal of action = ⊞; No effect = ⊖ .

	Action (normal)				Effect of Ergotamine on Response				Effect of Atropine on Response.			
	Duodenum	Ileum	Colon	Uterus	Duodenum	Ileum	Colon	Uterus	Duodenum	Ileum	Colon	Uterus.
								Slight augmt.	Inhibit. or augmt.	Inhibit. or augmt.	Inhibit or augmt.	Augmt.
ren-ine	Inhibit.	Inhibit.	Inhibit.	Augmt.	++	+++	+	++++ ⊞	⊖	⊖	⊖	++++ ⊞
hed-ne	do.	do.	do. and augmt.	do.	⊖	⊖	+?	+?	⊖	⊖	⊖	⊖
ramine	Augmentor throughout				⊖	⊖	⊖	⊖	⊖	⊖	⊖	⊖ ++
lo-rpine	do.	do.	do.	do.	⊖	⊖	⊖	⊖	++++	++++	++++	+++
ysos-gmine	"	do.	"	"	⊖	⊖	⊖	⊖	++++	++++	++++	+++
etyl oline	"	do.	"	"	⊖	⊖	⊖	⊖	++++	++++	++++	+++

Uterus (augmentor)	0.025
Ileum (inhibition of pendulum movements	0.2
Duodenum (inhibition of tonus).. ...	2.5
Colon (inhibition of tonus)	50
Uterus (inhibitor	100

Ergotamine has very little power to antagonise ephedrine. Some antagonism may be produced in the case of the uterus and colon but none is demonstrable in the case of the ileum and duodenum. Ergotamine does not antagonise the action of tyramine in any tissue to any measurable extent.

Antagonism by Atropine.

Atropine is a weaker antagonist of adrenaline than is ergotamine. In the case of the uterus the ratio between the antagonistic power of ergotamine and atropine is about 1000 to 1. Atropine resembles ergotamine in that it antagonises the motor effect produced by adrenaline on the uterus but does not affect its inhibitor action. Atropine has no measurable antagonistic action against ephedrine, it produces a slight antagonism to the action of tyramine on the uterus but not on the gut.

These antagonistic actions suggest that ephedrine and tyramine act in a manner different from/

from adrenaline. According to Loewi's theory stimulation of the sympathetic nerves of the uterus and gut cause the liberation of very minute amounts of adrenaline and this drug produces the response observed.

Ergotamine presumably prevents adrenaline from uniting with certain specific receptors and hence paralyses the sympathetic nerves and also antagonises the action of adrenaline. It is however only a partial antagonist for in the uterus it paralyses augmentor actions without affecting inhibitor actions and in the gut it paralyses the action on the pendulum movements much more readily than it paralyses the action on the tonus.

Atropine appears to act on the same receptors as does ergotamine, but in a much weaker manner, and hence an antagonism can be demonstrated in the case of the uterus but not in the case of the gut.

Ephedrine and tyramine appear to produce their action chiefly on the receptors other than those occupied by ergotamine and atropine, and hence these drugs produce no antagonism in most cases and a slight antagonism in a few cases.

Antagonism/

Antagonism of Parasympathomimetic Drugs.

The parasympathomimetic drugs produce on the uterus effects similar to those produced by adrenaline. This resemblance has for long been a pharmacological puzzle. Rohrig (1874), Langley and Anderson (1895), and Cushny (1906) all found that atropine did not paralyse the action of the hypogastric nerves on the uterus. Cushny (1910) found, however, that pilocarpine produced an effect similar to stimulation of the hypogastric nerves in many cases, and that this effect of pilocarpine was antagonised by atropine. He also observed that some of the effects produced by pilocarpine were antagonised by ergotamine. This puzzling observation was explained by Dale and Laidlaw who showed that in the intact animal pilocarpine not only acted directly on the uterus, but also caused liberation of adrenaline by the suprarenal glands. It was this secondary action of pilocarpine that was antagonised by ergotamine.

According to Loewi's theory the parasympathetic nerves exert their action by causing the liberation of acetyl choline. This theory postulates that acetyl choline ought to produce all effects that are produced by the parasympathetic nerves. It does not however/

however follow that acetyl choline and other para-sympathetic drugs cannot produce actions other than those produced by the nerves.

Adrenaline acts on a limited specific group of receptors, some motor and some inhibitor, whilst acetyl choline acts on another group of motor acceptors. Ergotamine antagonises all the motor and some of the inhibitor actions of adrenaline. Atropine antagonises the action of acetyl choline in a very powerful manner and has a less powerful action on the motor actions of adrenaline. Ephedrine and tyramine produce a generalised feeble action on a wide group of receptors,only a few of which are affected by either atropine or ergotamine.

SUMMARY of CONCLUSIONS.

(1) Ephedrine resembles adrenaline in producing a purely inhibitory action on the duodenum and ileum, whilst it differs in that it produces a variable action on the colon, sometimes inhibitor and sometimes augmentor.

(2) None of these actions of ephedrine are antagonised by ergotamine, whereas this drug antagonises all the corresponding actions of adrenaline.

(3) Tyramine differs from both adrenaline and ephedrine because it invariably produces a stimulant action on all the various parts of the rabbit's gut. These actions of tyramine are not antagonised by ergotamine.

(4) Uteri of virgin and non-pregnant rabbits vary greatly as regards their sensitivity to both sympathomimetic and parasympathomimetic drugs. They are far more sensitive to the action of adrenaline than to that of all other sympatho- and parasympathomimetic drugs.

(5) a). All autonomic drugs (both sympatho- and parasympathomimetic) usually produce a stimulant effect on the rabbit's uterus.

(5) b). Of these drugs adrenaline acts chiefly on tonus by producing permanent rise with variable action on rhythmic movements which is mostly inhibitory, whilst all other drugs act chiefly on rhythmic movements which are usually increased both in rate and amplitude.

(6) Ergotamine while paralysing the action of adrenaline on rabbit's uterus differentiates between the stimulant action of the latter drug which gets paralysed and the inhibitor action which is not paralysed.

(7) When applied in high concentrations only, ergotamine is seen to antagonise partially the augmentor action of ephedrine on rabbit's uterus, but it has no such action of antagonising the uterine response to tyramine, pilocarpine, physostigmine and acetyl choline.

(8) Like ergotamine, atropine whilst antagonising the action of adrenaline on rabbit's uterus is seen also to differentiate the augmentor and inhibitor action of the latter, of which the augmentor action of adrenaline is antagonised by atropine and the inhibitor action, being uncovered, looks so marked as if it were increased rather.

(9) /

(9) Unlike ergotamine, atropine fails to antagonise the inhibitor action of adrenaline on the pendulum movements of rabbit's gut (i.e. ileum).

(10) The nature of the actions produced on the isolated gut and uterus of the rabbit by ephedrine and tyramine suggests that these drugs are not true sympathomimetic substances; this view is supported by the fact that these actions are not antagonised by either ergotamine or atropine.

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Appendix I.

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FACTORS INFLUENCING THE RESPONSE OF PLAIN MUSCLE TO DRUGS. By T. C. Nanda. From the Department of Pharmacology, University of Edinburgh. (With four figures in the text.)

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FACTORS INFLUENCING THE RESPONSE OF PLAIN MUSCLE TO DRUGS.

By T. C. NANDA. From the Department of Pharmacology, University of Edinburgh. (With four figures in the text.)

(*Received for publication 4th May* 1931.)

STRAUB (11) in 1907 put forward his well-known theory of the potential action of drugs, according to which a drug only produces an action when there is a difference in its concentration within and without the cells. This theory is capable of explaining a large variety of effects, and has been extensively applied by many authors. In particular JENDRASIK (3, 4, 5, 6, 7) claims to have proved that the common kations and anions, the alcohols and adrenaline all produce potential actions on the isolated gut and uterus, and he has concluded that (8) "potential actions are widespread. There is scarcely any substance which exercises no such action."

The chief reason for the popularity of this theory is that it is a means of explaining both the apparently transient action produced by many drugs on isolated organs and also the curious effects often observed when drugs are washed out of such tissues. RENTZ (10) recently revised the literature of this subject, and concluded that the potential theory could only explain a small fraction of the effects of this character that occurred. He suggested that the polyphasic actions so frequently observed when drugs act on plain muscle are due to the muscle undergoing some form of slow change. Polyphasic actions have been observed chiefly in the case of drugs acting on plain muscle, and the recent work of WINTON (12) shows that the response of plain muscle to drugs is complex, since two mechanisms are involved which he terms the phasic contractile mechanism and the postural contractile mechanism. The possible complexity of a drug response is illustrated by the curves obtained by CLARK (1) for the response of the *rectus abdominis* of the frog to excess KCl. The isotonic records show the response as a hyperbola, that is to say, a contraction that rises rapidly at first, then approaches asymptote, and is maintained indefinitely. The isometric record, however, shows a twitch of short duration. The response must therefore consist of two components, namely, an initial contraction that is associated with the development of a considerable tension, and a slow

process that develops very little tension but is capable of producing and maintaining extensive changes in length.

I have found that the response of the isolated gut to acetyl-choline is of the same nature, namely, a transient but relatively forcible initial contraction followed by a sustained rise of tonus. Consequently the apparent duration of response to acetyl-choline depends on a number of factors. If the gut is enfeebled by prolonged isolation, the lever heavy, or the dose of the drug small, then only the initial contraction is seen and the action of acetyl-choline on the gut appears to be transient. On the other hand, a sufficient dose of drug records the slow rise of tonus, which lasts as long as the drug is in contact with the tissue. Fig. 1

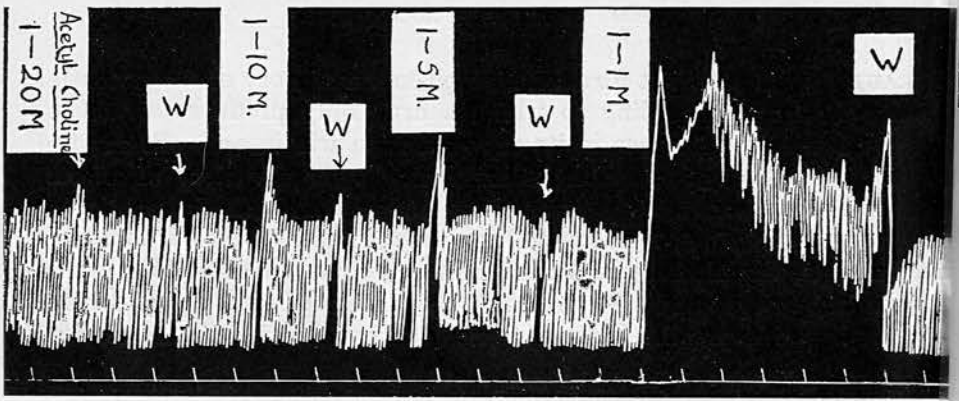

FIG. 1.—Rabbit's ileum. Low concentrations of acetyl-choline hydrochloride produce a tr sient contraction, whilst higher concentrations also produce a sustained rise of tonus. this and all other figures the tracing reads from left to right; upstroke shows contracti time in minutes.

shows a graded transition from the "transient" action of acetyl-choline to the continuous action. The so-called transient action of acetyl-choline appears, therefore, to be due to faulty experimental conditions.

It must be admitted that sometimes it is impossible to account for the vagaries of isolated tissues. Fig. 2 shows two responses of the same piece of rabbit's uterus to physostigmine. When the tissue was freshly isolated (fig. 2, *a*) the response consisted of an increase in frequency of contractions which lasted as long as the drug was present, whereas after prolonged isolation (fig. 2, *b*) the response consisted o a contraction which rapidly passed off.

The so-called transient action of adrenaline on isolated tissues has been frequently advanced as evidence in favour of the potential actio of drugs. JENDRASIK and MOSER (9) noted that the action of adrenalin on the isolated rabbit's gut rapidly passed off, and that the actio ceased long before any significant quantity of adrenaline had bee destroyed. GAUTRELET and BAIGY (2) also noted that after th

action has passed off, the gut was insensitive to further doses of adrenaline.

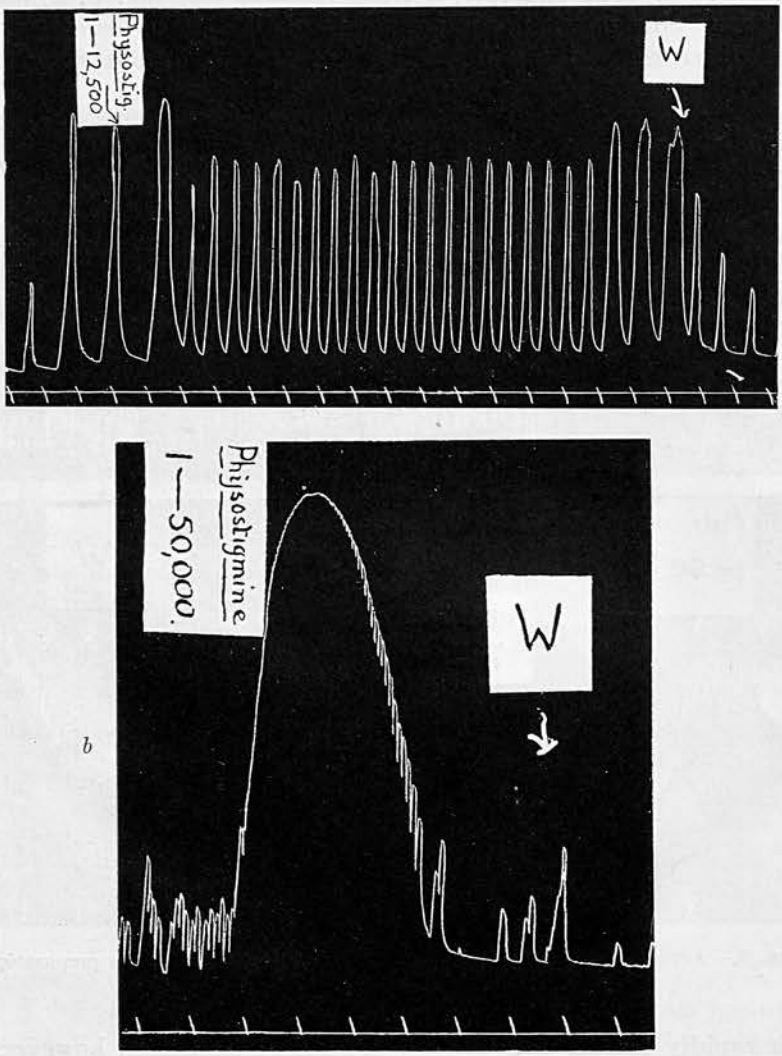

FIG. 2.—Rabbit's uterus. (*a*) Response of fresh tissue to physostigmine sulphate (1 in 12,500). (*b*) Response of same strip two hours later to physostigmine sulphate (1 in 50,000).

The writer has confirmed these observations as far as the action of adrenaline in depressing the pendulum movements is concerned. Adrenaline has, however, a double action on the gut—it depresses the pendulum movements and also reduces the tonus of the gut. The depression of pendulum movements is transient, but the action on the tonus persists as long as the adrenaline is present. The amount of

tonus change produced by adrenaline varies considerably, and hence it is a matter of chance whether the action produced appears transient or continuous. For example, a fresh piece of gut (fig. 3, *a*) showed little tonus change when adrenaline was added, and hence the only action observed was the diminution of the pendulum movements,

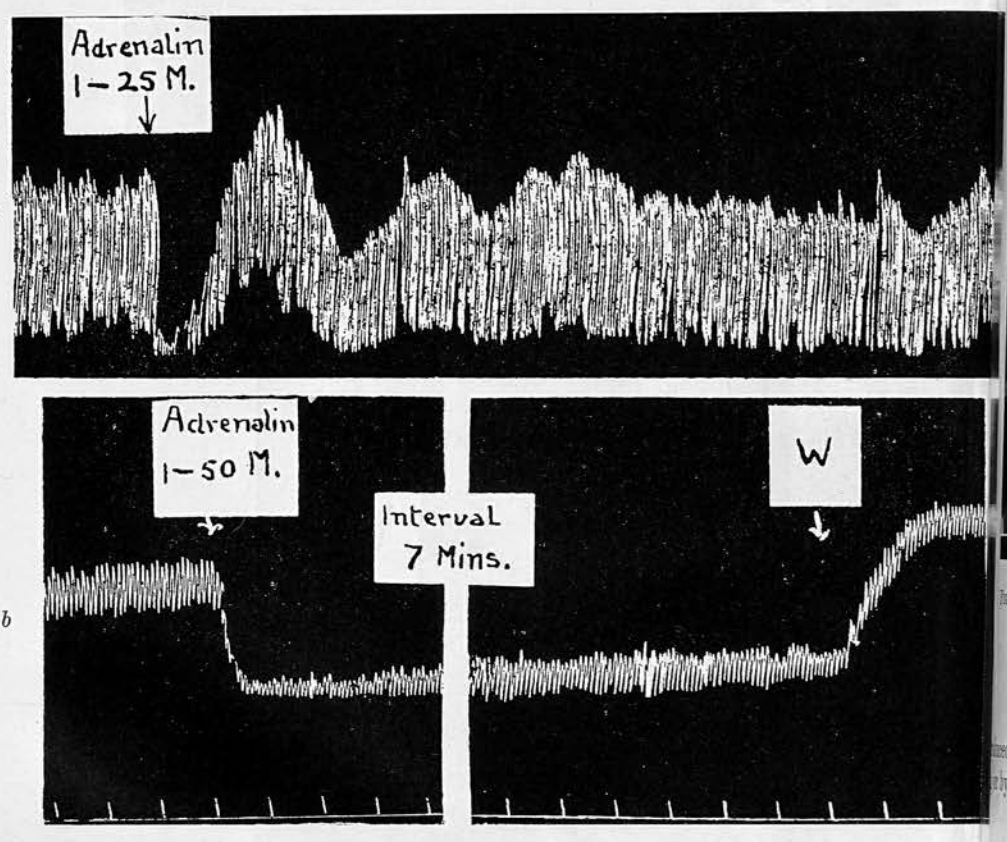

FIG. 3.—Action of adrenaline chloride on rabbit's ileum. (*a*) Fresh preparation. (*b*) Same preparation two hours later.

which rapidly passed off. After a few hours' isolation, however, the same piece of tissue with the same dose of adrenaline showed an extensive fall of tonus (fig. 3, *b*), and this was permanent as long as the drug was left in contact with the tissue.

The action of adrenaline on the gut is therefore only partly transient, and it seems absurd to suggest that the drug produces a potential action on the pendulum movements and an action of a different nature on the tonus. A much more reasonable line of explanation appears to be that put forward by RENTZ, that changes in the condition of the muscle may compensate for some of the effects produced by the drug.

A simple illustration of this type of action is the effect of increasing the filling of any hollow organ; the tension at once rises, and then the muscle relaxes and the tension falls. If the pressure is measured the action appears transient, whereas if the volume is measured the action is permanent. Fig. 4 shows that an exactly similar transient action

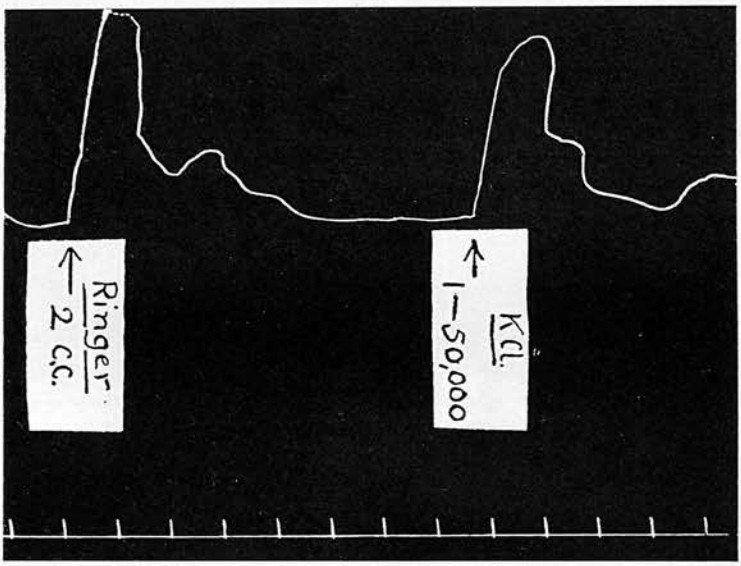

FIG. 4.—Pressure inside frog's stomach, recorded by tambour. Stomach filled with Ringer's fluid and immersed in the same. The first tracing shows the effect of increasing the filling with 2 c.c. Ringer's fluid. The second tracing shows the effect of adding KCl to the bath of Ringer's fluid.

is produced on the pressure in a frog's stomach either by increased filling or by adding excess KCl to the fluid around the stomach.

SUMMARY.

1. Many of the responses of plain muscle to drugs that are frequently described as transient appear so because the experimental conditions record only part of the response.

2. The phasic responses frequently observed can be explained on the assumption that the drug produces some rapid change, and that other changes, which proceed more slowly, modify the initial response.

3. These apparent transient actions do not necessitate the assumption of the potential action of drugs.

My thanks are due to Prof. A. J. CLARK for assistance and advice in carrying out this research.

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Appendix II.

An Examination of the Theory of Potential Action of Drugs as Evidenced by the Responses Observed in Plain Muscle.

Introduction.

Various workers have from time to time enunciated theories of the nature of drug action in the hope of providing a rational basis for pharmacology. It is improbable that any of these hypotheses represents the real truth, but many of these have been of service in that they have stimulated research. The fact that a theory has been of practical value does not, however, prove its truth. This has been well expressed by Dale (1923) in a criticism of Ehrlich's (1909) theories of chemotherapy. He, when dealing with the attempt on the part of Ehrlich to wean chemotherapy entirely from the parental pharmacology, observes/

observes as follows:

"Ehrlich's theory will always deserve the credit of having provided a vigorous stimulus to the investigation of problems which without some kind of working hypothesis might well have seemed beyond the reach of an experimental attack. That being admitted, it is necessary on the other hand, to admit that few of the successful results, hitherto obtained, have been obtained by a consistent application of the theory. Some of them to be the result of experiments which a serious acceptance of the theory would have discouraged. As new successful applications have become more frequent their basis has become increasingly empirical. It is difficult to resist the conclusion that a new theoretical foundation is required for further orderly building, and that this will have to take fuller account of the complexity of the therapeutic process and especially the co-operation therein of the infected host. And if this should mean some measure of reunion between chemotherapy and parental pharmacology, from whose rather unenterprising tradition it claimed to be free, the result can only be to the advantage of therapeutic science."

It appears to the author that this general criticism can also be applied to the theory of the potential action of drugs put forward by Straub in 1907. This theory has stimulated a large amount of research, but it is becoming increasingly clear that its fundamental assumptions are extremely doubtful.

The present work is an attempt to analyse by simple pharmacological process some of the chief evidence/

evidence that has been put forward in support of the theory of the "potential action" of drugs. Most of this evidence has been based on experiments on the isolated gut and uterus and hence the present work is concerned chiefly with these preparations.

Theory of the Potential Action of Drugs.

Straub perfused the excised hearts of aplysia and torpedo with muscarine and found that they took up large quantities of the drug which gradually disappeared from the perfusion fluid as its action on the heart waned. Moreover he noticed that the hearts after they had taken up a certain amount of the drug, became immune to further additions of the drug into the perfusion fluid. The frog's heart, however, did not show any spontaneous recovery, except when exposed to a very high dilutions of muscarine. He also found that atropine prevented the entrance of muscarine into the torpedo heart.

According to Straub the effect of some drugs e.g. digitalis glucosides and veratrine is proportional to their concentration within the cell/

cell but with others such as muscarine he found the relation to be quite different for he found the effects depended on the concentration outside the cell, or rather, on there being a higher concentration outside than inside the cell. From these observations Straub concluded that the action of certain drugs depended on a differential gradient of concentration between the outside and inside of the cells. He termed this "potential action" and this idea of Straub has been adopted by other workers and applied to a wide range of phenomena. For example Jendrassik (1929) concluded recently: "Potential actions are widespread. There is scarcely any substance which exercises no such action."

General Criticism of the Potential Action Theory.

(1) Digitalis Glucosides.

Jendrassik's wide application of this theory is obviously unjustified, for Straub himself pointed out that the theory could not hold in the case of such drugs as digitalis and strophanthin, for in these cases the greater the fixation of drugs/

drugs by the cell, the greater is the action. There is a large body of evidence that supports this view.

Clark (1912-13) found that a chemical union between heart muscle and the drug was certainly suggested by the fact that systemic effects, due to the action of digitoxin, may first appear when digitoxin is no longer in contact with the heart.

Dealing with the question of tolerance of digitalis glucosides the same author (1913) in one of his subsequent papers points out that there is no evidence to show that the heart muscle of any of the animals as grass-snake, frog, rat, rabbit can specifically absorb strophanthin or that any of the tissues of these animals have any power to destroy it.

Hatcher and Eggleston (1912-19) find that ouabin disappears rapidly from circulation in cat and dog, which occurs in two or three minutes, only less than 50per cent. of a massive intravenous dose being present in blood at death. They hold that they can offer no other explanation of this disappearance of large amount from blood than that the/

the normal liver decomposes the drug more rapidly than the perfused liver, or that the ouabin is widely distributed in various tissues of the body.

A noteworthy point concerning the action of digitalis is that it is mainly the accumulative effect of the drug which counts in therapeutics as it is a matter of common observation daily by the bed-side that even moderate doses of digitalis preparations administered at unusually longer intervals practically come out to be of no benefit to the patient, i.e. they fail to show much therapeutic effect until the dosage approaches the toxic stage - a fact for which no explanation is coming forward except that the drug acts only on getting into tissue cells (the heart muscle) as it is seen to disappear in no time when injected into the blood stream.

(2) Narcotics.

Many theories and hypotheses have been advanced from time to time to explain the mode of action of different groups of narcotics; those which chiefly concern us here are those enunciated to understand the nature of action of the drugs belonging/

belonging to aliphatic group. The most popular idea held at present about the mechanism of their action is based upon their selective solution-affinity or what is commonly known as Meyer-Overton law (1899) of partition coefficient which helps this group of narcotics to keep on showing their action side by side with their concentration outside the plasma membrane or cell membrane.

Narcotics act quickly but enter the cell slowly, i.e. Tissot's figures(1906) indicate that almost 8 hours is needed for equilibrium to be established between the inhaled chloroform and the body tissues.

Many authors have shown, however, that in full narcosis the concentration of narcotics in the brain is equal to that in the blood, as Nicloux (1906), Storm van Leeuwen (1916), Gensler (1914), Harris (1914), Southgate and Carter (1926). The last mentioned authors (Southgate and Carter) come to the conclusion that the concentration of alcohol in the blood is the best measure of alcohol intoxi-cation and that concentration of alcohol in the urine is proportional to that of blood under all conditions, so far examined, and thus a fairly good idea/

idea of intoxication can be made out from urine analysis.

Many attempts have been made to demonstrate tolerance to narcotics in isolated tissues, but on the whole the results so far obtained have proved to be negative. Davidson (1925-26) observed that during experiments with nitrous oxide gas a slight degree of tolerance to the drug appeared to be acquired. The effect of an active concentration of the gas seemed to be somewhat greater after abstention for several weeks from exposure to the gas. He frequently performed two or more experiments with N_2O on the same or successive days, but found that the results were too variable to determine whether tolerance did or did not occur.

In the case of acetylene he used inhalations on consecutive days of a concentration just sufficient to induce intoxication (i.e. unconsciousness); and also with a somewhat smaller concentration he recorded the reflex time in the choice of colours and in arithmetical and writing tests. With each successive inhalation he noted a slight diminution in reaction time.

From/

From these experiments and from his general impression he concluded that some degree of adaptation to intoxication occurred, and that in a continued inebriate condition the work performed, if enforced, might slightly improve. About the effect of ethyl chloride on reaction time he concluded that with concentrations allowing of prolonged administration a diminution in the reaction time eventually occurred. He remarks that two experiments with 2.5 per cent. ethyl chloride in air made on successive days are worthy of mention as they illustrate how slight the tolerance is that occurs with ethyl chloride.

Dealing with methyl ether in an experiment he observes that a point of interest in this experiment is the shortness of time taken to type the four sentences three minutes after recovery from a brief inhalation lasting five minutes and forty seconds and notes that this has been the only occasion in a considerable number of experiments that so marked an improvement had occurred.

Graham, H.T.(1929) comes to the conclusion that the narcotic gases, nitrous oxide, ethylene and acetylene increase the threshold of muscular contraction in response to break shocks exactly in the/

the same manner as ether vapour affects it, but at much higher tension. This increase or rise in threshold especially when rapid and marked is designated as "narcosis" by him. He holds that the gases cannot be separated from other narcotics on the basis of any different action on anaerobic processes, nor can muscle be distinguished from nerve because of its supposed resistance to narcosis by gases.

According to Graham's findings isolated frog sartorius, on being already exposed to fairly low tensions of narcotic for some time and then allowed to recover, is less easily narcotised than the fresh muscle. This decreased susceptibility of the muscle to narcotics is shown in(a)relatively increased period of exposure to narcotic before failure of response to sub-maximal break shock, (b) rise of threshold of contraction and (c) a rise in threshold of narcosis.

(3) Acids. etc.

The experiments of Jacobs (1920), Beerman (1924) and Bordine (1924) on the physiological action of CO_2, H_2S and cyanides prove that the drugs enter the/

cells and continue to act after entry. Jacobs (1920) observes that while in certain respects CO_2 in aqueous solutions behaves as an ordinary acid, acting through its ions, in other respects it may be said to have a specific action different from that of other acids. Further on he explains his point that the physiological behaviour of CO_2 probably depends to a considerable extent on two of its chemical and physical peculiarities, i.e.

(a) on the weakness of H_2CO_3 as an acid, permitting the existence of a relatively large amount of the free but undissolved acid (as well as dissolved CO_2) in the equilibrium that exists at neutrality or slight alkalinity, and

(b) on the readiness with which the undissolved acid or its anhydride (CO_2) enters living cells, perhaps in virtue of its lipoid affinity.

Beerman (1924) in his paper on the action of CO_2 and H_2S on protozoa re-affirms Jacob's findings noted above. Brodine (1924) in his experiments on the action of cyanides on protozoa concludes that the physiological action of HCN and its salts appears to be due (a) to the ease with which HCN molecules penetrate living cells and then, on ionizing, exert their/

their influence by H ions and CN ions, (b) HCN in acid, neutral or slightly alkaline media produces intracellular acidity because of the rapid penetration of HCN molecules into the cell.

In the cases considered, there is therefore direct evidence that the drugs enter the cells and continue to act upon them after their entry, and therefore their action cannot be due to a potential gradient between the concentration of drug within and without the cells.

(4) Micro-injection Experiments.

Straub postulated that the action of drugs depended on a concentration gradient which drove the drug through the cell wall. He considered this might be produced by a gradient in either direction i.e. a high concentration either within or without the cells.

The work already quoted shows that in many cases the drug continues to act after the cell has been filled up and any concentration gradient abolished. On the other hand the micro-injection experiments of Chambers and his school prove that in many cases drugs/

drugs injected into a cell fail to produce their typical action. These experiments can easily be explained on the theory that many drugs exert their specific action on the external surface of the plasmatic membrane but are very difficult to explain on the potential theory.

Chambers and Roznikoff (1924-25) studied the reactions of the protoplasm of living amoebae to Na, K, Ca and Mg salts injected with the help of Chambers' micromanipulator into the interior of the cells as compared with the reactions seen on immersion and also on tearing of amoebae in the solutions of their salts. They found that the liquifying action of Na and K on one hand and the solidyfying action of Ca on the other, seen on giving micro injections inside the cell (the amoebae) were also produced on immersing the animal in solutions of these salts. They also found that Na and K could penetrate amoeba much more effectively than Ca and Mg. On comparing their results of immersion experiments with those obtained by micro-injections they hold that both NaCl and KCl are much more toxic to amoebae when in contact/

contact with their external surface than when injected inside and that in the case of Ca and Mg salts (i.e. $CaCl_2$ and $MgCl_2$) the reverse is true - injection toxicity $MgCl_2$ and $CaCl_2$ > NaCl and KCl. Also they hold that since they believe the penetrating power of Ca into the amoebae is negligible, it is suggested that the antagonism of Ca to lethal action of Na and K occurs on the surface of the amoeba.

Hiller (1927) has tried narcotics on amoebae both by micro-injection as well as by immersion methods respectively. The drugs tried by him included ethyl alcohol, ether, chloroform and chloretone of aliphatic series. On comparing his results obtained by immersion experiments with those of micro-injection experiments, he concludes that no narcotic effect could be observed by injections into the interior of the amoebae.

Brinley (1928) deals with the effects of cyanides on the protoplasm of amoebae tried by both these methods of micro-injections and of immersion respectively. In these experiments he used HCN and KCN solutions varying from N/10 to N/3,000 concentrations. His findings are that the toxicity of HCN and of KCN for amoebae is due to their action on/

on the cell membrane and not on internal protoplasm. He bases his findings on the fact that while injections produce a reversible decrease in the viscosity of the protoplasm, in immersion experiments the decrease noticed in the viscosity of the protoplasm is of a lasting nature and may ultimately lead to the disintegration of the cell, say within the period of three days.

The same author (1928) also tried the action of H_2S on the protoplasm of amoeba and found that both the micro-injections as well as immersion experiments with sulphurated hydrogen led to the increase of the viscosity of the protoplasm, but the increase due to injections was only temporary. Accordingly he concluded that the toxicity caused by the gas to amoebae was largely dependent upon its action on the cell membrane.

Evidence/

Evidence in Favour of the Potential Theory of Drug Action.

This theory has received support from two series of observations:

(1) The apparent transient action of certain drugs.

(2) The stimulation effects observed in isolated tissues when the drug is washed out by immersing the tissue in Locke's solution free from drugs (washout phenomena).

The whole subject of the polyphasic action of drugs has recently been revised by Rentz (1929). He shows that an enormous variety of phenomena have been reported by various workers. In general the isolated frog's heart shows a fairly uniform response to drugs but in the case of plain muscle and particularly in the case of the isolated gut, the reaction of the tissue to the drug usually shows a remarkable variation, for drugs often produce first one effect and later the reverse effect.

Rentz concludes that the potential theory of drug action can only explain a small proportion of the effects observed and he suggests that probably the/

the presence of the drug produces slow changes in the tissue and that such changes are the reason for the variation in response observed.

The fact that polyphasic responses are so much common with the isolated gut than with the isolated heart suggests ~~that~~ the probability that they depend on some peculiarity of the tissue rather than on any general law of drug action.

The recent work of Winton (1930) indicates the nature of the changes that may occur in plain muscle. Winton shows that plain muscle contains at least two components, namely a phasic contractile and a postural contractile mechanism, and these may be acted on differently by drugs. For example, in the case of the retractor penis adrenaline causes a contraction and at the same time increases the elasticity of the tissue, hence the response, under appropriate mechanical conditions may be a lengthening followed by a sustained contraction.

Clark (1926) analysed the response of the rectus abdominis to excess of KCl. This muscle reacts to drugs in a manner more resembling plain muscle than ordinary skeletal muscle. Isotonic records showed a sustained contraction, but

isometric/

isometric records showed a twitch of relatively short duration. In this case apparently the drug stimulated a mechanism which caused the production of a considerable tension and this action was transient, and at the same time stimulated a second mechanism which produced a sustained contraction associated with very feeble tension.

Brocklehurst (1926) noted the effect that alterations of initial length produced on the tension of a plain muscle, under the influence of histamine. He notes that a plain muscle cannot be said to have any definite "unloaded length" but exhibits very great variations in length on the application of minimal loads and considers it to be an initial difficulty in the experiments with that muscle. By using an isometric lever he first lengthened the muscle (rabbit's ileum) by stages and then allowed it to shorten back to its original length. His findings are that the resting curve recorded by the muscle when it is allowed to shorten back to its original length shows a smaller tension at the greater length than does the resting curve during/

during lengthening process. This difference in tension he ascribes to the viscosity of the muscle and to the stretching of the non-muscular elements of the gut. He also found that the tension developed on histamine contraction depended on the amount of initial tension.

These facts help to explain the great variations noted in the responses of plain muscle to drugs. It seems obvious that the response of a plain muscle to drugs depends on a large number of factors that are at present only partly understood, and that it is highly unsafe to base any theory of drug action on the vagaries of plain muscle behaviour.

Transient Action of Adrenaline.

Many authors have observed that the rise of blood pressure produced by the injections of adrenaline passed off at a time when it was still present in a considerable concentration, e.g. Weiss and Harris (1926), Ehrmann (1905), Meltzer and Meltzer(1903), Dixon (1925), Gaddum (1926), Harris and Lipkin (1930), Kretschmer (1907)

Kretschmer, however, showed that if adrenaline was/

was injected into a rabbit slowly at a constant rate, a rise of blood pressure could be maintained for a prolonged period. Therefore the transient action of adrenaline on blood pressure appears to be due to circulatory adjustments made to meet a sudden change in the pressure. Similar re-adjustment is also seen if instead of raising blood pressure, it is reduced by nitrites, venesection etc.

Harris and Lipkin in their paper on high blood pressure describe the following experiment:-

A cat was given an injection of adrenaline (by Harris) and the pressure registered. After the systolic pressure had returned to normal, the blood from this cat was taken and injected into another cat. The adrenaline effect was demonstrable in the second one, although when the blood was taken from the first animal, the effect of adrenaline showing rise of blood pressure had already disappeared.

This experiment of Harris demonstrates conclusively that although a large amount of adrenaline is present in circulation for some time, it is unable to raise blood pressure for any length of time, for the simple reason that the organism counteracts/

counteracts any disturbances of the equilibrium in the level of blood pressure.

Other authors have observed the transient action of adrenaline on the isolated gut, e.g.

Jendrassik and Mosen (1924) showed that the action of the drug passed off at a time when active concentration of adrenaline was still present in the bath.

Gautrelet and Bargy (1925) noted that if repeated doses of adrenaline were given to an isolated intestine the tissue gradually acquired tolerance. These results will be discussed later, but it is important to note that they are not observed with all isolated tissues. For instance in the case of the isolated rabbit's uterus, Gaddum found no such tolerance. He concluded that adrenaline was very easily destroyed even in an empty bath, so that the (uterine) contraction was maintained at its full height only for a few minutes. If Ringer solution was transferred to another bath with a fresh piece of uterus when the contraction of the first piece had decreased to half its maximum value, the second uterine piece only gave half the contraction/

contraction of the first one, so that fatigue or tolerance was not a very important factor in the falling off of the contraction. However, if the concentration of adrenaline were maintained by perfusing the bath with Ringer containing adrenaline, the height of contraction was maintained.

Elsewhere he observed that if adrenaline was left in the bath until it had practically exhausted its effect on one piece of uterus, then on transferring the solution (containing that adrenaline) in the upper bath to the lower one it produced a large contraction of the other muscle.

Other authors express the following opinions regarding the transient or fugitive effect of adrenaline, seen both in vivo as well as in vitro:

Dixon (1925) says: "The introduction of adrenaline into circulation at all times produces a very fugitive effect, and adrenaline is destroyed. This destruction apparently goes on at the "nerve endings" until these are saturated; for we know that after perfusing the drug through innervated vessels only a certain amount is destroyed. What apparently happens is a combination between adrenaline/

adrenaline and some constituent at the periphery, which results in the stimulation of the muscle and when all this latter substance is used up, the adrenaline circulates free in blood and produces no further effect."

Straub (1907) holds that adrenaline like muscarine acts only in the process of permeation into the cells affected by it.

Dale (1915) considers its action to cease because of adrenaline being oxidised in alkaline medium.

The transient action of adrenaline and acetyl choline has therefore been explained on three theories:-

(1) Destruction of the drug (Dale, etc.)

(2) Exhaustion of receptors (Dixon)

(3) Potential action (Straub and Jendrassik)

As regards these three theories the first is certainly not adequate because many authors have shown that an efficient concentration of adrenaline can persist after its apparent action has passed off. The other two theories therefore alone require/

require consideration, although the possibility of destruction of the drug is an experimental error which always requires to be controlled.

Acetyl choline is another example of drugs showing transient action in vivo, which according to Dale and Ewins (1914) is due to its being quickly hydrolysed into its constituents in the circulating blood, but which, also observed and emphasised by Dale and we shall see later on, can show its action for an indefinite period under certain conditions, when allowed to act on isolated organs.

(2) Wash-out Stimulation.

Neukirch (1912) noted that after a piece of isolated gut had been soaked in high concentrations of pilocarpine, a powerful stimulation occurred when the gut was placed in fresh Tyrode solution. He observed that this wash-out stimulation was abolished by atropine.

Kuyer and Wijsenbeek (1913) showed that similar effects were produced with numerous drugs and with various tissues such as uterus of rabbit and guinea pig as well as excised intestine. In all cases they found that the concentrations needed to/

to produce a wash-out effect were greatly in excess of that needed to produce stimulation.

Trendelenburg (1913) observed the same phenomena in bronchial mucosa which responded by strong contraction when put in solutions of muscarine, pilocarpine and arecoline. On washing the muscle its constriction did not go off, rather on the contrary further constriction developed.

This evidence seems to be of doubtful significance because the results obtained on washing off the drugs could also be produced if the drug exerted a specific action on the cell surface and in addition a paralytic action after it had entered the cell. In this case the drug in high concentrations would produce stimulation followed by depression and, on washing out the drug, the stimulation might be produced by the drug that emerged from the cell and maintained an excitant concentration for some time at the cell surface.

Experimental/

Experimental Methods.

Strips of tissue were set up in a bath of 25 c.c. and the movements were recorded with isotonic levers. The Locke's fluid used had the following percentage: NaCl 0.9, KCl 0.042, $CaCl_2$ (anhydrous) 0.024, $NaHCO_3$ 0.05, glucose 0.05; pH 7.4.

I. Action of Sympathomimetic Drugs.

(1) Mode of action of adrenaline on isolated gut.

It has already been mentioned that the action of adrenaline on the isolated rabbit's gut is usually considered to be a typical example of the transient action of this drug. I found, however, that the nature of the response obtained depended largely on the condition of the gut. Fig. A.1.a. shows a typical example of the transient action of adrenaline on the ileum of the rabbit. This was obtained from a freshly excised strip and the reaction consisted of a diminution in the pendulum movements followed by a rapid recovery. The same strip, however, after it had been isolated for an hour gave the totally different response in Fig. A, 1.b./

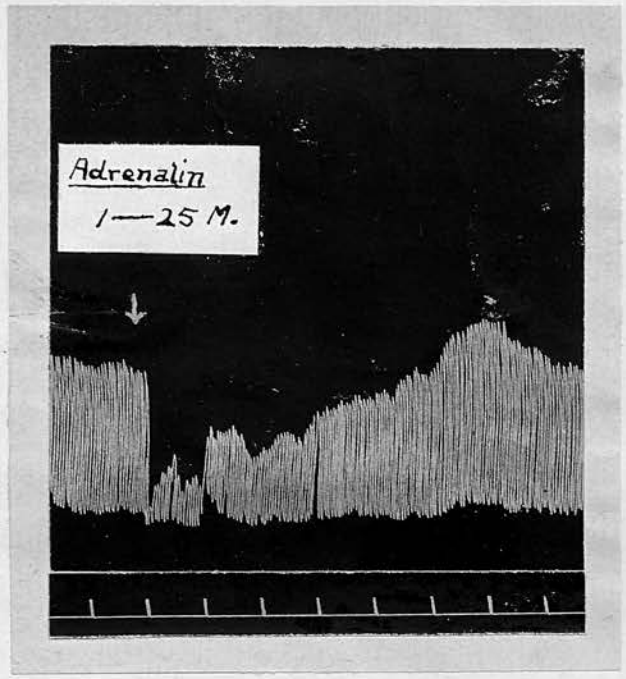


Fig. Ⓐ 1.a. showing a typical example of transient action of adrenaline on rabbit's ileum (fresh preparation).

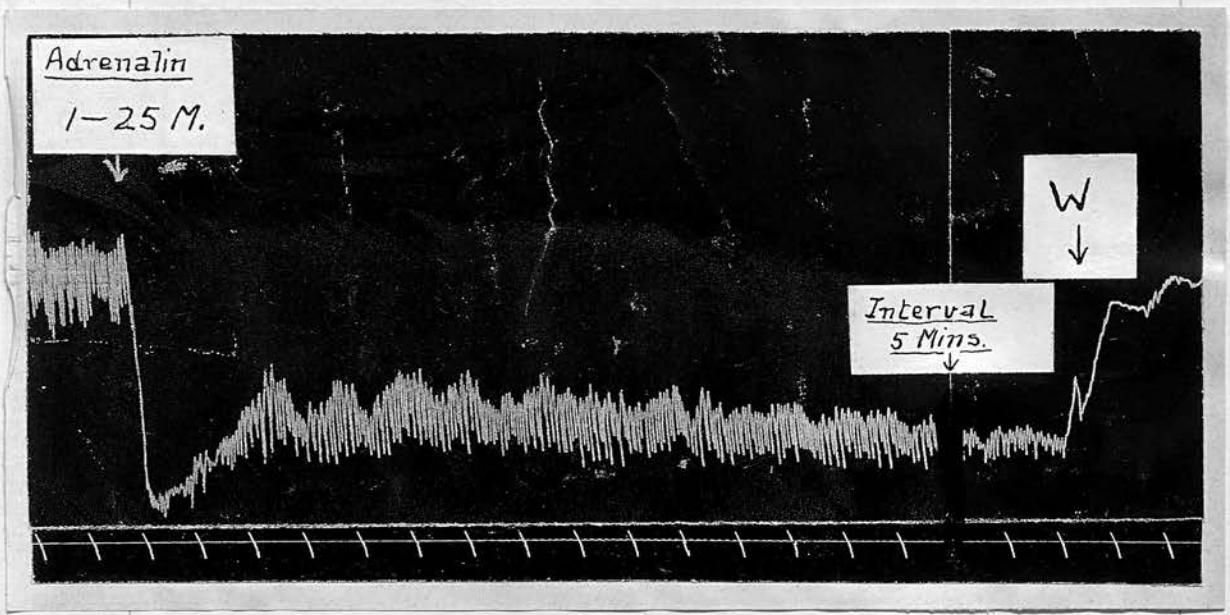


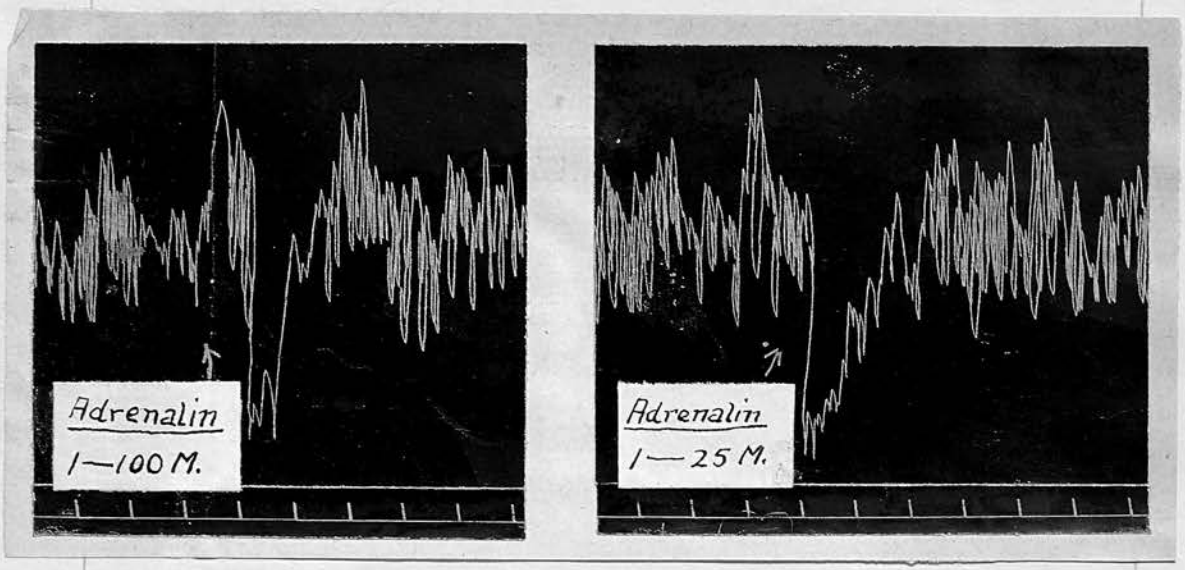
Fig. Ⓐ 1.b. The same preparation of ileum as in Fig. A.1.a. showing permanent lowering of tonus about an hour or so after.

The concentration of adrenaline was the same as before but in this case the drug produced not only a diminution in the pendulum movements but also a fall in tonus. The pendulum movements recovered quickly but the fall in tonus was maintained for 20 minutes.

In general I observed that the action of adrenaline in decreasing pendulum movements was always transitory, but that when the drug produced a fall in tonus, this fall was permanent.

Fig. Ⓐ 2.a-d shows the effect of increasing the concentration of adrenaline.

Fig. /



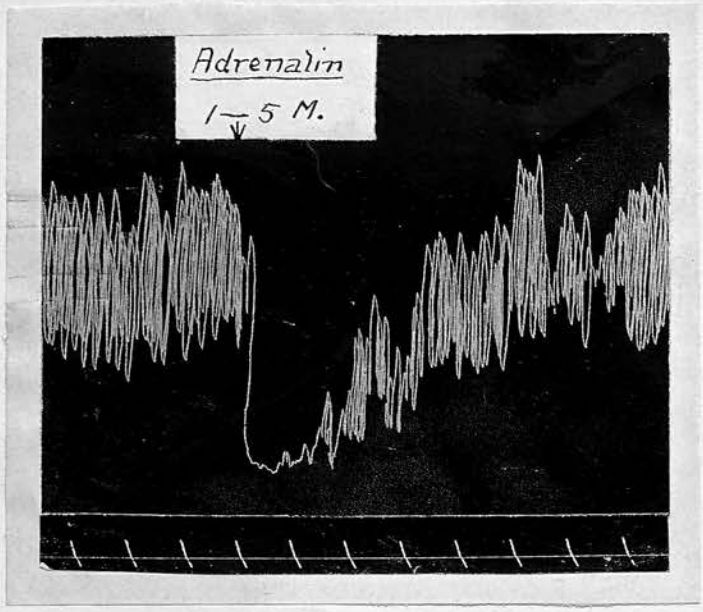


Fig. Ⓐ 2.a-d. showing the effect of increasing doses of adrenaline. Low concentrations produced a transient effect followed by recovery, both as regards fall in tonus and diminution in pendulum movements, while a concentration of 1 in 1 million adrenaline also produced a transient diminution in the pendulum movements but the fall in tonus kept permanent.

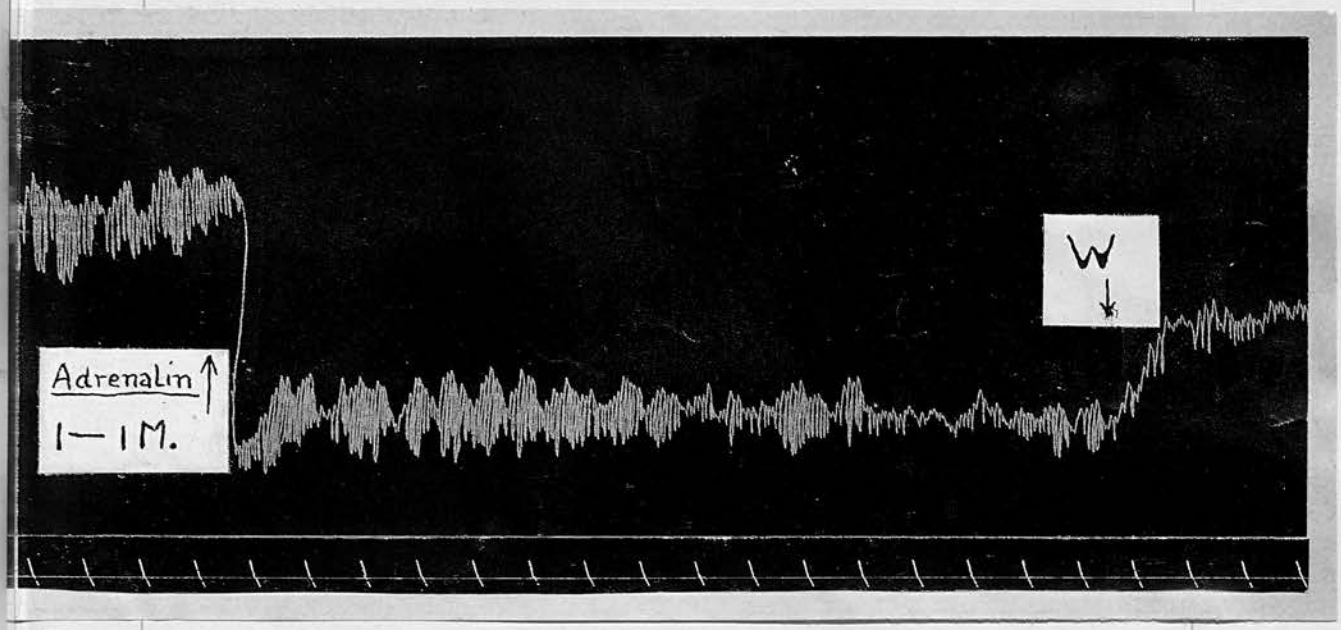


Fig. Ⓐ 2. d.

Low concentrations produced a transient effect followed by recovery both as regards fall in tonus and diminution in pendulum movements, a concentration of 1 in 1 million adrenaline also produced a transient diminution in the pendulum movements,but the fall in tonus was permanent.

The adrenaline action therefore appears transient if little fall of tonus occurs, but appears permanent if a large fall of tonus occurs. The amount of action on the tonus depends on a number of factors such as the weight of the lever and the freshness of the preparation. Moreover it depends upon the portion of the gut from which the strip is taken as is shown in Fig. Ⓐ 3.a-c. In this case the ileum (a) showed large pendulum movements and little change in tonus and hence the adrenaline action appeared to be transitory, whereas the colon (b) and duodenum (c) showed small pendulum movements and extensive tonus changes and hence the action of adrenaline appeared to be permanent.

Fig. /

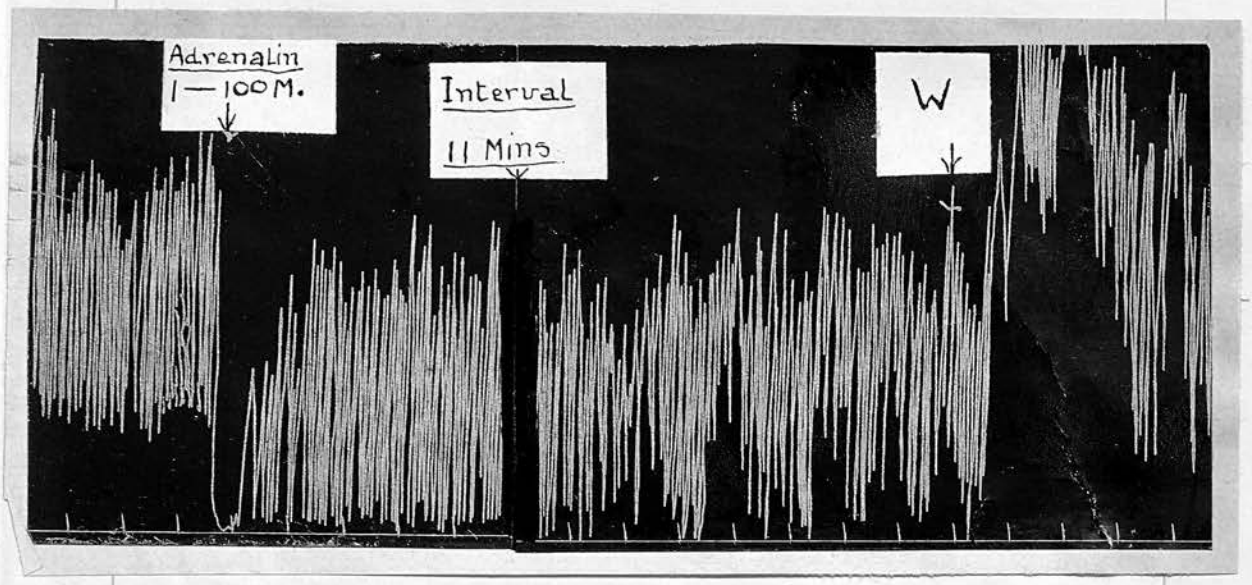


Fig. Ⓐ 3.a. Preparation from rabbit's ileum showing large pendulum movements. Adrenaline produced transitory action.

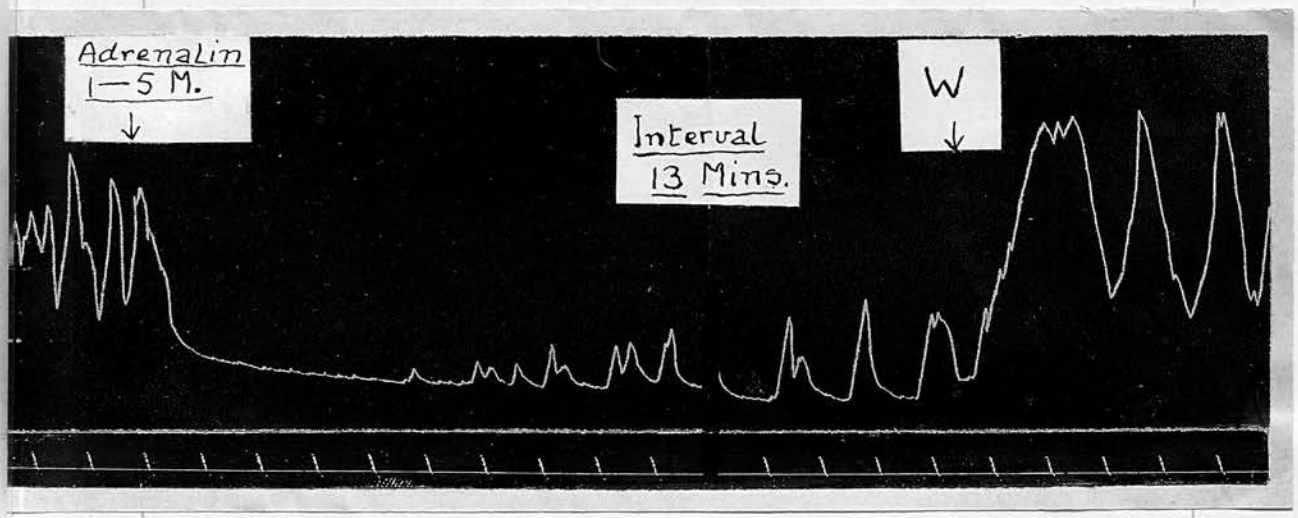


Fig. Ⓐ 3.b. Preparation from rabbit's colon showing small pendulum movements and extensive tonus changes. Adrenaline produced permanent action.

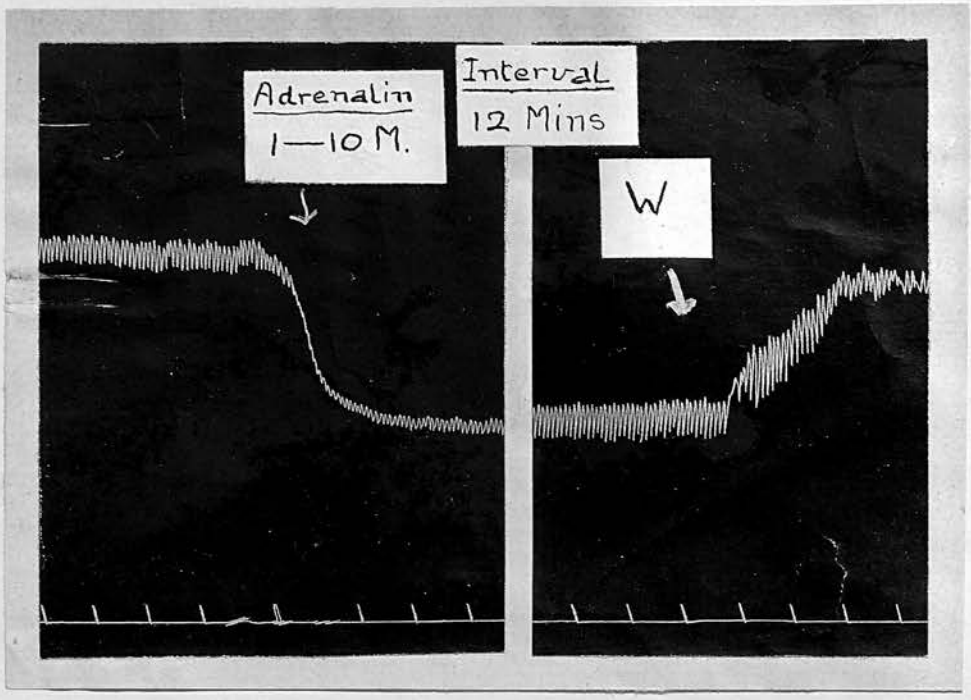


Fig. Ⓐ 3.c. Preparation from rabbit's duodenum showing small pendulum movements. Adrenaline produced permanent action.

(2) /

(2) The Transient Action of Adrenaline on Pendulum Movements.

I have confirmed the statement of previous authors that the pendulum movements of the gut recover long before there is any measurable loss of adrenaline by oxidation and that further addition of adrenaline produces little effect. The gut therefore shows a true tolerance to adrenaline as far as pendulum movements are concerned, but it shows no such tolerance as regards loss of tonus.

The pendulum movements and tonus of the gut show other differences in their pharmacological responses for I have shown (1931) that the ergotamine readily prevents the action of adrenaline on the pendulum movements of the gut, whereas it has little influence on the tonus lowering produced by adrenaline.

Clark and Gross (1922) also noted that in the rat's gut lack of oxygen produced profound changes in tonus but did not affect the pendulum movements.

This distinction cannot be easily explained on the/

the theory that one effect is neurogenic and the other effect myogenic because both the tonus and the pendulum movements appear to be myogenic.

Gasser (1926), however, while believing that Auerbach's splexus is not necessary for pendulum movements, hold that with the exception of nicotine, the other drugs as pilocarpine, physostigmine, atropine and adrenaline, produce their effects by direct action on the muscle fibres.

Neither the theory of occupation of receptors nor the potential theory of drug action is a satisfactory explanation of this partial tolerance of the isolated gut to adrenaline, for on both these theories a tolerance both as regards pendulum movements and tonus should occur.

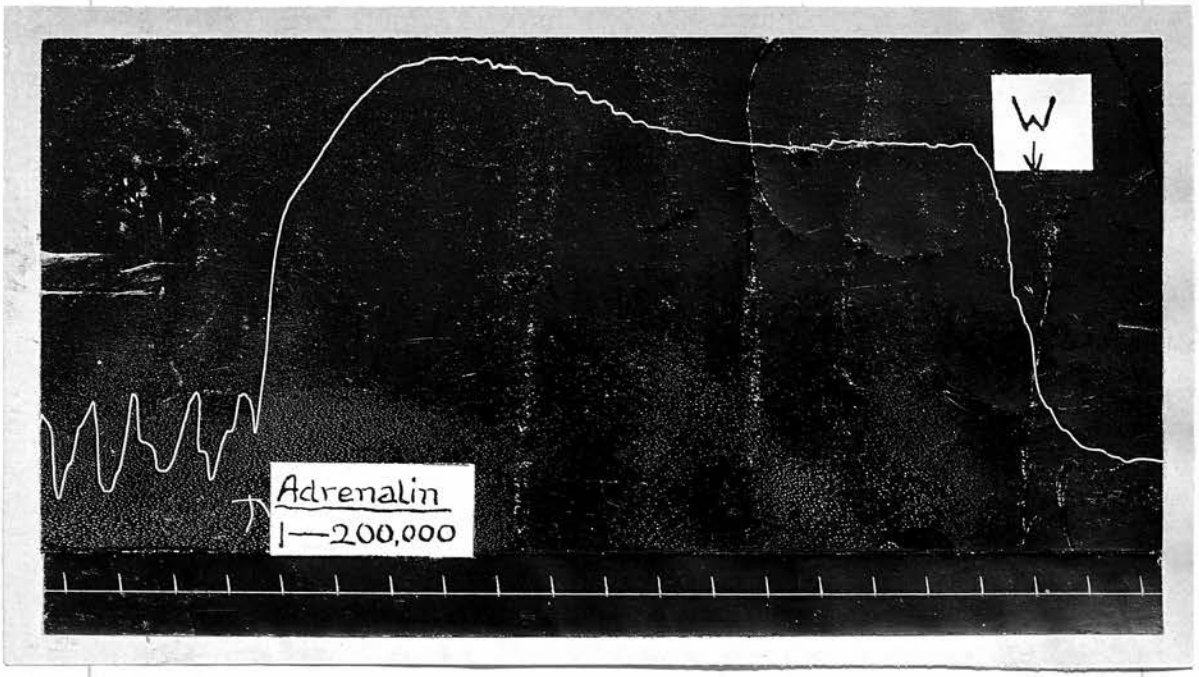
The most probable explanation appears to be that adrenaline produces some change in the gut, and this is followed by some secondary change that restores the pendulum movements but does not influence the tonus.

(3)/

(3) Action of Adrenaline on the Rabbit's Uterus.

I confirmed Gaddum's conclusion that the rise of tonus produced by adrenaline in the rabbit's uterus is practically permanent. The prolonged and steady nature of the response is shown in Fig. A 4.a and b.

Fig. /



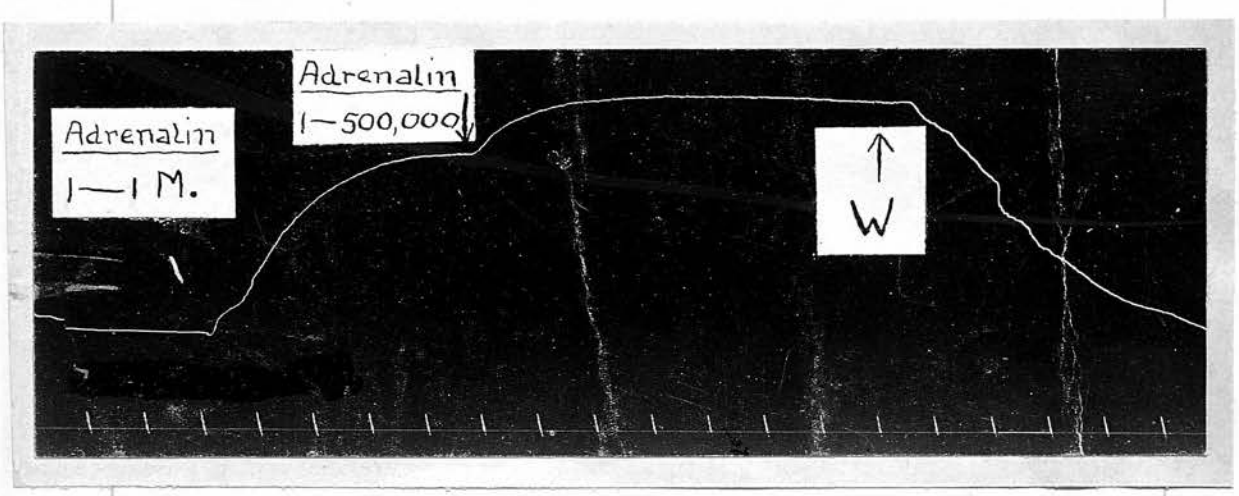


Fig. A 4.a and b. Rabbit's uterus. Adrenaline showed its permanent action by causing sustained rise in tonus; in the preparation (b) another dose of adrenaline given shortly after the first one led to further rise in tonus.

Action of Parasympathomimetic Stimulants.

(4) Action of Acetyl Choline on the Gut.

This drug is known to be broken down quickly by an enzyme when injected into the living animal and this explains its transient action in vivo.

Clark (1927) has shown that it is slowly destroyed by isolated tissues of the frog and the same is true in the case of mammalian tissues (Dale and Ewins, 1914).

The action of the drug cannot therefore be maintained indefinitely, but in addition it is possible under certain experimental conditions to produce what is apparently a transient action. This effect is shown in Fig. AC.1.

Fig. /

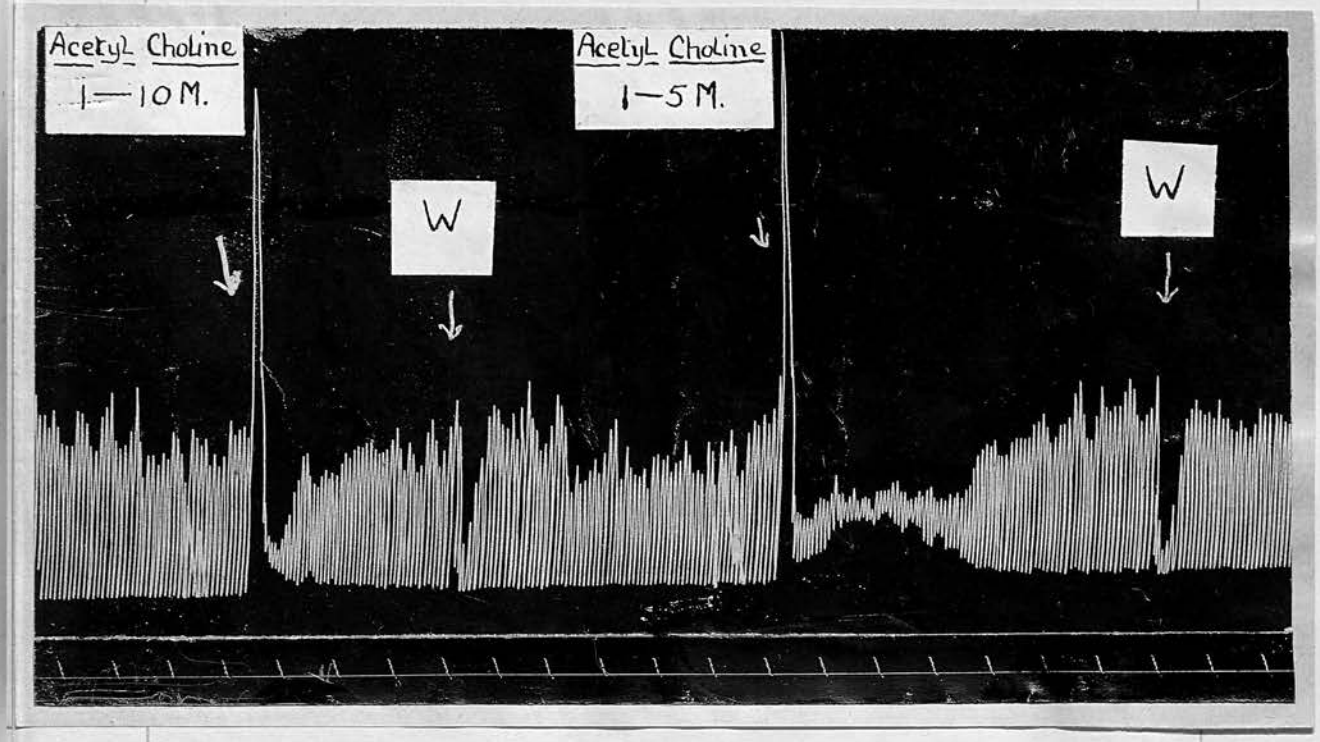


Fig. AC. 1. Preparation from rabbit's ileum showing transient action of acetyl choline, i.e. a twitch-like contraction accompanied and followed by temporary inhibition of its pendulum movements.

As is seen in this figure the first dose of acetyl choline produces an action that is apparently completed in about 2 minutes, and the second dose appears also to be transient. The reason for this appearance is shown in Fig. AC.2.

Fig./

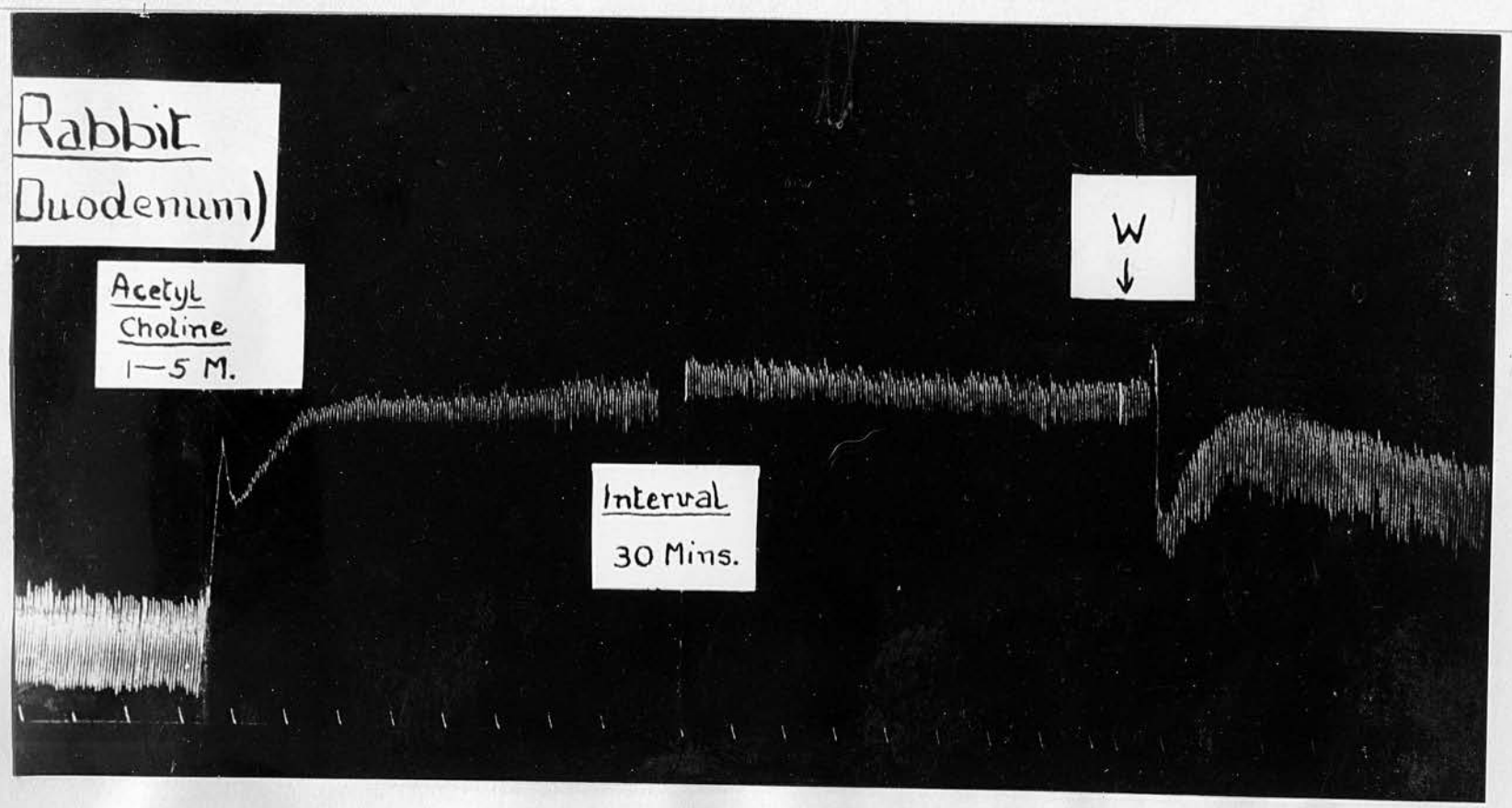


Fig. AC.2. Preparation from rabbit's duodenum showing double action of acetyl choline, a rapid twitch followed by a sustained rise of tonus. If the tissue is feeble or lever heavy, this second action seen in sustained rise of tonus is abolished and only the initial twitch is shown.

This shows that the action of acetyl choline is a double one, namely a rapid twitch followed by a slow sustained rise of tonus. If the tissue is feeble or lever heavy this second action is abolished and only the initial twitch is seen. Thence the transient action shown in Fig. AC.1. is produced. Fig. AC. 3 and 4 show the alteration in the response that can be produced simply by altering the weight of the lever. Fig. AC.5 shows how in an exhausted piece of gut the rise of tonus is not maintained and hence an apparently transient action results.

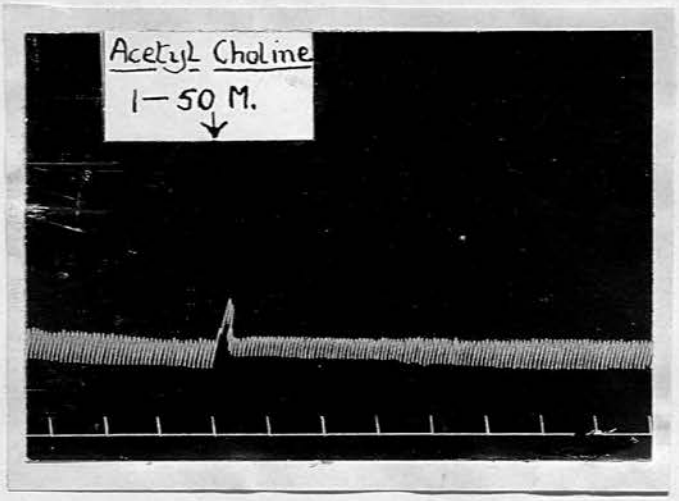


Fig. AC. 3. Preparation from rabbit's duodenum showing temporary action of acetyl choline seen in twitch-like response when loaded with heavy weight.

Fig./

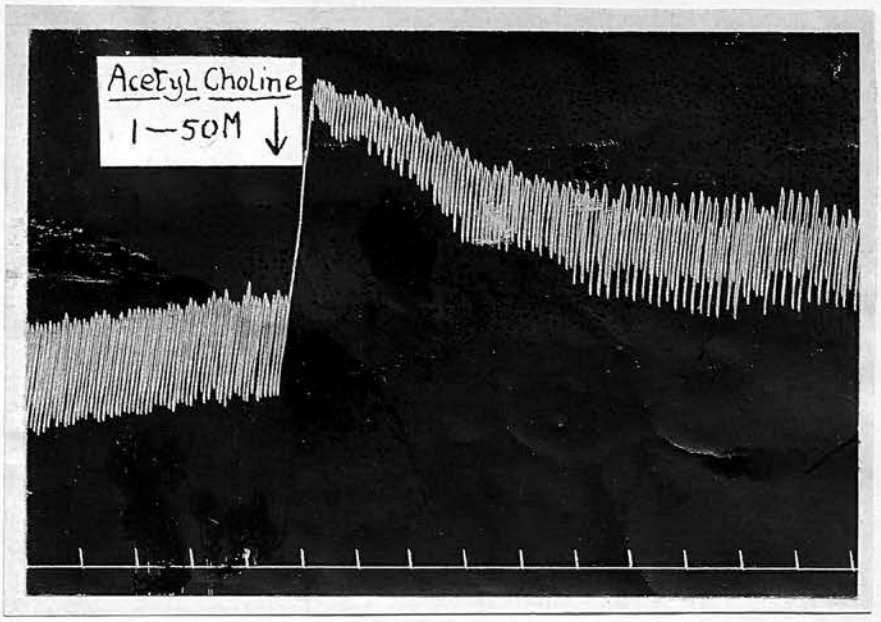


Fig. AC. 4. The same preparation as in Fig. AC. 3 showing permanent action of acetyl choline seen in sustained rise of tonus, when loaded with light weight.

Fig. /

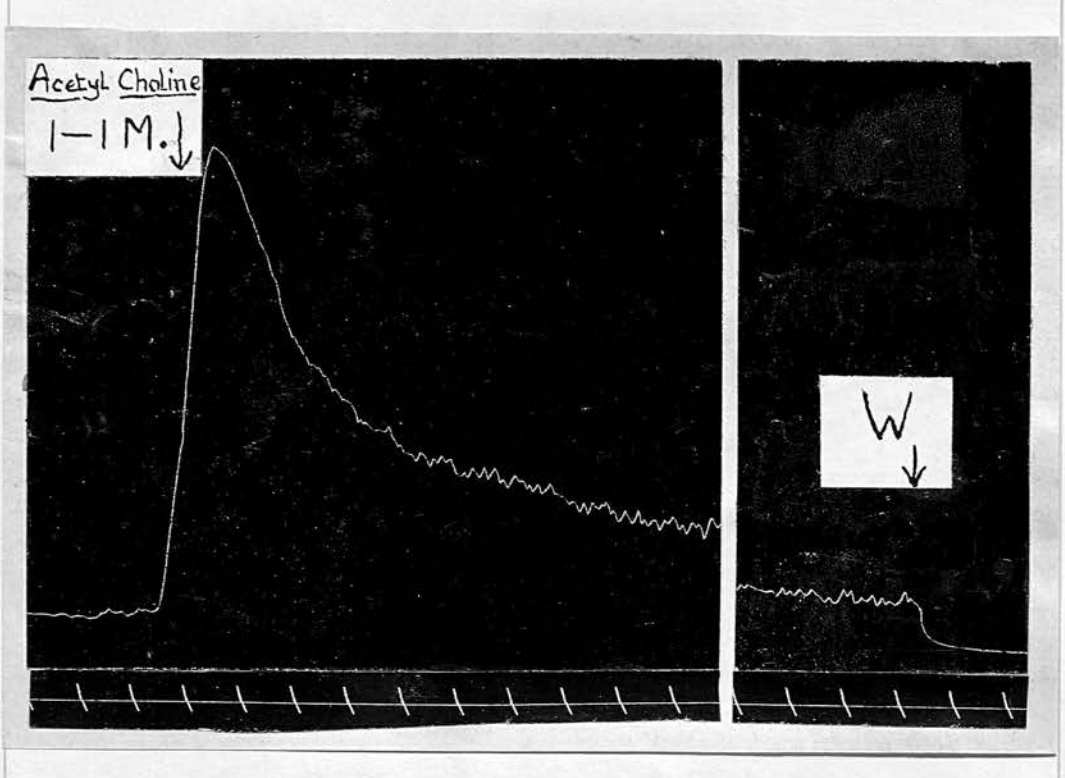
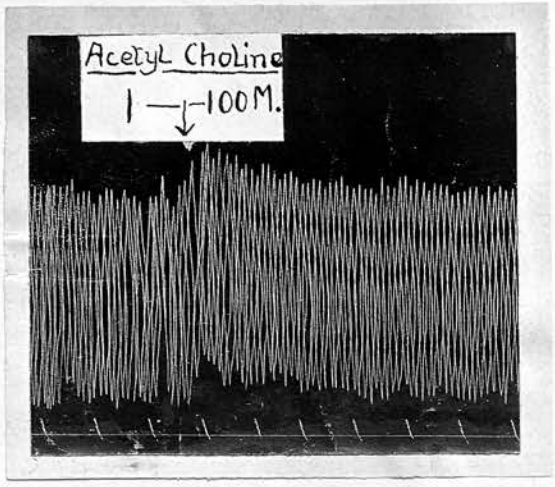


Fig. AC. 5. Preparation from rabbit's colon showing the transient action of acetyl choline when the strip is in exhausted condition.

Those portions of the gut which show most extensive tonus changes, show the clearest response to acetyl choline. This difference is shown in Figs. AC. 6, 7 and 8, which show the actions of acetyl choline on ileum, duodenum and colon respectively.

Figs./



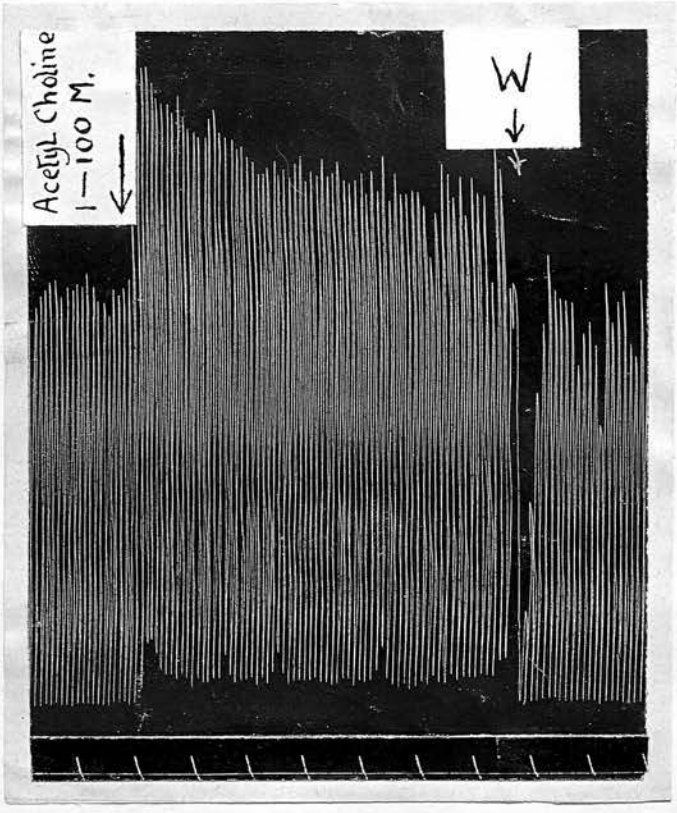


Fig. AC. 6 (a) and (b). Preparations from rabbit's ileum showing the action of acetyl choline on pendulum movements only with little change in tonus.

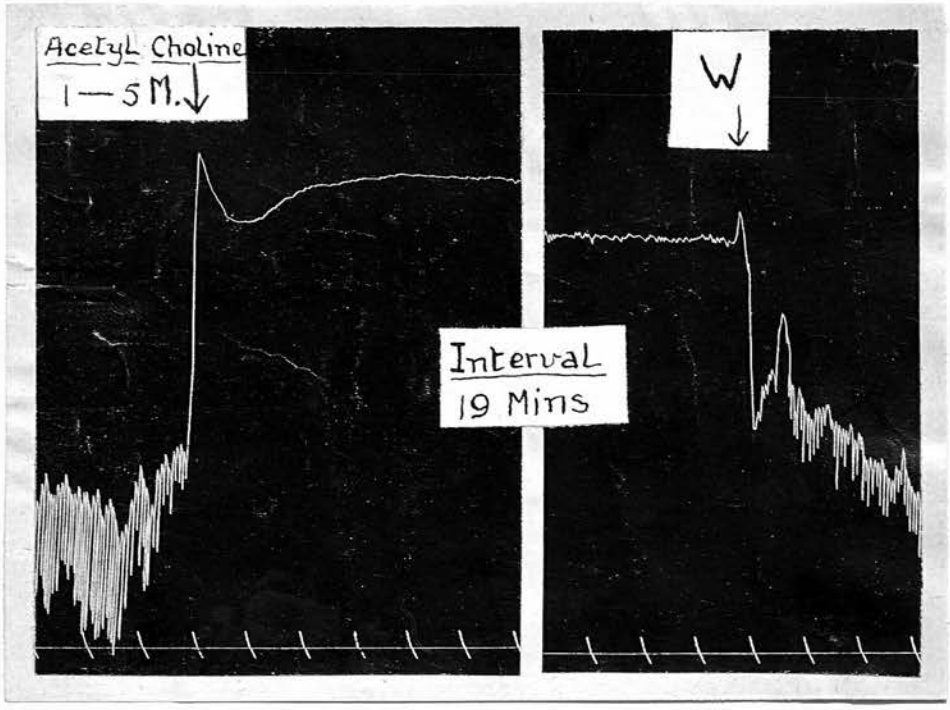


Fig. AC. 7. Preparation from rabbit's duodenum with extensive changes in tonus showing the action of acetyl choline on tonus of the gut along with the inhibition of pendulum movements. Note the high concentration (1 in 5 million) of acetyl choline as compared with low concentration (1 in 100 million) used in the case of ileum in the last figure.

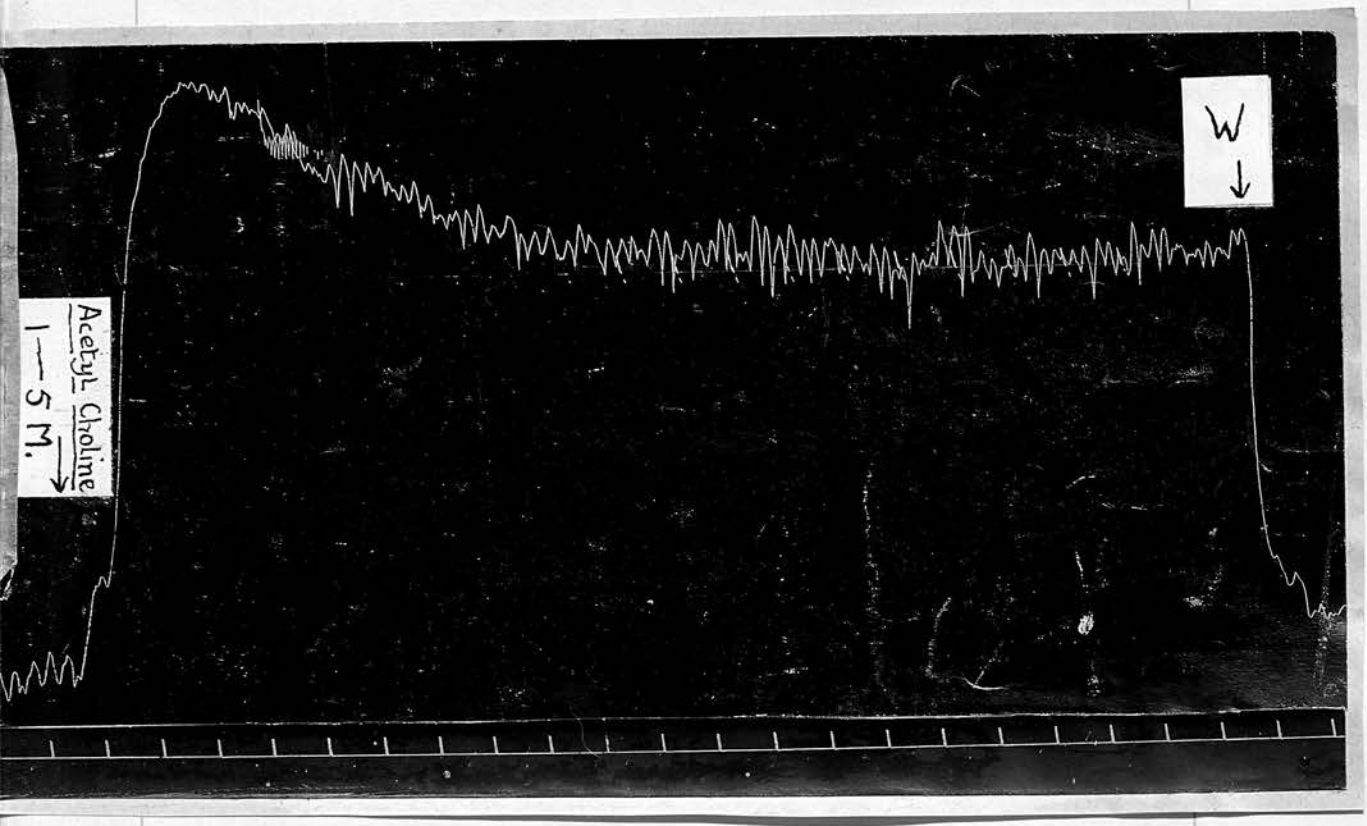


Fig. AC. 8. Preparation from rabbit's colon with extensive tonus changes showing the action of acetyl choline on tonus of the gut without much effect on pendulum movements. Note the high concentration (1 in 5 million) of acetyl choline as compared with low concentration (1 in 100 million) used in the case of ileum in Fig. AC.6.

The/

The response of the uterus to acetyl choline is very varied. Two extremes are shown in Figs. AC. 9 and 10.

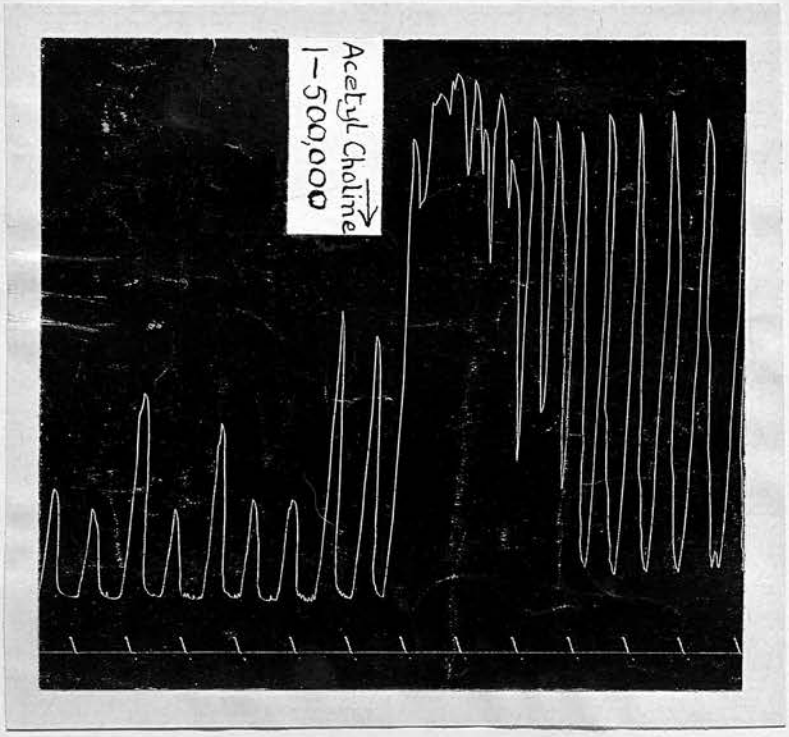


Fig. AC. 9. Preparation from rabbit's uterus showing transient action of acetyl choline on tonus followed by increased pendulum movements.

Fig. /

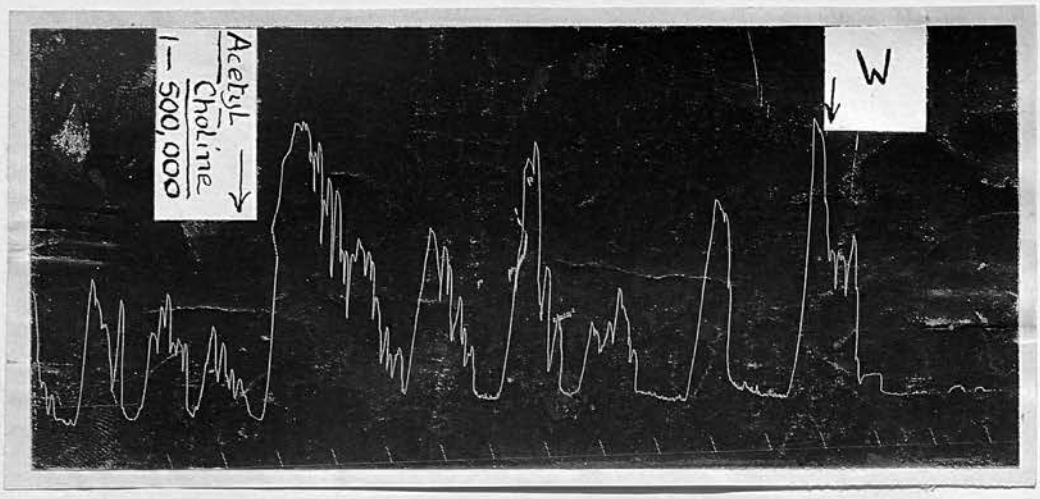


Fig. AC. 10. Preparation from rabbit's iterus showing temporary action of acetyl choline on tonus only without much effect on pendulum movements.

In the first case there is a rise of tonus which is temporary, and this is followed by increased pendulum movements. In the second case the tissue is in a poor condition and the action of the drug appears to be temporary.

The action of acetyl choline on the isolated tissues appears to consist in an initial powerful action followed by a sustained less powerful action. Under/

Under favourable conditions, when the whole response is recorded, the response continues as long as the drug is in contact with the tissue, but if the experimental conditions are imperfect then only the initial effect is recorded and the action appears to be temporary.

(5) Action of Pilocarpine on the Gut.

This drug is usually stated to have transient action, but I found that under suitable conditions the action was maintained indefinitely. Fig. Pil. 1. shows an experiment that was continued for nearly an hour and for the whole of this period the drug maintained a rise of tonus and this was rapidly abolished by washing out.

The result of this experiment seems completely antagonistic to the potential theory of action.

Fig. /

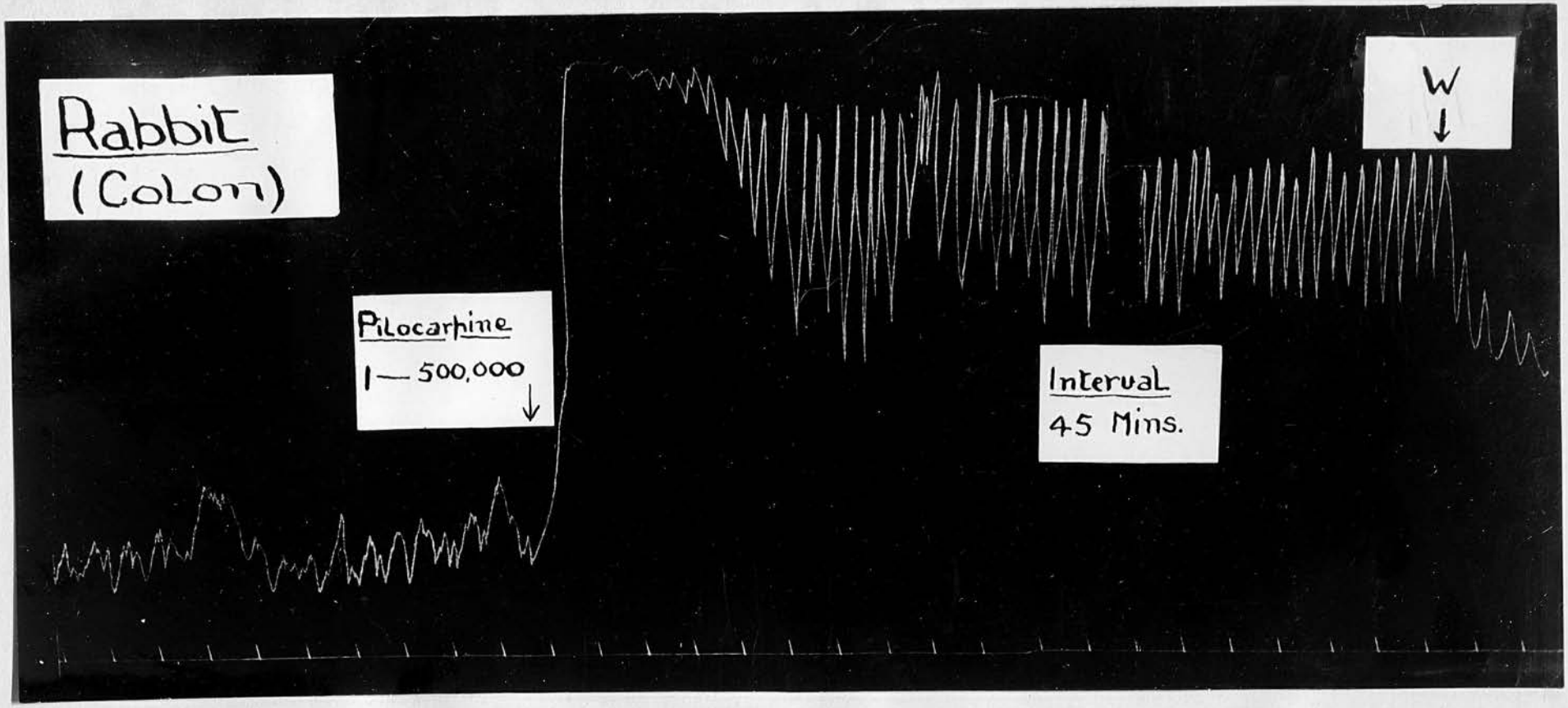


Fig. Pilo. 1. Preparation from rabbit's colon showing permanent action of pilocarpine both on tonus and on pendulum movements. The concentration of the drug, 1:½ million, caused sudden rise of tonus sustained over an hour or so till wash as well as increased pendulum movements simultaneously maintained all the time till wash.

(6) Action of Potassium (KCl.)

The author measured the tension in the frog's excised stomach by means of a tambour. The introduction of extra fluid (Locke's solution), e.g. 1-2 c.c. at a time into the stomach cavity caused an immediate rise of tension which fell rapidly. Exactly similar curves were obtained by adding KCl solution in excess to the bath containing frog's Locke solution in which the stomach was mounted.

These effects can be explained by Winton's theory of two components, i.e. a quick elastic component and a slow viscous component.

Injection of fluid into the stomach cavity causes a rise of pressure but the viscous component rapidly relaxes until pressure returns to normal.

Addition of potassium chloride solution in excess apparently stimulates the elastic component and under isometric conditions the tension set up by the rise of pressure causes viscous component to relax and the pressure falls. On the other hand under isotonic conditions shortening occurs and a more or less permanent equilibrium is maintained at a new length.

In/

In tracings vide Fig. KCl.1 is shown potassium action on frog's stomach side by side with the response of that organ to Locke's solution injected into the cavity. Sudden rise of tonus followed by quick fall is seen in one case, i.e. the so-called transient action; and sudden rise followed by plateau fall in the other.

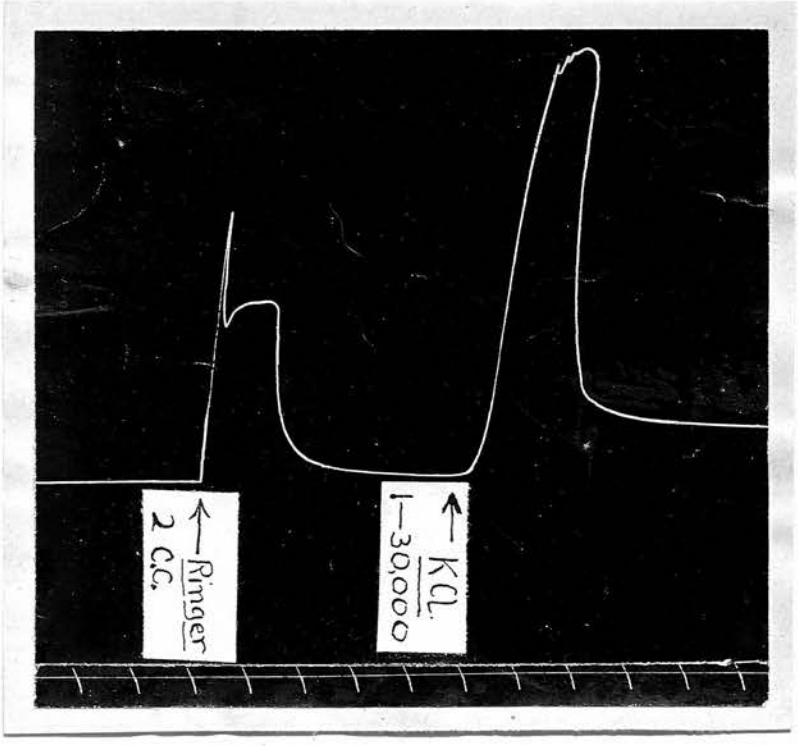


Fig. KCl. 1. The figure shows the changes in pressure in a frog's stomach filled with fluid and immersed in a bath. A sudden increase of filling causes a temporary rise in pressure. The same temporary effect is produced by adding excess of KCl to the bath.

Excess of potassium chloride produces in the rabbit's ileum an action similar to that produced in the frog's stomach, namely a transient contraction followed by relaxation. Prolonged exposure causes complete inhibition of the gut. This effect, namely, a paralysis preceded by a stimulation is very frequently produced by drugs and calls for no special comment. (See Fig. KCl.2.)

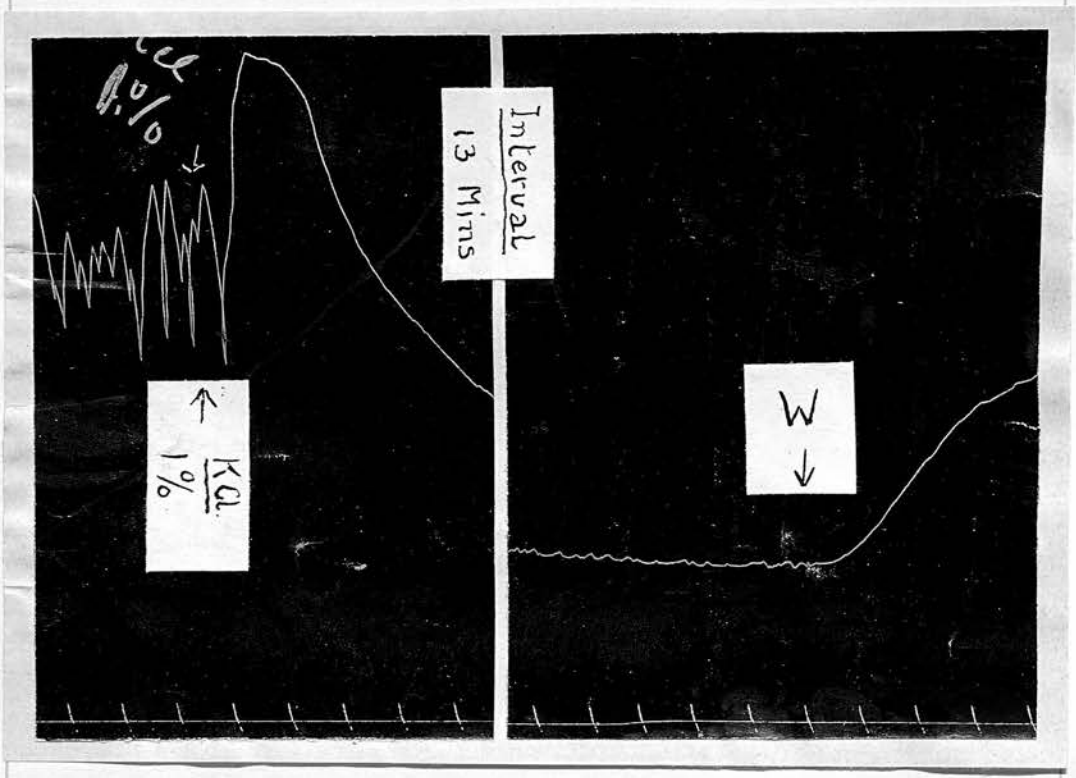


Fig. KCl.2. Preparation from rabbit's ileum showing the transient action of excess of potassium chloride on the tonus of the gut followed by paralysis of the tissue.

Wash-Out Phenomena.

The writer only used moderate concentrations of drugs, and although the tissues were left exposed to the drugs for long periods, yet in most cases the effect of washing out the drug was merely to restore the tissue to an approximately normal condition.

Occasionally, however, contraction on wash-out was observed after acetyl choline. An example of such an effect is shown in Fig. W.O.1. On the other hand some tissues always contracted whenever they were washed out, and the author believes that this contraction of wash-out is associated with the fact that prolonged exposure to acetyl choline renders the gut hyperexcitable. This effect is shown in Fig. W.O.2. The first dose of acetyl choline caused no certain rise of tonus but it caused irregularity of the pendulum movements which persisted until the drug was washed out when a rise of tonus occurred. The wash-out of later applications of acetyl choline caused an S shaped variation in tonus.

In general my observations make me agree with the conclusions of Rentz that the phenomena observed on washing out drugs are so variable that it is almost impossible to draw from them any certain conclusions.

Figs./

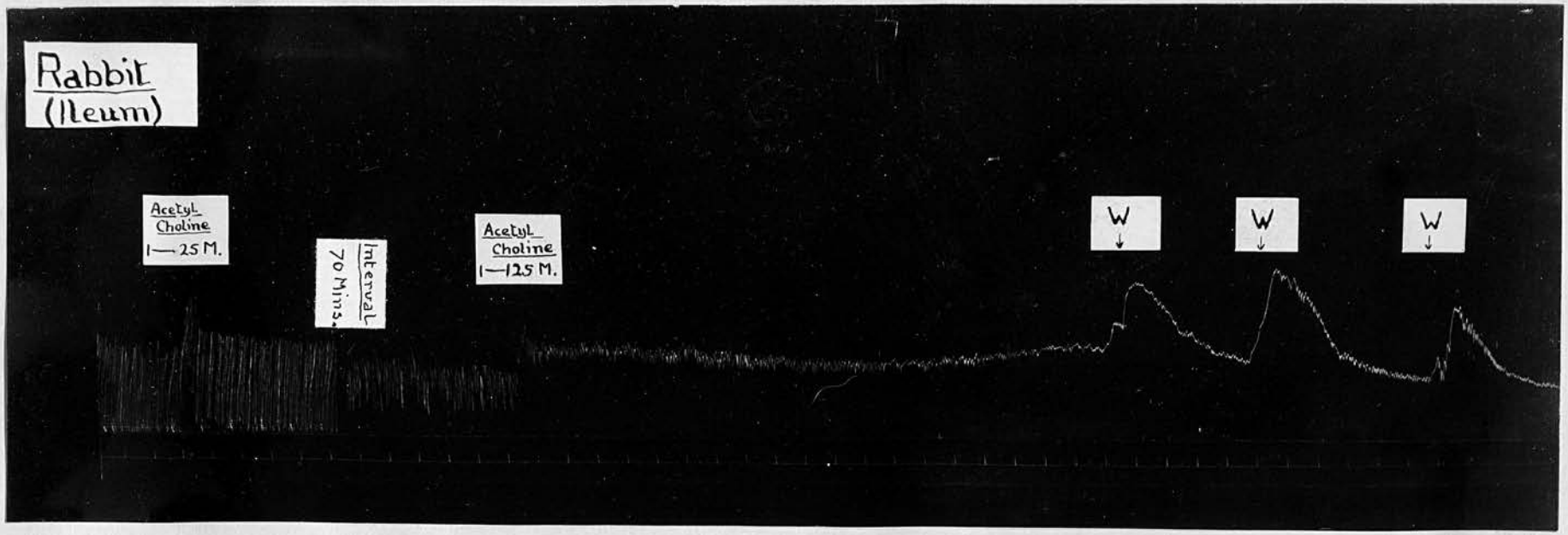


Fig. W.O.1. Preparation from rabbit's ileum exposed to acetyl choline more than two hours and every time after a dose of acetyl choline became more and more sensitive to the succeeding low concentration of the drug. Compare its response to the acetyl choline concentration of 1 in 25 million at the beginning of the experiment with the response to the same concentration about an hour and a half later. On repeating washings the gut always responded by showing stimulation to each wash.

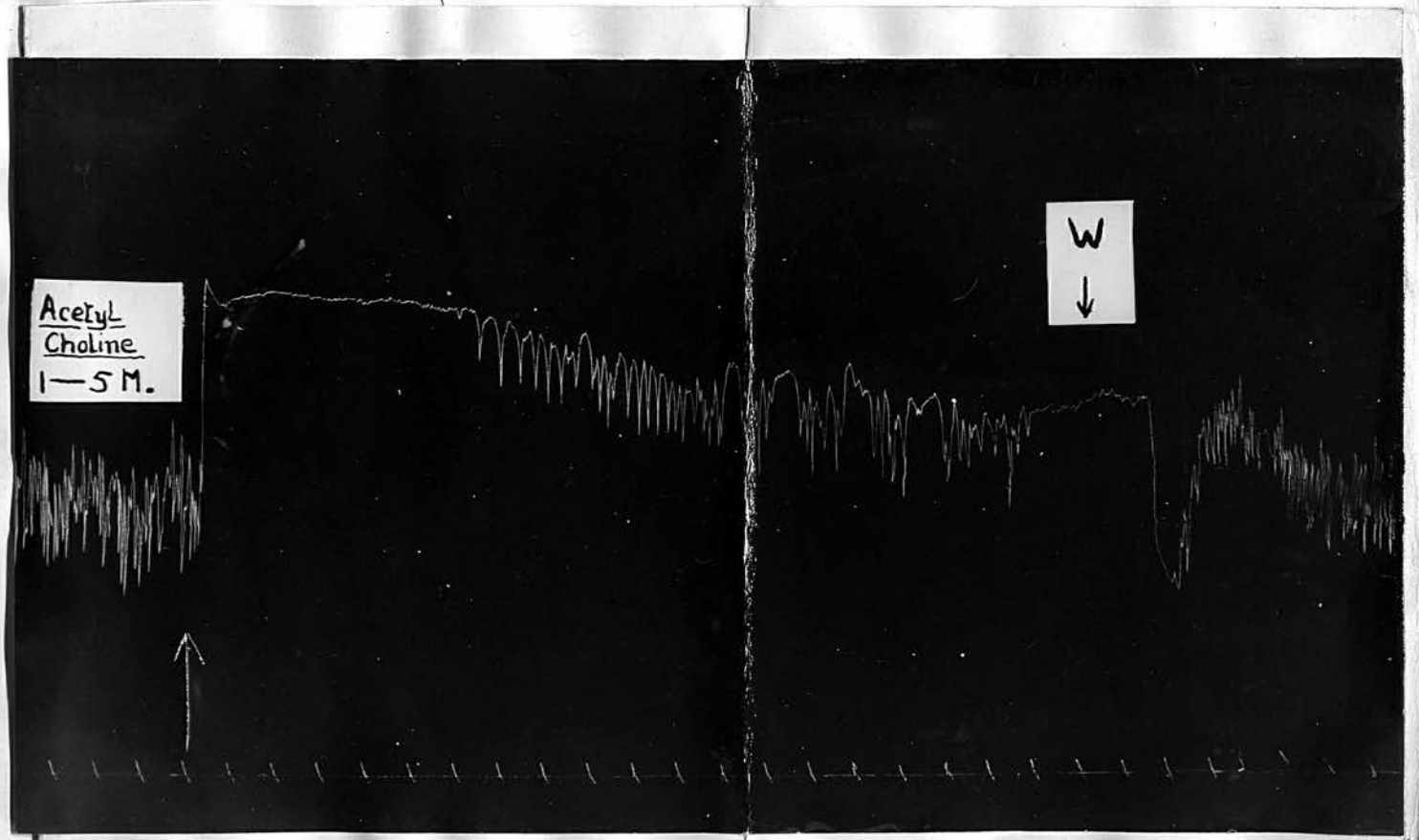
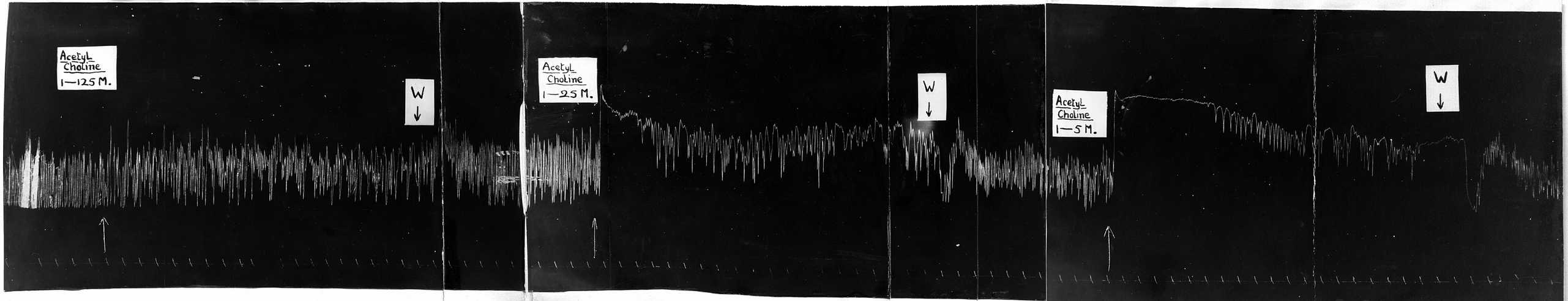


Fig. W.O.2. Preparation from rabbit's ileum showing its hyperexcitability after succeeding doses of acetyl choline. On wash-out only S shaped variations in tonus were observed.


Acetyl Choline 1—125 M.
W
Acetyl Choline 1—25 M.
W
Acetyl Choline 1—5 M.
W

Discussion and Conclusions.

As already noted Winton has shown that the length of plain muscle under any load depends partly on an elastic and partly on a viscous component. He calls one (1) phasic contractile mechanism, and the other (2) postural contractile mechanism.

(1) When the phasic or elastic contractile mechanism comes into play, during the contraction period of the plain muscle under any stimulus, there occurs a relatively rapid contraction of quite transient or temporary nature, in the form of a twitch, and the muscle seems to contract under considerable tension. Thus we see a rapid change in length only maintained for a short time and the action is said to be an apparent transient one.

(2) On the other hand, when the postural or viscous mechanism predominates, the reverse occurs. The muscle on being stimulated responds by contracting with relatively slow changes in length. The contraction is maintained permanently but only a very small tension is developed. All this depends on slow viscous change in the muscle tissue and the action/

action of the drug appears to be permanent or sustained. Brocklehurst has also held more or less the same view as noted above. From his experiments in connection with $\frac{\text{tension}}{\text{length}}$ curves obtained from rabbit's ileum he concludes that the resting curve recorded by the muscle when allowed to shorten back to its original length shows smaller tension, and vice versa. This difference in tension he ascribes mainly to be due to viscous changes in the muscle itself.

Experiments with sympathomimetic, parasympathomimetic and other drugs on the different parts of rabbit's gut and uterus etc. described above show both the temporary and the permanent action of a drug as held by Jendrassik and so the actions shown by these various drugs form a good example of the double actions pointed out by Jendrassik.

On closely studying the nature of these actions, temporary and permanent,one finds how gradually rather imperceptibly one passes into another according to the change or changes in experimental conditions. This observation leads us to conclude that there are valid reasons to explain the two actions/

actions temporary and permanent on the basis of two factors or Winton's two components existing in the plain muscle, i.e. a quick or phasic factor and a slow or postural factor. Accordingly on keeping these two factors in mind, we can interpret the various responses of plain muscle say to adrenaline and acetyl choline. Thus in the tracings shown in Fig. A 2.(a) and (b), quite a temporary action of the drug is seen up to the concentration of 1 in 25 million, then there follows a transition in (c) with 1 in 5 million, and finally a stage is arrived at which full permanent action ensues,(d) with 1 in 1 million).

This phenomena of a quick or phasic factor and a slow or postural factor is beautifully shown by a strip of rabbit's uterus in its response to physostigmine. In the early stage of the experiment after its being mounted in the bath, the uterus appeared to have low tension on account of slow viscous changes as the contraction set up by physostigmine stimulus was a permanent rhythmic or pendulum movements (Fig. PHyso. 1). The same strip of uterus towards the end of the experiment showed a rapid contraction (a twitch) with considerable/

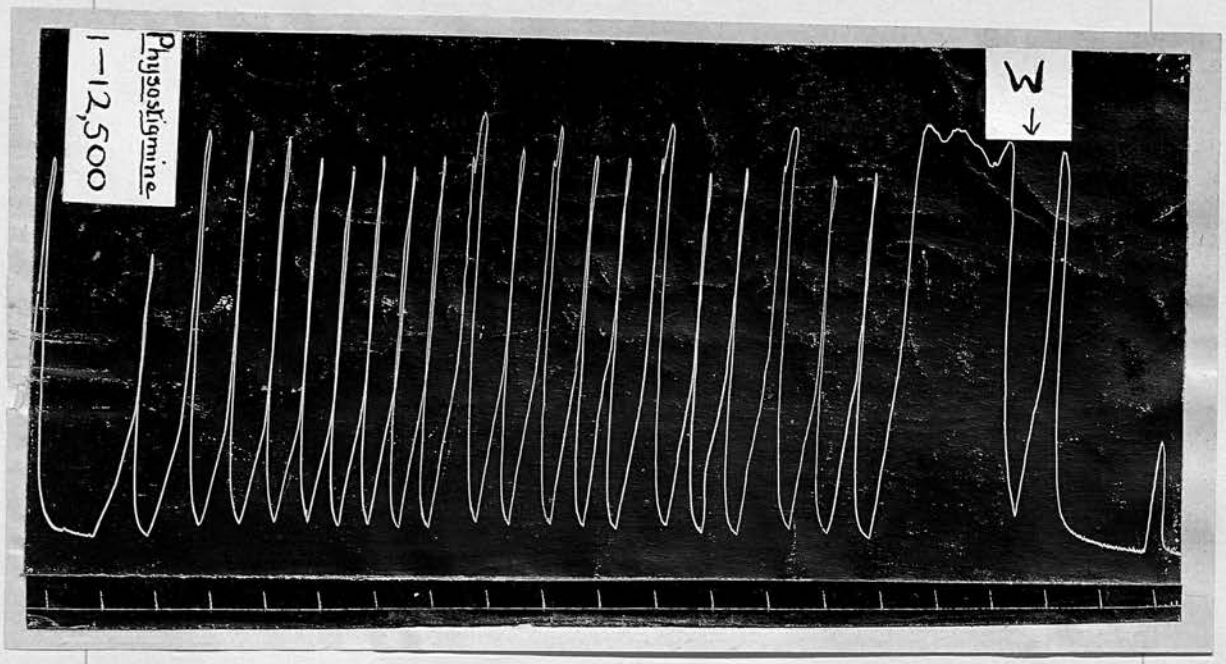


Fig. Physo. 1. Preparation from rabbit's uterus showing response to physostigmine (1 in 12,500) when in fresh condition.

Fig. /

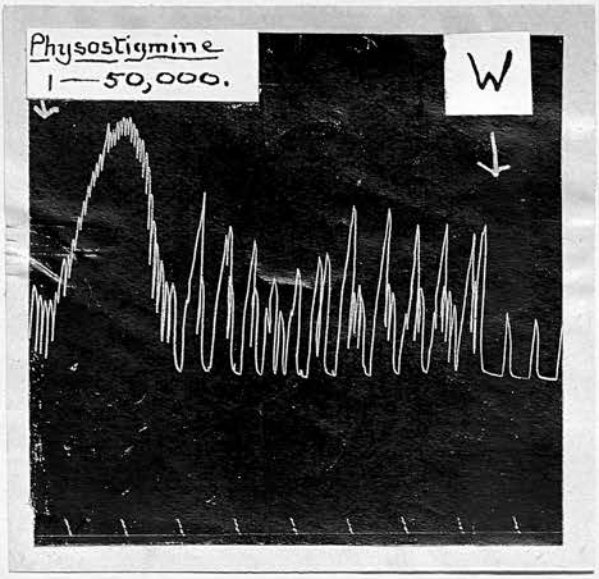


Fig. Physo. 2. The same showing in response towards the end of the experiment when in exhausted condition.

considerable tension, i.e. so-called temporary response (Fig. Physo. 2).

The records published by Clark (1926) of the isometric and isotonic records of contraction of the frog's rectus abdominis show clearly the double nature of the response obtained on treating the muscle with excess of potassium chloride. The isometric record shows an initial twitch associated with considerable tension which rapidly passes off. This type of response which is due to the quick factor or phasic contractile component, is shown in Figs. KCl. 1 and 2. On the other hand when light isotonic lever is used in recording the muscle contraction, the slow viscous component or postural contractile mechanism predominates and an apparent continuous contraction is obtained in tracing. The heavier the lever the nearer do the experimental conditions approximate to isometric.

The distinction between the initial twitch associated with considerable tension, and the slow sustained contraction associated with very slight tension are shown very clearly in the case of acetyl choline. Fig. AC.6 show pure twitch. Fig. AC. 2 shows the double response whilst in Figs. AC. 1 and AC.5 the distinction between the initial/

initial twitch and the subsequent sustained contraction are blurred.

The evidence gathered from these experiments leads to the conclusion that the transient effects seen with different drugs in their action on plain muscle, e.g. Fig. Physo. 2, are due to the fact that the lever weight or load is greater in proportion to the activity of the muscle, while with the permanent effects shown by them, e.g. Fig. Physo.1. the reverse is true.

Likewise these experiments also show that both the pendulum movements and tonic contractions can also be explained on this basis or hypothesis, i.e. the former being due to phasic or elastic contractile mechanism and the latter to postural or slow viscous contractile mechanism.

Winton believes that the phasic contractile mechanism may vary independently of any change in the postural one. Usually, however, it is observed that apparently the pendulum movements recover from the action of the drugs far more quickly than does the postural tonus or tonic contraction. But, with suitable apparatus, the change in tonus can be shown to be more or less permanent/

permanent even when the pendulum movements are affected in a transient manner.

A very interesting phenomena in connection with the action of drugs on pendulum movements as compared with that on postural tonus or tonic contraction has been noticed by the author (1931) in his experiments while trying antagonism of adrenaline by ergotamine on rabbit's gut. Adrenaline is seen producing a double action on the gut, i.e. an inhibition of pendulum movements and a general fall of tonus, the former being chiefly marked in the ileum and the latter in the duodenum and colon.

On trying antagonism of adrenaline action by ergotamine, it was noticed that ergotamine readily abolished the action of adrenaline on pendulum movements but showed much less action in affecting the fall in tonus, i.e. the fall in tonus caused by adrenaline persisted although ergotamine had abolished all adrenaline action on pendulum movements. Why this selective action on the part of ergotamine abolishing the action of adrenaline on pendulum or rhythmic movements only and sparing the fall in tonus due to adrenaline is not understood yet.

It has also been pointed out in that paper that the/

the extent of the antagonism observed between ergotamine and adrenaline on rabbit's gut depends on experimental conditions. Pieces from the upper portion of the small intestine show higher tonus than those from the lower. Furthermore the postural contractile mechanism is favoured by light loading and by alkaline Locke's solution. It is therefore easy to select conditions of postural or slow viscous contractile mechanism on the one hand or of phasic or elastic contractile mechanism on the other, any of which may affect the antagonism between these two drugs in their actions on the movements of rabbit's gut.

Thus it is quite evident that the various phases seen in the response of the different tissues to the actions of drugs and ascribed to their "potential actions" can be explained in other ways. Modes of action of digitaloids, narcotics, acids etc. have already been discussed in the preliminary part of this paper, and it has been shown that the action of any of these drugs cannot be explained on the basis of "potential action" theory of Straub emphasised by Jendrassik so much.

The/

The other evidence put forward by Jendrassik in favour of this theory on the basis of transient action of certain drugs and on the stimulation produced by washing out the drug from isolated tissues has also been duly weighed in the light of the action of drugs on plain muscle. The drugs as adrenaline, acetyl choline, etc. which are usually believed to produce transient actions, have been seen to show their permanent effects on various isolated parts of rabbit's gut and uterus. The wash-out phenomena of stimulation are seen so irregularly that it cannot be depended upon to bear a satisfactory evidence.

Winton's hypothesis of the presence of two components, on the one hand, and the transient and permanent records of contraction obtained from plain and skeletal muscles on using isometric and isotonic levers, on the other, go a good deal far off to make us understand the transient and permanent actions of the drugs shown on plain muscle.

Rentz on his surveying the polyphasic action of drugs comes to the conclusion that the "potential actions" theory can only explain a small proportion of/

of the effects of the drugs and he suggests that probably during the presence of the drug slow changes in the tissues are produced, which in their consequence are responsible for the variations in the response observed.

The original hypothesis of Straub based on the action of muscarine on aphysia and torpedo heart is opposed by Loewi (1912) by pointing out that the resumption of heart beat in the presence of muscarine appears to be analogous to the escape of the heart from vagus stimulation and is due to hypersensitiveness of the cardiac muscle.

Summary/

Summary of Conclusions:-

(1) Adrenaline produces two distinct actions on isolated gut, i.e.

(a) Inhibition of pendulum movements, and
(b) Fall in tonus.

(2) The writer has shown in another paper that these two actions are affected differently by drugs. Ergotamine abolishes the action of adrenaline on pendulum movements and has much less effect on the action of adrenaline on the tonus.

(3) The so-called "transient action" of adrenaline on the tonus depends on the fact that its action on pendulum movements is transient. If, however, the conditions are favourable for the measurement of change of tonus, then the action of adrenaline on tonus can be shown to be fairly permanent and to continue long after the pendulum movements have recovered. Therefore the action of adrenaline on gut is not really transitory, for only partial recovery occurs.

(4) /

(4) Parasympathomimetic stimulants appear to produce a double action on the gut and uterus, a transient action associated with considerable increase in tension and a permanent postural contraction.

(5) If the conditions are favourable the permanent postural contraction is recorded but with a feeble tissue or a heavy lever only the transient action may be recorded and hence the action may appear to be transient.

(6) The wash-out effects observed after administration of drugs are extremely irregular. The apparent rise in tonus sometimes seen after wash-out of the excitant drug may be due to two causes:-

(a) Increased sensitivity of the tissue causing it to contract in response to a slight mechanical stimulus set up by the change of fluid during wash, or

(b) The effect of some drugs in first causing stimulation and later producing depression. In this case wash-out causes the tissue to pass through a temporary stage of stimulation.

(7) The actions produced by drugs studied on the isolated gut and uterus of rabbit can be explained by other causes than the theory of "potential actions" of drugs.

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Appendix III.

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THE ACTION OF ERGOTAMINE ON THE RESPONSE OF THE RABBIT'S GUT TO ADRENALINE

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Several authors have shown that ergotamine abolishes the inhibition produced by adrenaline in the isolated rabbit's gut (Planelles (1), Rothlin (2, 3), Langecker (4), Tokieda (5), Issekutz and Leinzinger (6), Thiennes (7)). Mendez (8) working in this department concluded however that ergotamine either did not effect this inhibitor action of adrenaline or at the most affected it in a very feeble manner. The following experiments were undertaken to ascertain the cause of this discrepancy in experimental results.

METHOD

The Magnus preparation of the isolated rabbit's gut was used. The bath (25 cc.) was filled with Locke's solution of the following percentage composition: NaCl 0.9, KCl 0.042, $CaCl_2$ (anhydrated) 0.024, $NaHCO_3$ 0.05, glucose 0.05. The drugs used were ergotamine tartrate (Sandoz) and adrenaline hydrochloride.

RESULTS

My chief results are shown in figure 1. This figure shows that adrenaline produces a double action on the gut, an inhibition of pendulum movements and a general fall of tonus. The response of the duodenum to adrenaline (fig. 1, A) consists chiefly of a fall of tonus, whilst the response of the ileum (fig. 1, C) consists chiefly in inhibition of pendulum movements. Very frequently a double response is seen (fig. 1, B). Ergotamine readily abolishes the action of adrenaline on pendulum movements (fig. 1, B and C) whilst it has much less action on the fall in tonus, for this fall

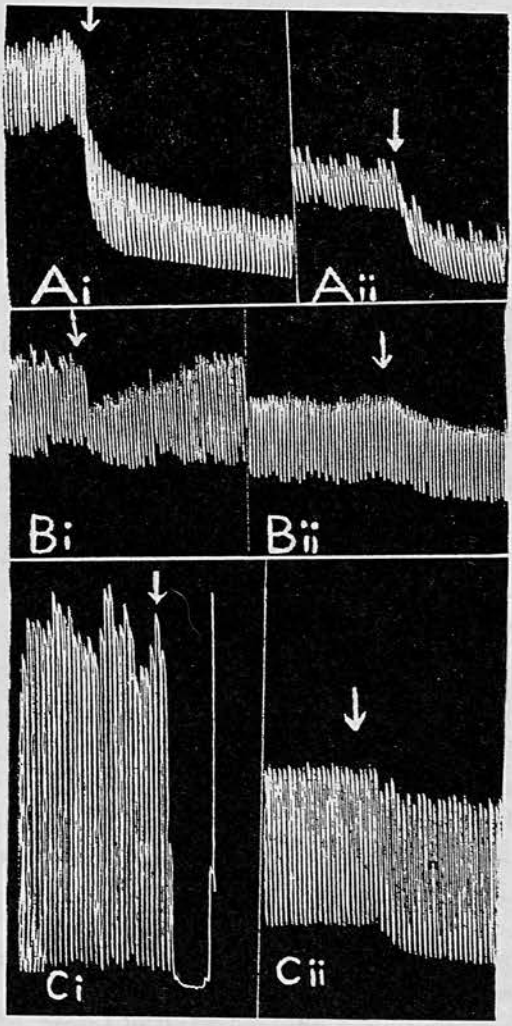

FIG. 1. THE ANTAGONISM BETWEEN ERGOTAMINE AND ADRENALINE IN ISOLATED STRIPS OF THE RABBIT'S DUODENUM AND ILEUM

A_i and A_{ii}. Response of duodenum to adrenaline HCl (20 parts per 10^8) before and after ergotamine tartrate (200 parts per 10^8).

B_i and B_{ii}. Response of duodenum to adrenaline HCl (10 parts per 10^8) before and after ergotamine tartrate (10 parts per 10^8).

C_i. Response of lower ileum to adrenaline HCl (10 parts per 10^8).

C_{ii}. Response to adrenaline HCl (200 parts per 10^8) after ergotamine tartrate (400 parts per 10^8).

persisted in all three cases in figure 1 although the ergotamine had abolished all the action of adrenaline on the pendulum movements. This selective action of ergotamine was observed by Issekutz and Leinzinger (6).

The extent of the antagonism observed between ergotamine and adrenaline in the rabbit's gut depends therefore on the experimental conditions. Pieces from the upper portion of the gut show higher tonus than those from the lower portion. Further-

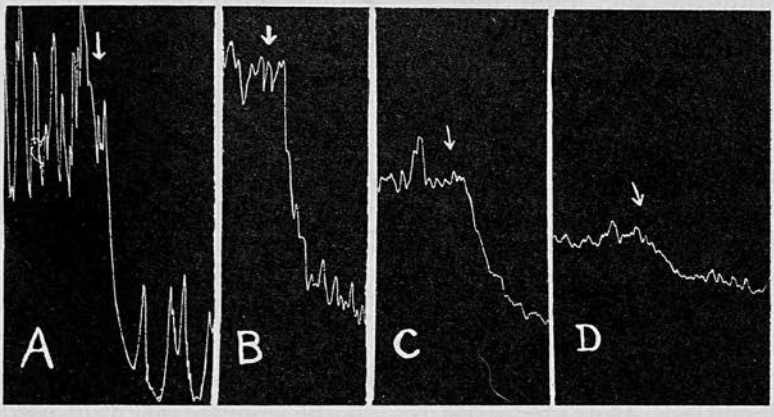

FIG. 2. ACTION OF ADRENALINE AND ERGOTAMINE ON RABBIT'S COLON

	PARTS PER 100 MILLION OF	
	Adrenaline HCl	Ergotamine tartrate
A	2	0
B	2	5
C	2	20
D	2	400

more tonus is favoured by light loading and by alkaline Locke's fluid, while alkalinity is readily produced by vigorous aeration. It is therefore easy to select conditions in which the antagonism is either well marked or very slight.

As regards the colon I found that very high doses of ergotamine were needed to affect the adrenaline response, which in this case consisted almost entirely of a fall in tonus (fig. 2). I noted some antagonism however, and therefore my results were intermediate

between those of Thiennes (7) who found that a concentration of 1:20,000 ergotamine did not influence the response of the rabbit's colon to adrenaline and those of Rothlin (3) who found that the antagonism in the rabbit's colon was produced by the same concentration of ergotamine as in the rabbit's uterus.

QUANTITATIVE ESTIMATION OF ANTAGONISM

I confirmed the conclusions of Issekutz and Leinzinger (4) and Rothlin (3) who found that ergotamine acted on the rabbit's intestine much more rapidly than it did on the rabbit's uterus and also was washed out much more rapidly. I usually allowed the ergotamine to act for half an hour, and always added the adrenaline in the presence of the ergotamine. This ensured that the ergotamine had produced its full action. For convenience of comparison I have calculated all concentrations of adrenaline and ergotamine in parts per 100 million. These figures can be converted into molar concentrations by multiplication by 5×10^{-8} in the case of adrenaline and by 1.6×10^{-8} in the case of ergotamine tartrate.

The quantitative estimation of antagonism presents certain difficulties. Rothlin (3) estimated the concentration of ergotamine needed to abolish the action of a concentration of adrenaline. This method is unsatisfactory, firstly because it is very difficult to estimate an exact end point and secondly because different pieces of gut vary extensively in their sensitivity to adrenaline. The most satisfactory method appears to be to determine the concentrations of adrenaline needed to produce a given action on the gut before and after the administration of ergotamine. This was the method adopted by Mendez (6) who showed that in the case of the rabbit's uterus a constant value was obtained with the formula $\frac{C_{A_2} - C_{A_1}}{C_E}$, where C_{A_1} and C_{A_2} represented the concentrations of adrenaline needed to produce a given action before and after the administration of any particular concentration of ergotamine (C_E). When the concentration of ergotamine is large, C_{A_1} is so much smaller than C_{A_2} that the formula may be simpli-

fied to C_{A_2}/C_E. It will be seen that the larger the quotient $\frac{C_{A_2}}{C_E}$ the greater is the power of ergotamine to antagonise adrenaline.

Estimation of this nature made on the ileum is shown in figure 3. A concentration of 2.5 parts per 100 million adrenaline

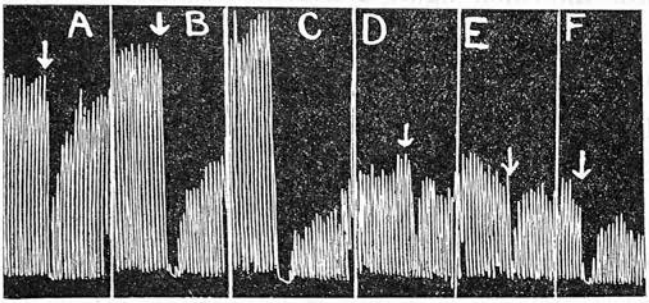

FIG. 3. ESTIMATION OF ERGOTAMINE-ADRENALINE ANTAGONISM ON RABBIT'S ILEUM

A, B and C. Action of adrenaline HCl 2.5, 5 and 10 parts per 10^8.
D. E and F. Action of adrenaline HCl 20, 40 and 100 parts per 10^8, after ergotamine tartrate 10 parts per 10^8.

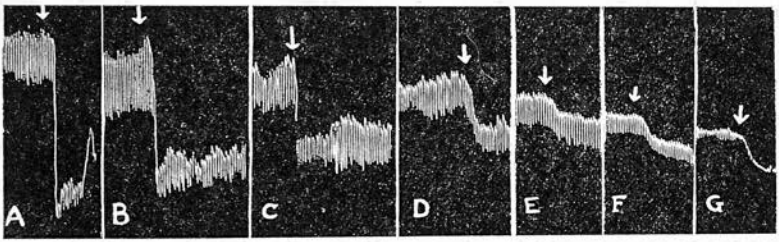

FIG. 4. ESTIMATION OF ERGOTAMINE-ADRENALINE ANTAGONISM ON RABBIT'S DUODENUM

A, B, C and D. Action of adrenaline HCl 10, 4, 2 and 1 parts per 10^8.
E, F and G. Action of adrenaline HCl 2, 8 and 20 parts per 10^8 after ergotamine tartrate 5 parts per 10^8.

(C_{A_1}) produces full inhibition of a single pendulum movement. After the application of 20 parts per 100 million of ergotamine (C_E) similar effects are produced by 40 parts per 100 million adrenaline (C_{A_2}). The formula $\frac{C_{A_2} - C_{A_1}}{C_E}$ becomes therefore $\frac{40 - 2.5}{10} = 4$. In six similar experiments the value of $\frac{C_{A_2} - C_{A_1}}{C_E}$ varied from 2 to 10 and the average value was 7.

The duodenum gave very different results from the ileum for figure 4 shows that with an ergotamine concentration of 5 parts the concentration of adrenaline required to produce the effect previously produced by 1 part per 100 million, lay between 8 and 20 parts per 100 million, which gives a figure of about $\frac{12}{5} = 2.5$ As regards the colon figure 2 shows how slight an antagonism there is in this case between ergotamine and adrenaline. Other experiments showed that the ratio $\frac{C_{A_2} - C_A}{C_E}$ was not more than 0.02. In the case of the uterus Mendez (8) found that the ratio $\frac{C_{A_2} - C_{A_1}}{C_E}$ was 40 and I confirmed this figure. Hence the power of ergotoxine to antagonize adrenaline as measured on different tissues of the rabbit can be represented by the following figures:

Motor response of uterus	40
Inhibition of pendulum movements of ileum	7
Fall of tonus of duodenum	2
Fall of tonus of colon	0.02

These figures are only approximations but they indicate the extent of the differences that exist in different organs in the power of ergotamine to antagonize adrenaline. There are moreover other effects produced by adrenaline which cannot be inhibited by any practicable concentration of ergotamine. Such effects are the inhibition of the uterus of the rat and guinea pig (Rothlin (3), Mendez (8)), and the inhibition of the uterus of the rabbit (Thiennes (7), Mendez (8)). The last effect is an inconstant one, for in many rabbits the smallest effective dose of adrenaline produces contraction, but in a certain number a small dose produces inhibition and a larger dose contraction. In these latter cases ergotamine causes a reversal of adrenaline action, and the inhibitor response to adrenaline thus obtained is not abolished by any practicable concentrations of adrenaline.

DISCUSSION

A large variety of drugs can antagonize the effects produced by adrenaline on isolated organs. As regards the rabbit's uterus Sugimoto (9) and Ogata (10) showed that the motor effects of

adrenaline were antagonized by atropine. The inhibitor effect produced by adrenaline on the guinea pig's uterus can be antagonized by a variety of changes, e.g., hypotonic solution (Dale (11)), excess of KCl (Tate and Clark (12)), barium (Lenz and Ludwig (13)), histamine (Frohlich and Pick (14), Tate and Clark (12)), pituitary extract (Gunn and Gunn (15), Sugimoto (16), Cow (17), Tate and Clark (12)), and alcoholic extracts of tissues (Berggren (18)). Loewe (19) examined the action of 33 synthetic cycloethylamines on the uteri of the common laboratory animals. He found that a large number of these substances antagonized adrenaline, and that not only the motor but also the inhibitory actions of adrenaline were antagonized quite frequently, and that in many cases the antagonism was better marked in the case of inhibitory than in the case of motor responses.

The reversal of adrenaline responses in isolated strips of the rabbit's stomach has been studied by Brown and McSwiney (20) who found that any drug that augmented tonus (e.g., pilocarpine, histamine or barium) changed the response to adrenaline from contraction to inhibition; and that ergotamine also produced this effect. In the case of the rabbit's uterus they found that histamine could change an augmentor adrenaline response to an inhibitor response.

The action of ergot alkaloids was originally considered to be an unique example of selective paralysis of the motor sympathetic. It is now known that the nature of the sympathetic response can be modified by a large number of drugs, some of which simply diminish the response, whilst others reverse the response; the reversal is usually a change from augmentor to inhibitor action, but sometimes reversal from inhibitor to augmentor occurs (e.g., pituitary extract and adrenaline on guinea pig's uterus). Furthermore the ergot alkaloids antagonize a large variety of adrenaline responses both augmentor and inhibitor. Important quantitative differences in the intensity of this antagonism can be demonstrated on the isolated tissues of the rabbit. The antagonism is most intense in the case of the motor response of the uterus, is barely demonstrable in the case of the colon, and cannot be demonstrated in the case of the inhibitor responses of the uterus.

CONCLUSIONS

1. Ergotamine antagonizes the inhibitory action produced by adrenaline on the pendulum movements of the rabbit's isolated gut, but has much less power to antagonise the fall in tonus produced by adrenaline.

2. The antagonism between ergotamine and adrenaline is easy to demonstrate with the ileum of the rabbit, but is much less obvious with the duodenum.

3. Ergotamine probably antagonizes the action of adrenaline on the colon but this antagonism is so feeble that its existence is doubtful.

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