M. Ed.

Quantitative Observations on the Antagonism between Ergotamine and Adrenaline.

By William Gilchrist Patterson. M.D. 1926

The object of the following experiments on the antagonism between adrenaline and ergotamine was to obtain a series of quantitative observations for comparison with the conclusions reached by Professor Cushny - with regard to the antagonism between atropine and pilocarpine. From experiments on the salivary secretion of dogs under atropine and pilocarpine he formulated the following statements.

1. "In different dogs a constant amount of atropine was necessary to oppose the action of a constant amount of pilocarpine, i.e. the antagonistic action did not differ in degree in different animals".

2. "In the same dog the ratio of the pilo-:carpine to that of the atropine necessary to oppose its action remained the same however much the

actual /

actual amounts injected might vary; i.e. the antagonism proceeds according to the laws of mass action and not according to those of chemical combination".

3. "There was evidence that when one poison had been allowed to act for some time its antagonist was less effective than if it had been injected simultaneously".

The antagonism between ergotamine and adrenaline was observed on the excised uterus of the rabbit. Adrenaline is said to stimulate all sympath-:etic myoneural junctions, whether motor or inhibitory. Ergotamine, on the other hand, while thought to have no action on the inhibitory sympathetic myoneural junctions is supposed to stimulate the motor ones in small doses and to paralyze them in larger amounts. As the paralyzing effect exerted on these myoneural junctions by a large dose of ergotamine is able to inhibit the contractor response to subsequent doses of /

of adrenaline, it seemed feasible that observations on this apparent antagonism might throw some light on the laws governing the antagonistic action between drugs.

The following technique was followed. The rabbit having been killed by a blow on the neck, was bled from the carotids: its uterus was immediately removed and placed in cold 0.9% sodium chloride solution.

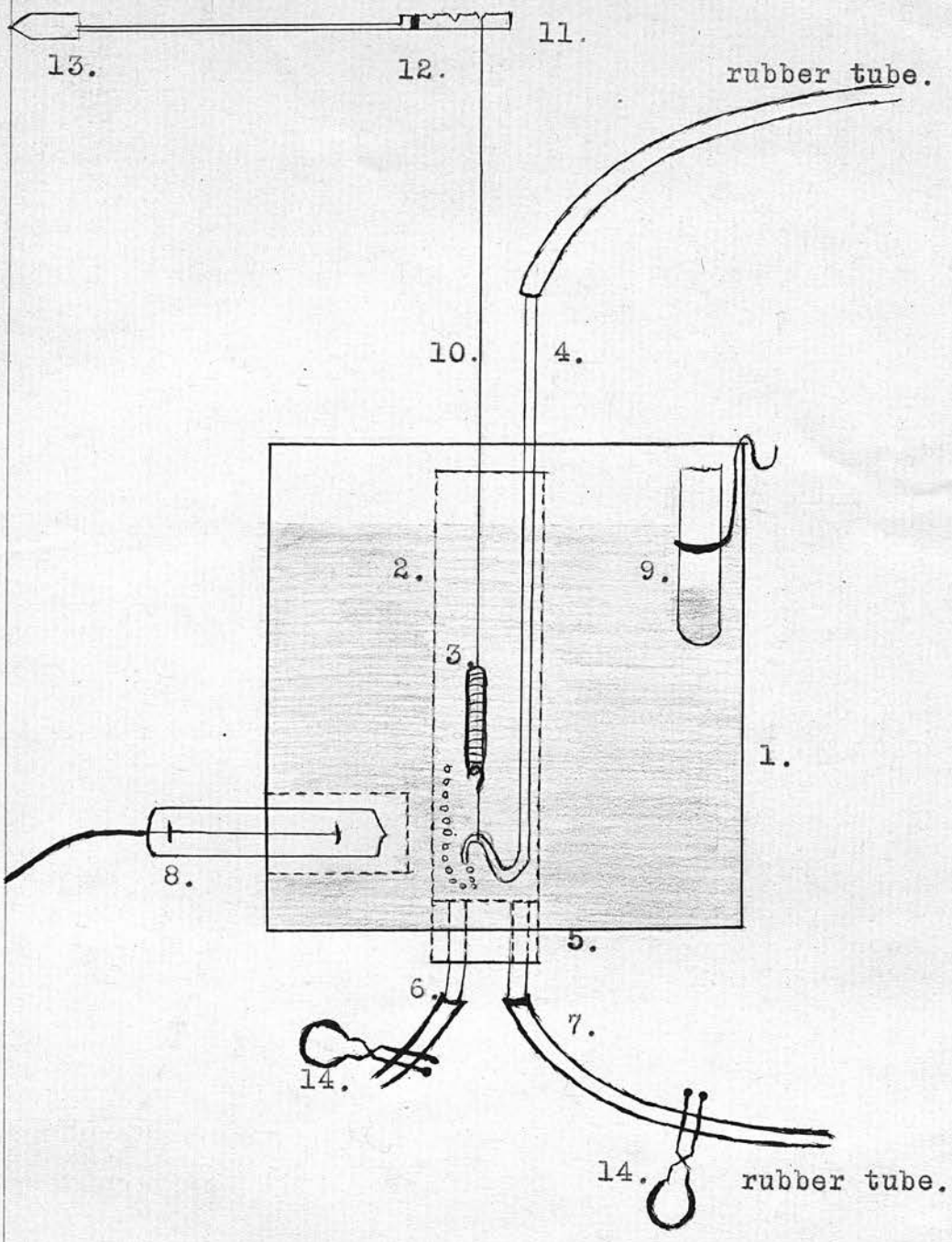
Successive uterine strips obtained from this uterus were suspended in a glass funnel of Ringer's * solution kept constantly at 37° C. by a surrounding copper water bath which was heated by an electric lamp. Oxygen was bubbled at a constant rate through the uterine chamber. One end of the uterine strip was fixed to the oxygen tube's terminal part, which approached the bottom of the funnel: to the other end /

* Note: The Ringer was made according to the formula given by Broom & Clark (Reference 2).

end was attached a thread which passed up to the short arm of a lever, of which the long arm wrote with a paper point on a slowly revolving smoked paper. (See diagram on page 4a). As a time tracing was taken simultaneously there was thus obtained a magnified record of all changes in the length of the uterine strip, and also a record of the time at which such variations occurred.

The ergotamine and adrenaline were employed in concentrated solutions, the respective doses of which were added at the appropriate times, through finely graduated pipettes to the Ringer's solution in the uterine funnel; the resulting concentration of the particular drug immersing the uterine strip was recorded on the smoked paper over an arrow at the point corresponding to the time of addition of the drug. Fresh solutions were prepared for each experiment in order that the preparations used in different experiments might be, as far as possible,

of /



1. Water bath.
2. Uterine funnel.
3. Strip of uterus.
4. Oxygen tube.
5. Rubber bung.
6. Tube for emptying uterine funnel.
7. Tube for refilling uterine funnel from reservoir of heated Ringer's solution.
8. Electric lamp for heating water bath.
9. Test tube for heating ergotamine dose to 37°C.
10. Thread connecting uterine strip to 11, i.e. short arm of lever.
12. Fulcrum.
13. Writing point.
14. Clips for rubber tube.

of constant strength. Thus of the ergotamine tartrate, which was kindly supplied gratis by Sandoz Company Limited, there was weighed out and dissolved in 0.9% sodium chloride solution, immediately before the experiment began, only such quantity as was deemed necessary for that experiment. The fresh specimen of 1: 1000 Parke Davies adrenaline hydrochloride solution employed on each occasion was likewise diluted down with 0.9% sodium chloride solution, just prior to the commencement of the experiment, so that the doses required might be of volume convenient for accurate measurement by pipette. The Ringer's solution in the uterine funnel was raised to the 25 c.c. level immedi-:ately after the addition of each dose and the drugs were made up in the concentration of 1 in 10,000: hence a dose of 1 c.c., for example, gave a concentra-:tion of 1 in 250,000 of the particular drug immersing the uterus. Since the adrenaline dose was of small volume the solution of this unstable drug was added unheated./

unheated. When the volume of the ergotamine dose was large enough, relatively to the uterine funnel, to make an appreciable temperature change if added cold to the fluid bathing the uterine strip, it was previously heated to 37°C. in a test-tube suspended in the outer water-bath.

The sensitivity of the uterine strip to adrenaline having been demonstrated, the Ringer's solution was replaced by fresh fluid from a heated reservoir kept at 37°C. Ergotamine was then added and followed after a stated interval by adrenaline. Contrary to the method of Broom and Clark*, the Ringer's solution was not changed in the interval between ergotamine and adrenaline. Their method would appear to introduce an unnecessary variable factor for one can not guarantee that the effect of each washing is the same. As is illustrated in the following experiment (See page 8) however, the paralyzing effect exerted by ergotamine on the sympath-

:etic /

sympathetic motor myoneural junctions diminishes only slightly after repeated washings; so the variable factor introduced by Clark's method may not have much effect.

* Reference 2.

Concentration of ergotamine solution surrounding uterine strip.	Excursion of writing point as response to successive doses of adrenaline of concentration 1: 1,000,000.	
	Before uterine funnel had ergotamine solution added to it.	After uterine funnel had ergotamine solution added to it.
$1 : \frac{1}{16}$ million.	5.5 centimetres.	(a) Before any washings and 6 minutes after ergotamine's addition. Nil.
		(b) After 7 washings and 48 minutes after ergotamine's addition. 0.5 centimetre.
		(c) After 8 more washings and 2 hours 4 minutes after ergotamine's addition. 0.75 centimetre.

Inference:

The paralyzing effect exerted by ergotamine on the sympathetic motor myoneural junctions diminishes only slightly after repeated washings.

The response to adrenaline could be obtained unaltered for an indefinite period from a strip of uterus which had not been treated with ergotamine. Thus in one experiment 21 successive doses of adrenaline, each giving a concentration of 1 : 1,000,000, gave successive writing point excursions of about 8 c.ms.: the doses were added at intervals of 3 minutes, and the Ringer's solution was replaced by fresh fluid 2 minutes after each dose. The pro-:longed absence of response after ergotamine is therefore due to the ergotamine action, not to a natural failure in the ability of the uterus to respond to successive doses of adrenaline. As it was impossible to wash out the ergotamine in a reasonable time each piece of uterus could be used for only one reading. The object of each reading was to find what concentration of ergotamine, allowed to act for a certain time, was necessary to antagonize almost completely a particular concentration of adrenaline: the /

the standard adopted for this antagonism was that after the uterine strip had been acted on by ergotamine the adrenaline should produce only a slight contractor response: complete inhibition of adrenaline's motor action was not desired as such complete inhibition would have eliminated the control against the use of an excessive concentration of ergotamine.

Section I.

With reference to the antagonism ratio between ergotamine and adrenaline in different uteri only two experiments are quoted below: for, as will be pointed out later, the numerous faults which appeared in the method, rendered the vast majority incapable of furnishing any data whatsoever concerning the antagonism between these two drugs.

Experiment A.

Pieces of Uterus.	Concentration of Ergotamine bathing uterine strip.	Concentration of Adrenaline H.Cl. bathing uterine strip.	Writing point's excursion corresponding to contraction of uterus as response to adrenaline.	
			To adrenaline before addition of ergotamine.	To adrenaline 6 minutes after addition of ergotamine.
2nd	1 in $\frac{1}{2}$ million.	1 in 1 million	5 cm.	0.5 cm.
3rd	1 " $\frac{1}{4}$ "	1 " $\frac{1}{2}$ "	2.8 cm.	0.8 cm.
4th	1 " 1/6 "	1 " 1/3 "	9 cm.	2.8 cm.

Inference: Ergotamine tartrate 2: X allowed to act for 6 minutes barely antagonizes completely adrenaline hydrochloride 1: X.

Antagonism Ratio 2 : 1.

Experiment B.

Ratio	Pieces of Uterus	Ergotamine	Adrenaline H.Cl.	Writing point's excursion after Adrenaline.	
				Before Ergotamine	6 minutes after Ergotamine
2:1	1st	1 in ½ million.	1 in 1 million.	15 cm.	12 cm.
8:1	(5th	1 in 1/8 "	1 " 1 "	4.5 cm.	6 cm.
	(7th	1 " 1/16 "	1 " 1 "	5 cm.	
	(		1 " ½ "		4 cm.
16:1	(2nd	1 in 1/16 "	1 in 1 million.	8 cm.	1 cm.
	(3rd	1 " 1/8 "	1 " 2 millions.	2.5 cm.	0.2 cm.
	(4th	1 " 1/8 "	1 " 2 "	7 cm.	1 cm.
	(8th	1 " 1/12 "	1 " 4/3 "	2.5 cm.	0.5 cm.

Inferences:

1st piece:- Ergotamine 2:X allowed to act for 6 minutes does not antagonize appreciably adrenaline hydrochloride 1:X.

5th and 7th pieces:- Ergotamine 8:X allowed to act for 6 minutes prior to addition of adrenaline does not antagonize adrenaline hydrochloride 1:X.

2nd, 3rd, 4th & 8th pieces: Ergotamine 16:X allowed to act for 6 minutes barely antagonizes completely adrenaline hydrochloride 1:X. Antagonism ratio 16:1.

In experiment A the antagonism ratio between ergotamine and adrenaline was 2 : 1: in experiment B it was 16 : 1. That is, in different uteri a constant concentration of ergotamine does not antagonize a constant concentration of adrenaline: i.e. the antagonistic action does differ in degree in different uteri.

In this respect, therefore, the quantitative observations on the antagonism between ergotamine and adrenaline differ from those made by Professor Cushny on the antagonism between atropine and pilocarpine. Is the variation fundamental to the nature of the antagonism, or is it an adventitious difference introduced by the method of these experiments on ergotamine and adrenaline?

The following points negative the suggestion that change in the strength of the ergotamine may be at fault. On each occasion the ergotamine was dissolved in fresh 0.9% saline immediately before the experiment /

experiment was begun: and the constancy of the ratios obtained in individual experiments indicates that the ergotamine solution did not deteriorate appreciably during the course of an experiment. That the ergotamine powder itself had not deteriorated during the period of the series of experiments was indicated by a comparison of a 'vaso-reversal' experiment performed after their termination, on a decerebrated cat, with several such experiments carried out some months before: it was found that the dose of ergotamine, per kilo of cat, required to inhibit the vaso-pressor action of a certain dose of adrenaline, was no larger in the last vaso-reversal experiment than in the earlier ones; viz. 0.010 grm. milligrammes of ergotamine per kilo, for 0.0001 grm. adrenaline. (Reference 3). It may be mentioned as an aside that an attempt to utilise such vaso-reversal experiments as a source of data concerning the antagonism ratios between ergotamine and adrena-

adrenaline proved fruitless.

To return to the quest for the origin of the variable factor in the antagonism ratio between ergotamine and adrenaline in different uteri, the adrenaline employed throughout was 1 : 1,000 Parke Davis solution, a fresh specimen of which was diluted down with 0.9% sodium chloride solution at the beginning of each experiment. The use of different specimens of this stock solution introduced a fallacy: but, as a fresh bottle was used each time, this could not account for such an extreme variation as that between the 2 : 1 ratio of experiment A and the 16:1 ratio of experiment B.

The technique of the experiments as regards preparation of specimens, Ringer's solution tempera-:ture and oxygenation of bath, and so on, was the same throughout the series.

These considerations indicate that the uteri themselves are the site of the variable factor or factors /

factors. Owing to this variation the antagonism ratio has to be determined anew for each uterus; and after it has been worked out for one particular concentration of adrenaline, the number of pieces available from the uterus is often exhausted, so that the completion of a series of concentrations for comparison is impossible. This proved a most formid-:able hindrance to the obtaining of a reasonable amount of data.

The variation in the antagonism ratio between ergotamine and adrenaline in different uteri, may be related to the presence in the uterus of a complex sympathetic system, of which both the motor and the inhibitory components are stimulated simultaneously by adrenaline. The rabbit's uterus is generally regarded as not having an inhibitory mechanism. In the case of several young virgin uteri in this series of experiments, however, the response to adrenaline

was /

was relaxation. But in none of the ergotamine - adrenaline experiments did a uterine strip after treatment with ergotamine respond to adrenaline by relaxation instead of the contractor response which it gave before being treated with ergotamine: the only effects obtained from the ergotamine were either reduction or complete abolition of the contractor response to adrenaline: so there was no definite proof of the existence of an inhibitory mechanism in those rabbit uteri which originally gave a motor response to adrenaline. Hence the suggestion that the cause of the variation in the antagonism ratio in different uteri, may be the presence of a double sym-:pathetic mechanism, motor and inhibitory, the relative strengths of which may vary in different uteri with corresponding variation in the response to adrenaline, is not substantiated. But as it is possible that such a mechanism is present, the lack of agreement between Professor Cushny's results and

those of the present experiments can not be taken as definitely negativing the applicability of his first conclusion as a general law for antagonism of drugs. Rather it demonstrates certain limitations of the uterine method.

Besides this theoretical limitation a serious practical difficulty was that many of the uteri, when immersed in the warm oxygenated Ringer's solution, exhibited, at quite irregular intervals, spontaneous contractions of a character indistinguishable from that of the apparent responses to adrenaline. Such uteri were quite unsuitable because with them it was uncertain whether a contraction occurring after the addition of adrenaline bore any relation, other than that of time, to the adrenaline or not. Unfortunately those uteri which were valueless on this account were the large ones which could have furnished enough pieces for an extensive series of comparisons. The necessary type of uterus was one practically /

practically quiescent, or at least exhibiting no spontaneous contractions not readily distinguishable from responses to adrenaline. But this variety was generally of such small size that it was divisible into only a few pieces. Thus even Experiment B, though actually the most complete of a large series, was incomplete because of the limited number of uterine strips available; while the control of increasing the adrenaline proportion beyond that of the antagonism ratio was performed, the number of uterine strips available did not permit the reverse control of increase of the ergotamine beyond this ratio.

SECTION 2.

Experiment A gives the antagonism ratio between ergotamine and adrenaline in successive strips from the same uterus as 2 : 1 for different concentrations of adrenaline; experiment B gives the ratio in successive strips from another individual uterus as 16 : 1 for different concentrations of adrenaline. That is, in one individual uterus the ratio of adrenaline to the ergotamine necessary to antagonize it almost completely is the same for different concentrations of adrenaline. In this respect therefore the uterine experiments on the antagonism between ergotamine and adrenaline agree with those of Professor Cushny on the salivary secretion under atropine and pilocarpine.

No light is thrown on the question whether the law of multiples in the antagonism of ergotamine and adrenaline is that of chemical combination or that of mass action: for essential data are lacking.

For /

For example, the reaction of different pieces of the same uterus, equally weighted and of approximately equal size, to any particular concentration of adrenaline are not quantitatively constant. Thus in experiment B the respective writing point excursions produced by different fresh pieces after adrenaline 1 : 1,000,000 were 15 c.m., 8 c.m., 5 c.m., and 4.5 c.m. The method, therefore, gives no mathematical constant from which one could state that any individual contraction was equivalent to that produced by a certain concentration of adrenaline. Such a constant is necessary for the method by which Professor Cushny reached his conclusion that the law of multiples in the antagonism between atropine and pilocarpine was that of mass action; his reasoning was that if after X of atropine, y of pilocarpine produced salivation equivalent only to that produced by 'a' of pilocarpine, then, should the law of multiples in the antagonism be that of chemical combination, after

50 X of atropine, 50 Y of pilocarpine should produce salivation equal to that produced normally by 50 'A' of pilocarpine; as his experiments disproved this he concluded the law of multiples in the antagonism between atropine and pilocarpine was that of mass action. In addition to the absence of the necessary constant the difference in the concentrations of ergotamine, e.g. 1 : 1/16 million and 1 : 1/8 million in experiment B, are too small to give such a decisive result as the 1 : 50 ratio of the atropine - pilocarpine experiments.

SECTION 3.

As is illustrated in the following experiment (See page 25.) the antagonistic action of ergotamine to adrenaline increases with lengthening of the interval between the addition of the drugs to the uterine bath. In this third point then the uterine experiments on the antagonism between ergotamine and adrenaline agree with the salivation experiments on the antagonism between atropine and pilocarpine.

Experiment.

Pieces of Uterus.	Ergotamine.	Adrenaline H. Cl.	Writing point's excursion as response to adrenaline	
			Before Ergotamine	After Ergotamine
1st	1: 1/16 million	1:1/4 million	9 c.m.	6 minutes after ergotamine 2 c.m.
2nd	1: 1/16 "	1:1/4 "	13 c.m.	30 minutes after ergotamine Nil.

Summary.

I. Experiments on the antagonism between the respective actions of ergotamine and adrenaline on excised rabbit uterus present so many difficulties and fallacies that there is little likelihood that such experiments can furnish much conclusive information with regard to the nature of antagonistic action between drugs.

II. In different uteri a constant amount of ergotamine does not oppose the action of a constant amount of adrenaline: i.e. the antagonistic action does differ in degree in different uteri. Because of possible complications in the shape of a double mechanism, motor and inhibitory, with relative strength varying in different uteri, and consequent variation in the degree of adrenaline's contractor action, this finding does not definitely negative the /

the applicability of Professor Cushny's first statement as a general law in the antagonism of drugs.

III. In different strips of the same uterus the ratio of the ergotamine to the adrenaline which it antagonizes is the same for different concentrations of the respective drugs. No evidence was obtained as to whether the antagonism between ergotamine and adrenaline proceeds according to the laws of mass action or according to those of chemical combination.

IV. The antagonistic action of ergotamine to adrenaline increases with lengthening of the interval between the addition of drugs to the uterine bath.

References.

1. Quantitative Observations on Antagonism.

A.R. Cushny.

Journal of Pharmacology (1914-15) page 439.

2. Standardisation of Ergotamine Preparations.

Broom & Clark, Journal of Pharmacology,

Vol. 22 (1924) page 59.

3. Dale. Journal of Physiology, No. 46,

1913 - page 293.

William G. Patterson, M.B.Ch.B.,

Whitburn,

Westlothian.
