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THE ACTION OF ADRENALIN AND ERGOTAMINE ON THE UTERUS OF THE RABBIT.

By J. H. GADDUM.

(*From the Wellcome Physiological Research Laboratories.*)

When a rabbit's uterus is cut in pieces and tested with ergot alkaloids and adrenalin, according to the technique introduced by Broom and Clark(1) as a test for ergot alkaloids, it gives reactions which are notably consistent over long periods. These reactions have been further considered in the hope of arriving at a more precise idea of their nature.

The action of varying concentrations of adrenalin. The rabbits used weighed from 1·8 to 3 kilos. Some of them were pregnant, some were not. The volume of the baths was about 20 c.c. each and they were contained in a water bath similar to that used by Dale for assaying pituitary extracts. The oxygen was delivered in small bubbles through a fine jet. The tension on the muscle was about 1 gram weight. About 1 mg. of Sandoz ergotamine was weighed out each morning and dissolved in water to a strength of 1/5000 by the addition of 2 drops of 3 p.c. acetic acid. The adrenalin used was Burroughs, Wellcome and Co.'s 1/1000 solution. The drugs were diluted with water and added to the bath in volumes which varied from 0·15 to 1 c.c. The Ringer solution used was: NaCl ·9 p.c., KCl ·042 p.c., $CaCl_2$ ·024 p.c., $NaHCO_3$ ·05 p.c., glucose ·1 p.c.

According to Clark a uterine horn should be cut into pieces about 1 cm. long and each piece should be divided in the plane of the mesentery and secured in the apparatus by its anti-mesenteric border.

The value of this was confirmed. At the attachment of the mesentery the uterus seems unable to relax. When pieces of large uteri were divided into four quarters, the quarters free of mesentery worked well and the others did not. The pieces of uterus kept well in an ice box on glass, but after they had been in the bath some time they began spontaneously to produce a series of short-lived contractions returning always to the same base line. This rhythm was particularly marked in the pregnant uteri. The addition of adrenalin had the effect of raising the base line

and the height to which it was raised was thought to be the best indication of the size of the contraction.

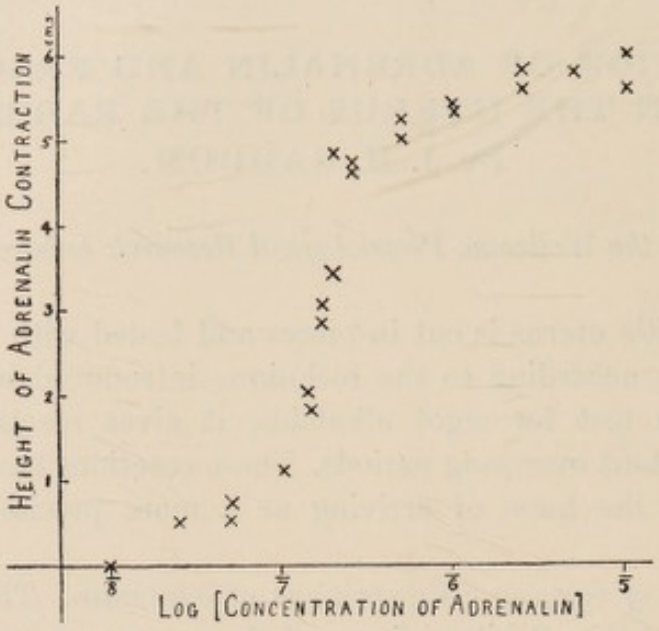

Fig. 1. See Text. Ordinates show double the actual shortening of the muscle.

This gave fairly constant readings independently of the spontaneous rhythm. In Fig. 1 the height so measured is plotted against the logarithm of the concentration of adrenalin. Shackell has obtained curves of similar form for the effect of adrenalin on arterial rings (2) and also by plotting the percentage mortality among a number of individuals against the dose of toxic substance (3). These results have been confirmed in experiments with a number of different drugs in this laboratory, and a paper on the subject by J. W. Trevan is in preparation. The form of the curve is that which would be obtained on the assumption that the drugs are acting on a number of units whose susceptibility is distributed about a mean in accordance with a probability curve—it is the curve of the integral of the normal distribution.

It was thought that if the mechanism producing changes in tone was quite distinct from that producing the rhythmic contractions, it might be possible to demonstrate a difference in the shape of the tracings or of the above curves when an isometric lever was used. Tracings obtained with such a lever were directly compared with those obtained with an isotonic lever. They were very similar.

Adrenalin is very easily destroyed under these conditions, even in an empty bath, so that the contraction is maintained at its full height only for a few minutes. If the Ringer solution be transferred to another

bath with a fresh piece of uterus when the contraction of the first has decreased to half its maximal value, the second piece of muscle only gives half a contraction, so that fatigue is not a very important factor in the falling off of the contraction. If the concentration of adrenalin be maintained by perfusing the bath with Ringer solution containing adrenalin the height of the contraction is maintained.

Effect of some cations. Magnesium chloride in a strength of ·005 p.c. or less was found to have no appreciable effect on the adrenalin contraction. In a strength of ·05 p.c. it slightly diminished the height of the contraction.

The effect on the adrenalin contraction of varying the percentage of calcium in the Ringer solution was studied. In the absence of calcium the spontaneous rhythm of the muscle was diminished. In the presence of an excess (·05 p.c. of $CaCl_2$) the rhythm was increased and after a few adrenalin contractions the muscle went into a state of tone which disappeared when the calcium was washed out again.

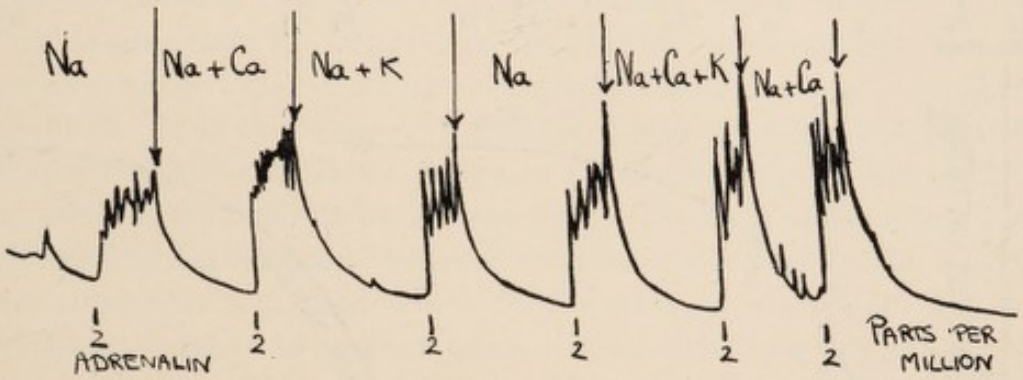

Fig. 2. The effect of the presence and absence of K and Ca in the bath. The salts were added to NaCl 0·9 p.c. in the following strengths: $CaCl_2$ ·024 p.c., KCl ·042 p.c.

The absence of both calcium and potassium had little or no effect on the adrenalin contraction—the same heights of contraction were obtained for the same doses. No reversal of the adrenalin contraction was obtained even with small doses of adrenalin as might have been expected from the results of Burridge(4), Wehland(5) and others. A drop of 10 *N* acetic acid added to the bath reduced the *p*H to about 5, and greatly diminished the adrenalin response.

The presence of a phosphate buffer ($pH = 7{\cdot}4$) had the effect of completely abolishing the spontaneous rhythm without much affecting the adrenalin contraction. Relaxation was delayed. This may be the result of the precipitation of calcium.

The action of ergotamine on the adrenalin contraction.

The test for ergot alkaloids is as follows:

The reaction of the muscle to adrenalin is tested and then ergotamine is added to the bath, left for 5 minutes and washed out. After 5 minutes in Ringer solution the muscle is again tested with adrenalin to see if it has been paralysed or not. This duration of stay in Ringer is

Fig. 3.

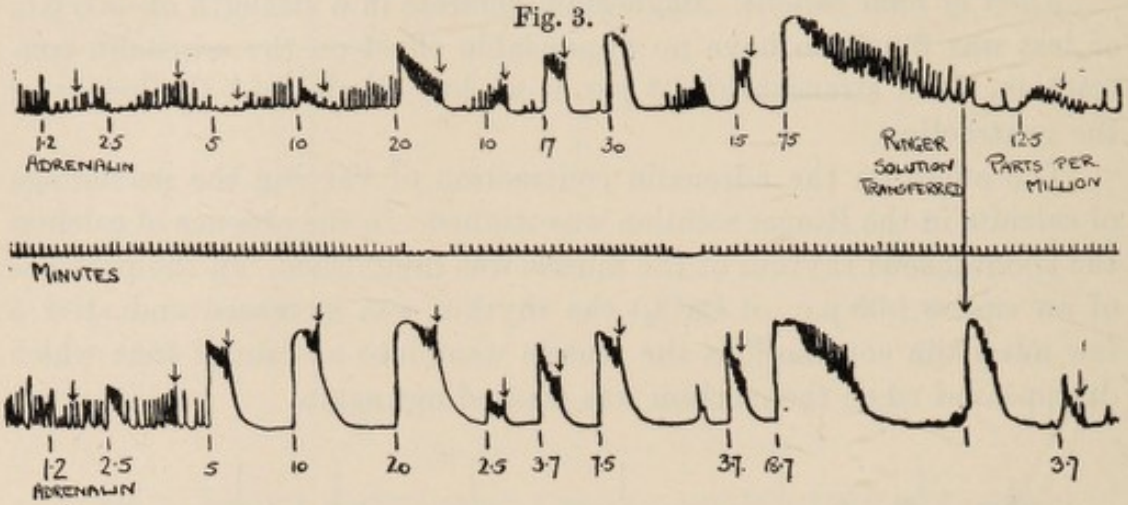

Fig. 4.

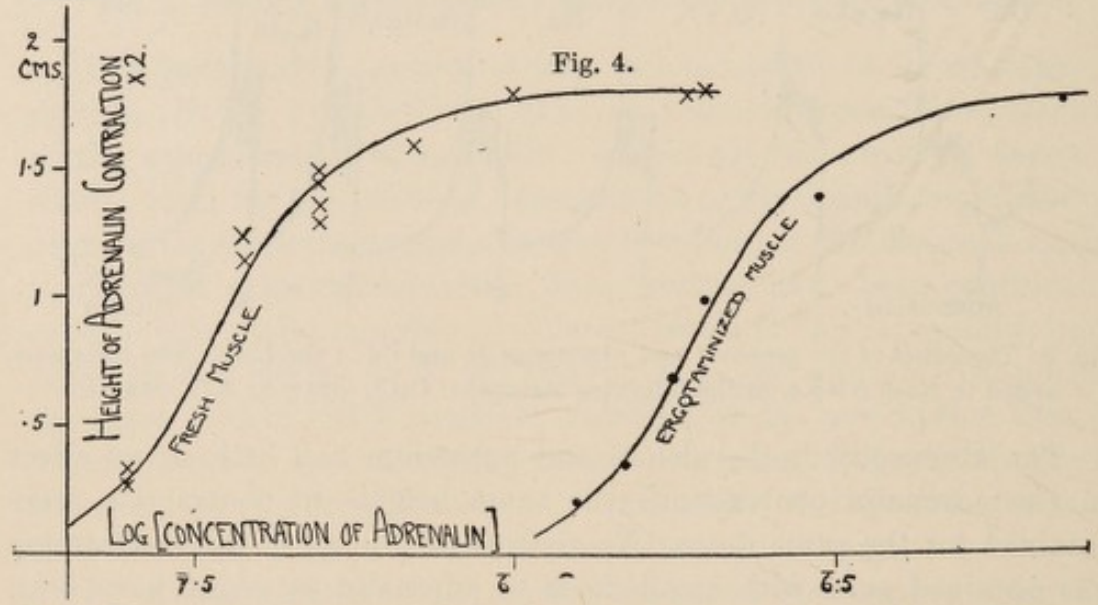

Fig. 3. Two pieces of muscle originally giving contractions of similar height. The upper one has been paralysed with ergotamine (five parts per million) for 5 minutes and it now requires four times the concentration of adrenalin to produce contractions of the same size as the lower one. Later the adrenalin was left in the bath until it had practically exhausted its effect on the upper tracing. The Ringer solution was now transferred to the other bath and produced a large contraction of the other muscle.

Fig. 4. Curves obtained from the tracing shown in Fig. 3 (in the first curve all the heights are reduced in the ratio 2 : 1·8 which was the ratio between the height of maximal contractions when neither piece was paralysed). The curves are drawn exactly the same shape.

essential. The paralysis appeared to take 5 minutes or more to develop its full force. It was irreversible in that it generally remained constant for at least an hour. During this hour, contractions were obtained by giving larger doses of adrenalin. Contractions of any height up to the maximum could be produced by increasing the dose of adrenalin in a definite proportion—a proportion which was the same for the different sizes of contraction and was thus, in some sort, a measure of the degree of paralysis produced. These facts are shown in Figs. 3 and 4. It will be seen that the proportion between the doses necessary to produce contractions of the same height, as represented by the distance between the curves (Fig. 4), is roughly constant. It is a little difficult to get two complete curves from the same portion of uterus, but a number of such curves have been obtained both before and after the action of adrenalin, and they are all of the same general shape. If the elements of muscle which are most susceptible to adrenalin were also most susceptible to ergotamine, it might be expected that the small responses to small concentrations of adrenalin would be more affected by ergotamine than the larger ones, because the nerve endings concerned in small contractions would be more paralysed than those concerned in large ones. Thus the slope of the curve would be increased. Similarly if there were any correlation between the distribution of susceptibility to the two drugs it would be expected that the shape of the curve would be altered by ergotamine. No such alteration was found. On the other hand, it was noticed that when uteri were kept overnight they were sometimes sensitive to smaller doses of adrenalin than before and these same uteri were also more sensitive to ergotamine, in that the same concentration produced a higher degree of paralysis. It is as if the route to their common site of action had become more permeable. This variation in the susceptibility of the whole muscle is thus probably determined by factors different from those which determine the variation among individual fibres, as the former case shows correlation between the susceptibility to the two drugs, and the latter does not.

When similar pieces of the same uterus were tested with varying concentrations of ergotamine, it was found that over as wide a range as was convenient the adrenalin proportion bore a linear relation to the concentration of ergotamine.

Similar results were obtained by Langley(5) in his study of the effect of curari and nicotine on the tone of the rectus abdominis of a frog, and by Cushny(6) with pilocarpine and atropine. Cushny points out that the results do not fit in with the conception of a simple chemical

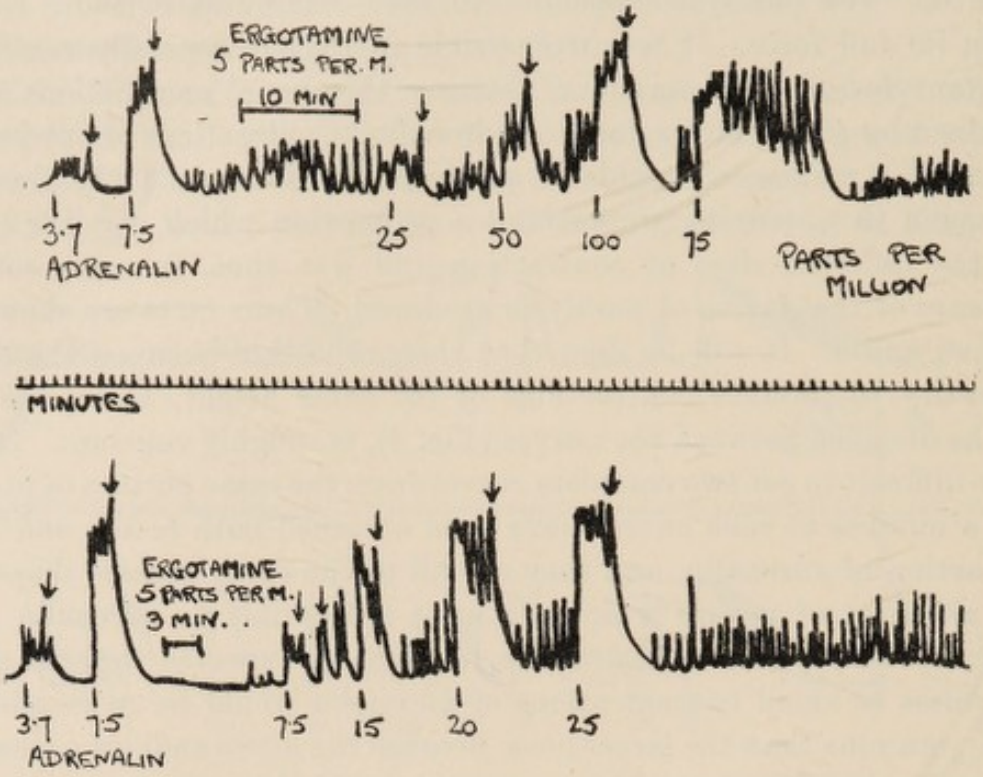

Fig. 5. Tracings showing the paralysis when ergotamine was left in the bath for different times. In the upper tracing the response to 50 parts per million of adrenalin is intermediate between that to 3·7 and that to 7·5. Thus the adrenalin proportion lies between 50/3·7 and 50/7·5 or 13·2 and 6·6. It is probably about 10 because the response to 7·5 is equal to that to 75. Similar reasoning applies to the bottom tracing where the proportion lies between 2 and 4 and is probably about 3.

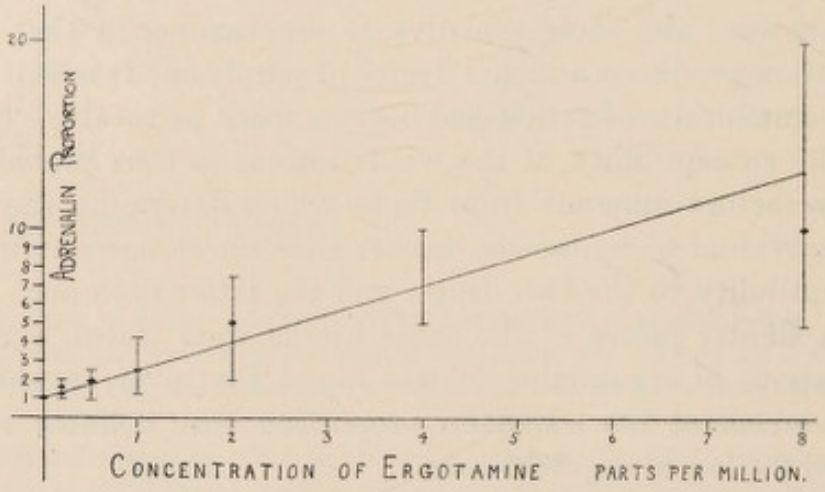

Fig. 6. Adrenalin proportion when different concentrations of ergotamine were left in the bath for 5 minutes. The vertical lines show the limits between which one can be certain of the proportions. (See discussion under Fig. 5.)

combination of the two drugs—leaving a certain amount over. In confirmation of this it was not found possible to detect any action between ergotamine and adrenalin *in vitro*. A mixture of solutions of the two, incubated overnight, produced an effect on the uterus indistinguishable from that produced by the same solutions introduced independently into the bath—an adrenalin contraction falling rapidly after 3 or 4 minutes as the ergotamine came into action.

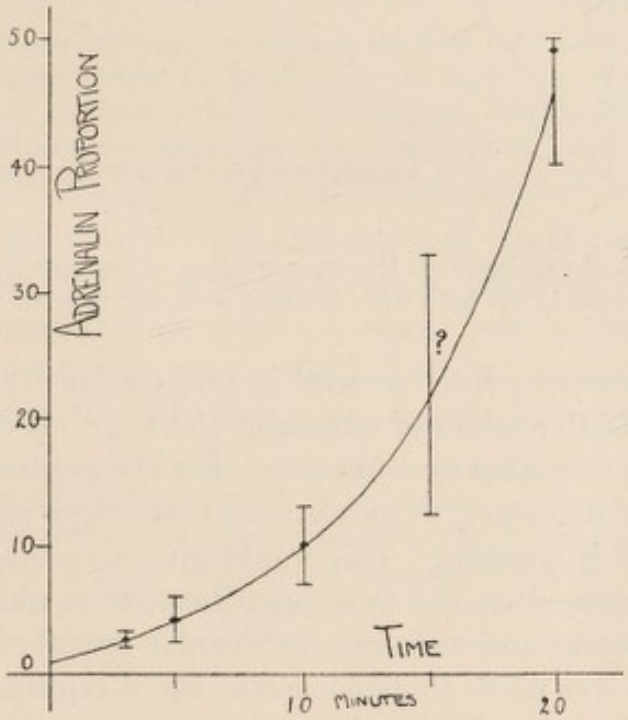

Fig. 7. Adrenalin proportion when five parts per million of ergotamine were left in the bath for different times.

The same dose of ergotamine was left in the bath for varying times and the adrenalin proportion determined. In all cases it was found that the degree of paralysis increased with time. The relation is shown in Fig. 7.

In some experiments with smaller concentrations of ergotamine there were indications that the paralysis reached a maximum value in from 3 to 20 minutes, but it was found impossible to get precise information as to the shape of the curves. When the ergotamine was left in the bath for a long time, the muscle developed additional tone which obscured all further results. It is possible by taking any one value of the adrenalin proportion and reading off in Fig. 8 the corresponding values of the concentration of ergotamine and the time it has had to act to get some idea of the relation between these two quantities.

The series of such curves obtained by taking different values of the adrenalin proportion are similar in general shape to those published by Sollman(7).

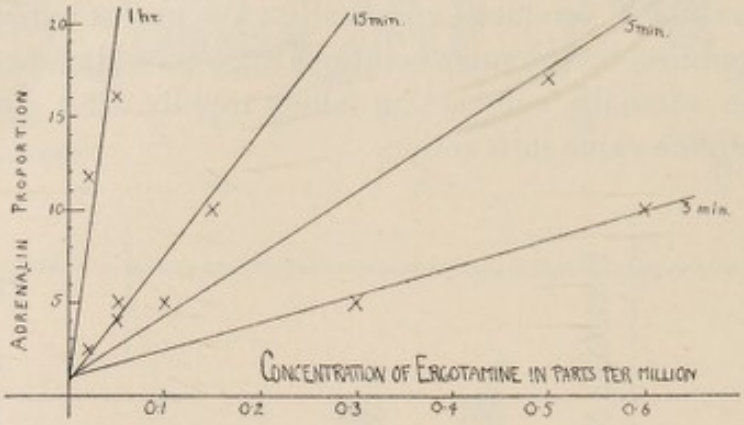

Fig. 8. Effect of varying both the concentration of ergotamine and the time it was left in the bath with different parts of the same uterus.

While the present paper has been in preparation Braun(8) has published a study of the action of adrenalin and ergot alkaloids on rabbit's uterus in which he makes the statement that the product of the concentration of ergot necessary to produce a given effect and the time it is left in the bath is constant. This would give a rectangular hyperbola as the curve connecting the two quantities. It is quite possible that Sollmann's curves and mine are rectangular hyperbolæ.

An attempt was made to demonstrate the disappearance of ergotamine from the bath by allowing it to act on one portion of muscle and then transferring the Ringer to another bath in which was a fresh bit of muscle. The second piece was paralysed to the same extent as the first, no measurable quantity of ergotamine having disappeared.

Thus the amount of ergotamine passing into the muscle is dependent on: (1) The concentration of ergotamine in the bath. (2) The time the muscle is in the ergotamine. Ergotamine appears to continue to pass in for over 20 minutes in some cases. (3) The temperature. Two corresponding halves were tested with adrenalin and then placed in ergotamine for 5 minutes—one at 37° and the other at 5°–2°. The muscles were then replaced in their baths and re-tested. The former was more paralysed than the latter.

It is independent of: (1) The bubbling of oxygen. Two corresponding halves were tested with adrenalin and then paralysed by being left in contact with ergotamine for 15 minutes. In one the oxygen was left bubbling all the time and in the other it was turned off after 2 minutes.

when mixing was probably complete. The degree of paralysis produced in the two cases was identical and corresponded with other values obtained for the same uterus. (2) The presence of adrenalin in the bath.

Ergotamine was not concentrated in the muscle sufficiently to produce a measurable alteration in the concentration of ergotamine in the bath.

After passing in, the ergotamine reacts in some way with the tissues. This reaction is not complete when the muscle is removed from the ergotamine. Its rate has a positive temperature coefficient.

Two corresponding halves were paralysed in the bath for 3 minutes and then immediately placed in Ringer solution for 12 minutes—one at 37° and the other at 2°. They were then replaced in the bath in fresh Ringer solution and retested 4 minutes later. The former gave a smaller contraction with adrenalin than the latter. After 10 minutes' further rest, the second stage was complete in both and they both gave the same result.

It was thought possible that this reaction might consist in the adsorption of ergotamine on some structure in the muscle. The evidence of the above experiment does not of course bear on this point, as adsorption, in common with most other processes, proceeds more quickly at higher temperatures. The following experiment is more relevant:

In a piece of muscle in which both stages had been completed in the bath, an attempt was made to change the equilibrium point by placing the muscle for 15 minutes in cold Ringer solution 2° and then replacing and retesting it. The degree of paralysis might have been expected to alter by about 20 p.c. under these conditions but no change could be detected. But of course it is very hard to say, what would or would not be the properties of such an adsorption and it is not possible to lay great stress on the result of this experiment.

A rough measure of the ergotamine content of a sample of ergot may be obtained with only two pieces of uterus by measuring the adrenalin proportions in the two cases. No improvement on Broom and Clark's technique for the final readings is suggested. The time must be accurately measured. If times longer than five minutes be used, the sensitivity of the test is increased, but the percentage accuracy is unchanged.

Theory of mode of action. A theory, which cannot be fully discussed here, has been formed on the following lines. It is assumed that there is an area in the muscle on which the adrenalin must act and that a fraction of this area is blocked by ergotamine so that in any given case the concentration of adrenalin must be increased in a certain proportion to produce the same effect. If it be assumed, as was found experimentally, that this proportion bears a linear relation to the concentration of ergotamine in the bath, the relation of this latter variable to the degree of action of the ergotamine may be calculated. The curve is of form similar to that found for the same relation in the case of adrenalin.

SUMMARY.

1. The effect of ergotamine and adrenalin on rabbit's uterus suspended in a bath has been studied. The relation of the concentration of adrenalin to the height of contraction has been measured.

2. If ergotamine be added it may take more than 20 minutes to develop its complete effect. This appears to take place in two stages —the first is a simple diffusion into the muscle. The second stage is some further change within the muscle.

3. Its effect is to increase in a certain proportion the concentration of adrenalin necessary to produce a contraction of given height.

4. Its degree in terms of this proportion bears a nearly linear relation to the concentration of ergotamine.

5. The effect of different temperatures on this paralysis has been studied. Both stages proceed more quickly at 37° than at 0°.

6. The simplest theory that can be suggested to account for these results entails a number of elements of varying threshold to adrenalin and of varying accessibility to ergotamine.

I wish to thank J. W. Trevan for his friendly criticism and advice.

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