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THE EFFECT OF VARYING TONICITY ON THE ANAPHYLACTIC AND OTHER REACTIONS OF PLAIN MUSCLE

BY

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THE EFFECT OF VARYING TONICITY ON THE ANAPHYLACTIC AND OTHER REACTIONS OF PLAIN MUSCLE

H. H. DALE

From the Wellcome Physiological Research Laboratories, Herne Hill, London, S. E.

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It was shown by Friedberger and Hartoch (1), that a preliminary injection of saturated sodium chloride solution enabled sensitised guinea-pigs to survive a reinjection of the sensitising antigen, which was sufficiently large to be uniformly fatal to identically sensitised control animals. Friedberger and Langer (2) have shown that sodium chloride given by the mouth in sufficient quantity has a similar protective effect. Friedberger and Hartoch attributed the effect of the salt to the well-known inhibiting action of hypertonic solutions on the fixation of complement, as studied *in vitro*. They found, indeed, that the mitigation of the symptoms following reinjection was attended by a diminution in the loss of complement, which accompanies a normal anaphylactic reaction. Since, according to Friedberger's theory, the complement plays an essential part in the production of "anaphylatoxin," any influence which inhibited complement-fixation would, on this theory, prevent the appearance of anaphylactic symptoms. Doubt has been cast on this explanation from more than one quarter. Bornstein (3) pointed out that intravenous injection of salt in such quantities caused a considerable dilution of the blood and increase of its volume, by the entry of water from the tissues. He regarded this as an important factor in the protective action. Ritz (4) showed that a preliminary salt injection protected against the action of "anaphylatoxin" prepared *in vitro*, which, on Friedberger and Hartoch's view, should not be affected. The demonstration of the anaphylactic reaction of isolated plain muscle, by Schultz (5), and by

myself (6), occurring under conditions in which the action of complement and the production of poisonous cleavage products appeared to be excluded, suggested to me that the protective action of salt injections might be due to a depression of the responsiveness of the plain muscle. By the use of isolated organs this possibility could be tested under conditions of ideal simplicity.

METHOD

As in my former experiments the uteri of young virgin guinea-pigs have been used throughout. Survivors from the standardisation of antitoxic (horse) serum have been used in all the experiments on anaphylactic response. Both horns of the uterus have been used in all experiments of this nature, the reaction of one being tested in Ringer's solution containing the usual 0.9 per cent of sodium chloride, to control the degree of sensitiveness, and the other being suspended in a similar solution, to which a further amount of sodium chloride has been added, in proportions varying in the different experiments. In some cases a preliminary perfusion of the uterus was carried out, as described in my previous paper. As perfusion fluid either the normal or the hypertonic Ringer's solution was used. The perfused horns were then transferred to separate vessels, one containing the normal, the other the hypertonic solution, kept warm and oxygenated. They were then tested in succession, each in the same volume of its appropriate solution. The choice of hypertonic or normal Ringer's solution for perfusion had no influence on the result, which was solely determined by the composition of the fluid in which the test was made. Identical results were obtained without preliminary perfusion. Care was also taken to perform the tests in the opposite orders in different experiments, so as to exclude any possible difference due to keeping one horn longer than the other in the solution. The temperature of the test solutions was kept uniform at 38°C. with special care. The use of the two horns of the uterus affords an opportunity of obtaining two anatomically and physiologically identical strips of uninjured plain muscle, such as can be obtained from no other organ.

The exact similarity of the curves obtained when the two horns were tested successively in solutions of the same composition, showed that any marked difference in the responses obtained with the two horns might with confidence be attributed to the only deliberate variation of the conditions, viz., the composition of the fluid in which the organ was suspended.

The Ringer's solution used for the controls was made up according to one of two formulae. The first was that which I used in my previously published experiments on the anaphylactic reaction, being Locke's formula, with the exception that the calcium content was reduced. It is as follows: NaCl, 0.9; KCl, 0.042; $CaCl_2$, 0.012; $NaHCO_3$, 0.05; dextrose, 0.1; water (distilled in glass), 100. This will be referred to as Ringer 1.

The second was modified from a formula given by Tyrode (7), and has proved an extremely effective medium for preserving the excitability of the isolated guinea-pig's uterus, without the spontaneous development of high tonus or large rhythm. It may be mentioned incidentally that it serves admirably for the method of standardising pituitary extracts which Laidlaw and I recently described (8). It is essentially Locke's solution, with the addition of a small proportion of magnesium chloride. The magnesium appears to possess a marked power of restraining the development of irregular automatic rhythm. The formula is: NaCl, 0.9; KCl, 0.042; $CaCl_2$, 0.024; $MgCl_2$, 0.0075; $NaHCO_3$, 0.05; dextrose, 0.1; water (glass distilled), 100. This is referred to as Ringer II.

Neukirch (9) has used Tyrode's solution with like effect for preserving the regular rhythm and excitability of isolated intestinal loops from the rabbit.

The results obtained with these two Ringer's solutions, with varying proportions of additional sodium chloride, have been of the same general type.

MODIFICATION OF THE ANAPHYLACTIC RESPONSE

Friedberger and Hartoch's protective action was obtained by injecting as much as 1 cc. of saturated sodium chloride intra-

venously into a small guinea-pig (200 to 250 grams). Taking the concentration as 37 per cent, and assuming 15 to 20 cc. as the blood-volume of such a guinea-pig, it is easy to calculate that the injection must raise the sodium chloride in the blood to as much as 3 per cent. Such a concentration would, of course,

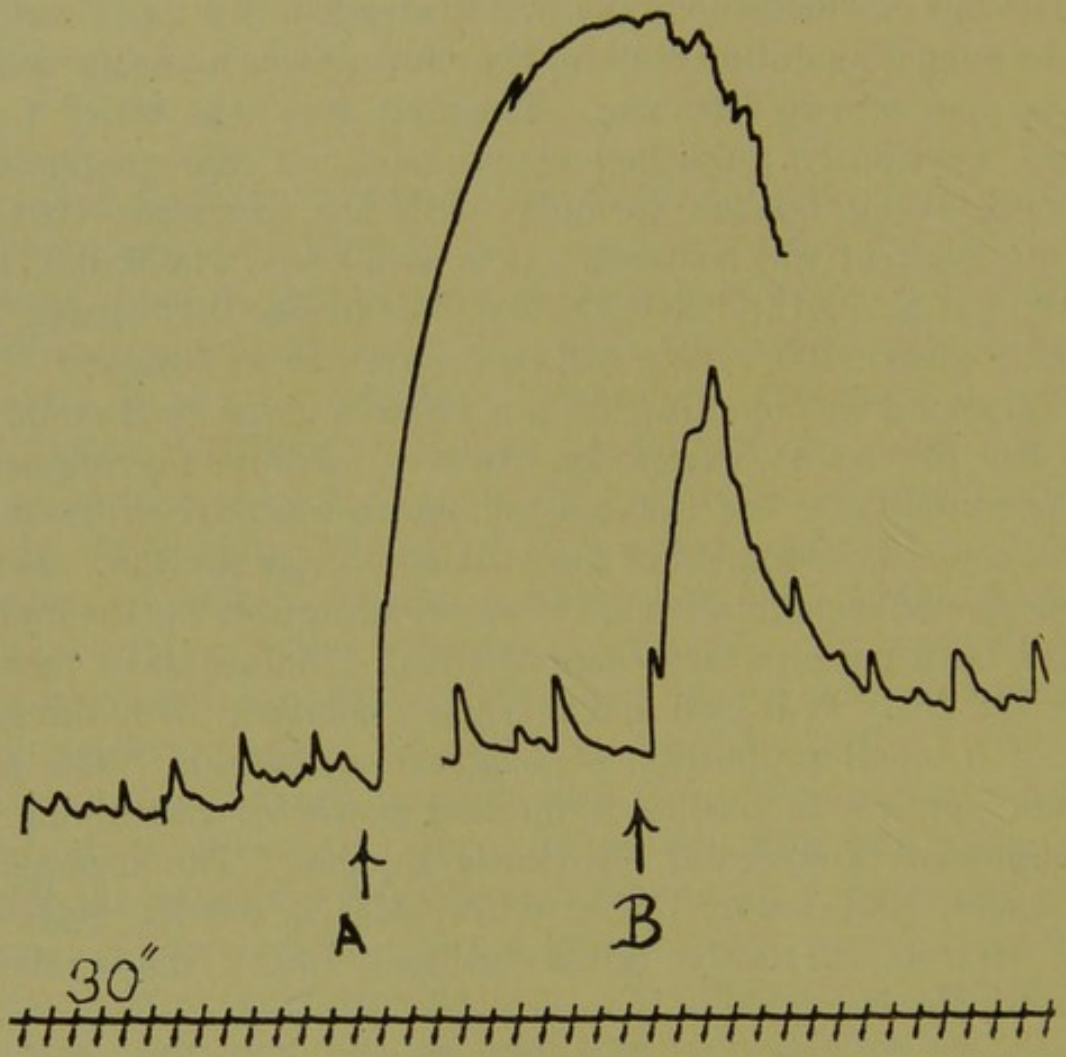

FIG. 1. UTERUS OF VIRGIN GUINEA-PIG (BOTH HORNS)

Sensitisation 1/500 *cc.* diphtheria antitoxic (horse) + 1 test dose toxin: eighteen days. Perfused with Ringer I. Bath volume (in all experiments illustrated) 250 cc. First horn in Ringer I, second horn in Ringer I + 0.2 per cent NaCl (1.1 per cent in all). At *A* and *B* 0.5 cc. horse-serum added to bath.

be only momentarily attained; but it is probable that the injection of antigen in such experiments is made while the tonicity of the blood is still at such a level that the isolated plain muscle could not long survive in an equivalent saline solution. It was quite unnecessary, however, to try the effect of such concen-

trated solutions on the response of the isolated organ, since a few experiments made it clear that a comparatively slight increase in the saline content of the fluid very markedly depressed the extent of the anaphylactic reaction.

Figure 1 shows the effect of raising the sodium chloride content from 0.9 to 1.1 per cent, 2 grams of salt being added to 1 liter of the Ringer's solution. Figure 2 shows a similar pair of

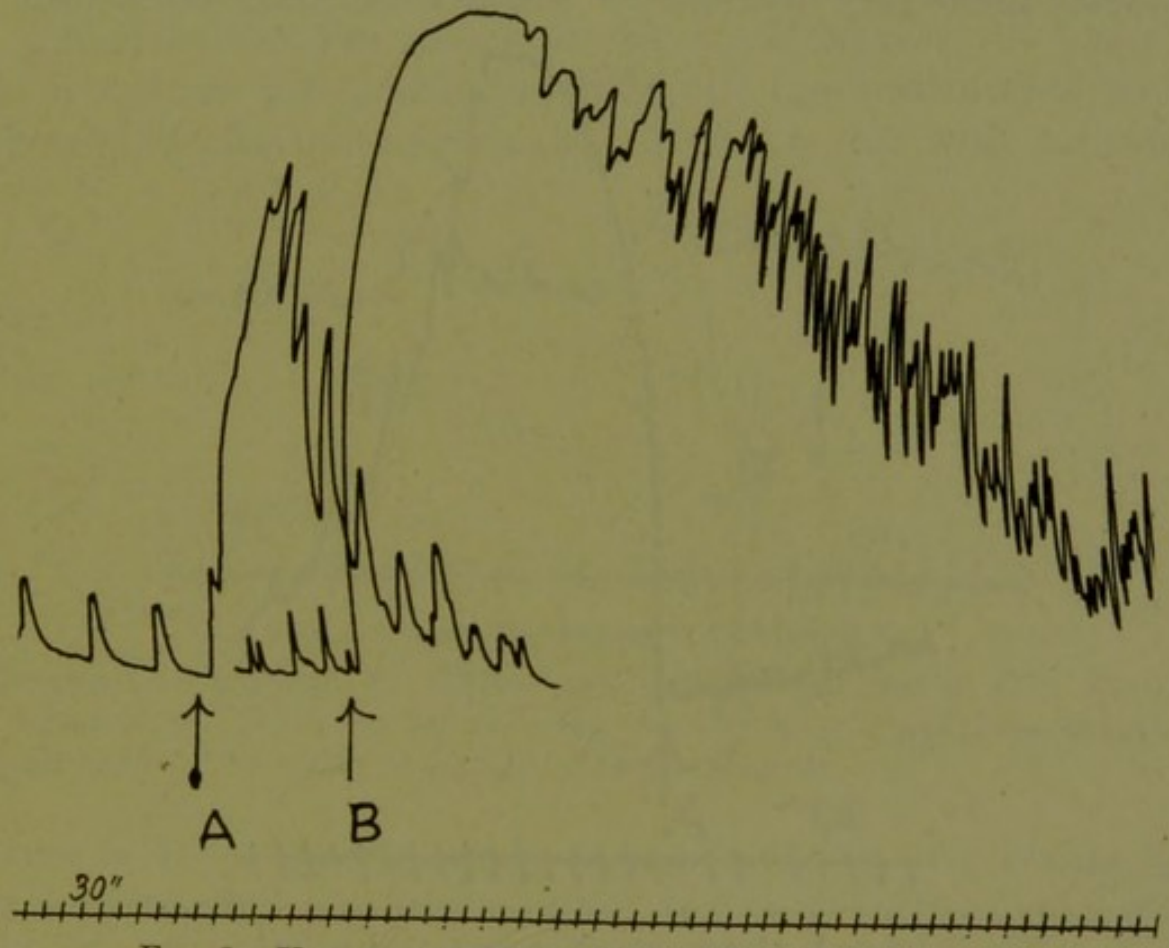

FIG. 2. UTERUS OF VIRGIN GUINEA-PIG (BOTH HORNS)

Sensitisation 1/45 cc. antitoxin + 1 test dose toxin: sixteen days. Perfused with Ringer I + 0.3 per cent NaCl (1.2 per cent in all). First horn in 1.2 per cent NaCl Ringer, second in normal Ringer I. At *A* and *B* 0.5 cc. horse-serum.

responses, taken in the reverse order, from one horn in Ringer's solution with 1.2 per cent NaCl, the other in the ordinary solution with 0.9 per cent NaCl. A rise to 1.3 per cent of sodium chloride produced almost complete extinction of the response to the antigen in the case illustrated in figures 3 and 4. Figure 4 illustrates another point of some importance. After the dose of antigen had been given at *A*, with minimal effect, the solution

was run off, and after repeated washing of the uterus with fresh quantities of the hypertonic solution, the bath was filled with the ordinary Ringer's solution, containing 0.9 per cent NaCl. This change produced a small rise of tonus, with the cause of which we shall deal in a later section. Here it is only necessary to mention that it has no connexion with the previous addition of antigen. A normal uterus, in the absence of any drug, shows a similar temporary increase of tonus when transferred from a

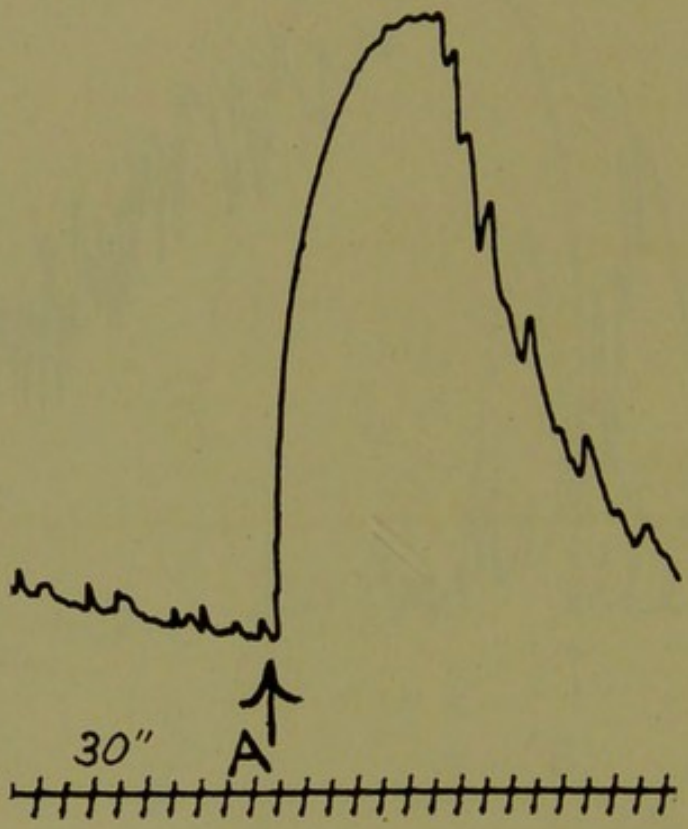

FIG. 3. UTERUS OF VIRGIN GUINEA-PIG

Sensitisation 1/600 antitoxin + 1 test dose: seventeen days. Perfused with Ringer I. At *A* 0.5 cc. horse-serum.

solution of higher to one of lower tonicity. After this change a further dose of the antigen was given at *B*, and was completely ineffective, although the control horn, receiving its first dose in the same solution, gave a typical anaphylactic response (fig. 3). This observation has been made repeatedly and always with the same result; though the tonicity of the solution was raised to such a point that addition of a full dose of antigen caused practically no contraction, the plain muscle was none the less effec-

tively desensitised to further doses, given in a solution in which contraction, apart from such previous treatment, occurs. Mere preliminary suspension of the organ in, or even prolonged perfusion with hypertonic Ringer, has no depressant effect on its subsequent response to the antigen in normal Ringer. The desensitisation can, therefore, only be attributed to the previous dose of antigen, and we are driven to the conclusion that the union of fixed antibody with antigen, and consequent desensitisation, is unhindered by the comparatively small increase of tonicity, which suffices practically to obliterate the contraction which normally results from such union. This is in line with the obser-

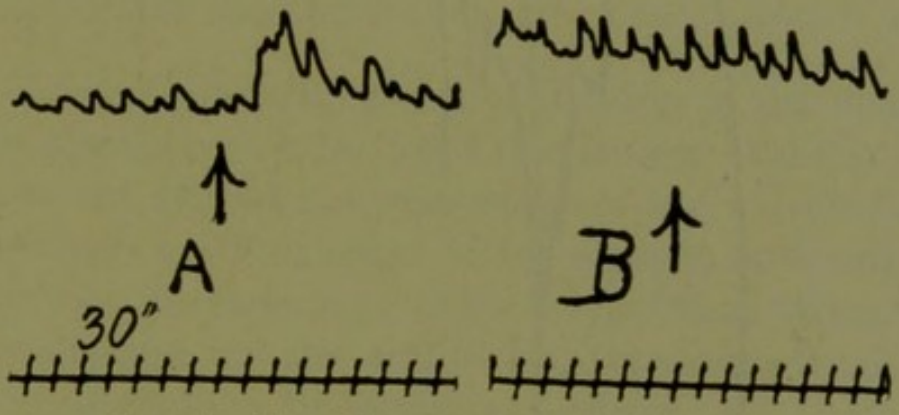

FIG. 4. SAME EXPERIMENT AS FIGURE 3.

Second horn in Ringer I + 0.4 per cent NaCl (1.3 per cent in all). At *A* 0.5 cc. horse-serum. Wash out several times with hypertonic and then change to normal Ringer I, to which, at *B*, add 0.5 cc. horse-serum.

vation of Ritz, that injection of strong saline protects against "anaphylatoxin" prepared *in vitro*, and leads to the expectation that the effect of added salt is to depress the responsiveness of the muscle to motor stimuli in general. In the following section it will be shown that this is, indeed, the case. Two further points, however, first need mention.

Firstly, the depressant effect is not due to excess of sodium ions in relation to others. If the concentration be increased by making up the Ringer's solution with a smaller proportion of water, while retaining the normal relation of the saline constituents to one another, the effect is identical with that produced by adding an extra quantity of sodium chloride.

Secondly, the customary solution with 0.9 per cent NaCl,

though it presumably represents approximately the saline composition of the body fluids, does not furnish the condition for maximum responsiveness of the isolated plain muscle. If we

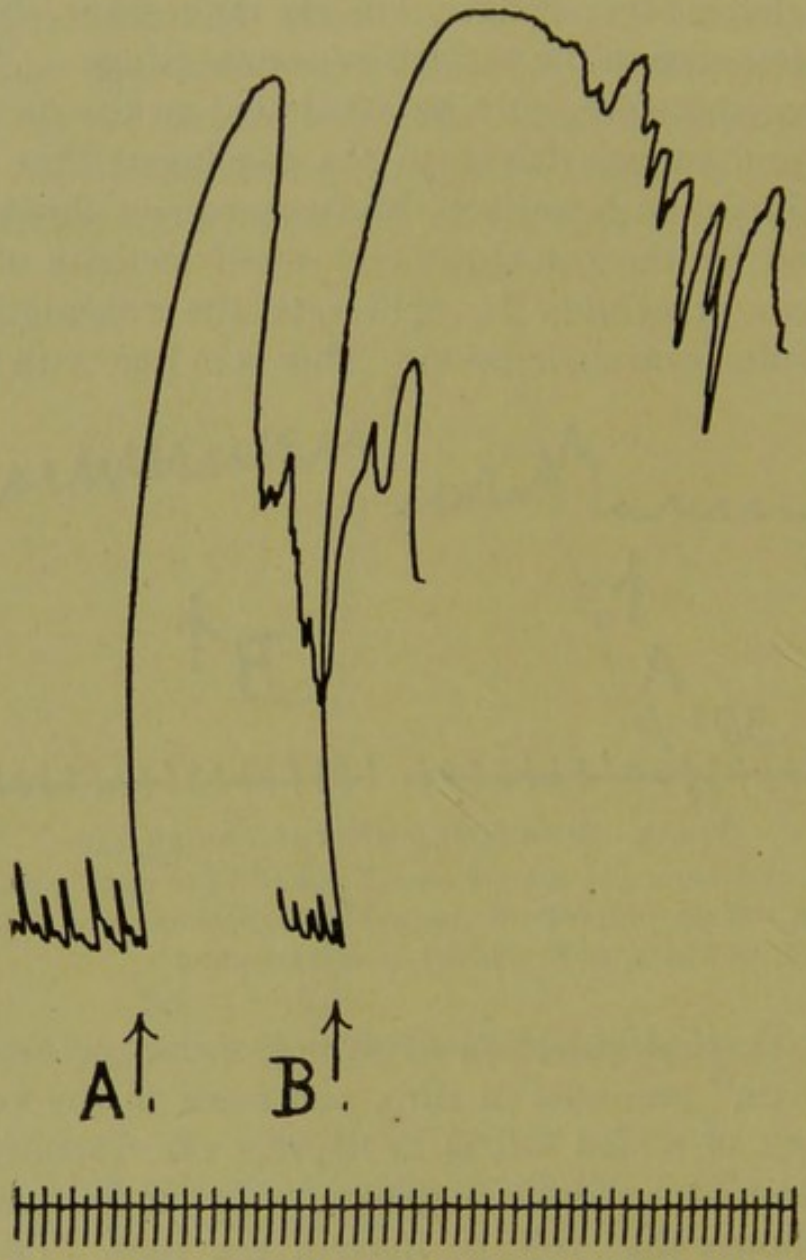

Fig. 5. Uterus of Virgin Guinea-Pig (both horns)

Sensitisation 1/400 antitoxin + 1 test dose: fourteen days, Not perfused. First horn in Ringer II, second horn in Ringer II diluted with one-eighth volume of water. At *A* and *B* 0.01 cc. horse-serum.

regard the responsiveness in this solution as representing the normal condition, a supernormal excitability is obtainable by lowering the concentration of the bath by addition of distilled water. The increase of anaphylactic response produced by thus

lowering the concentration of sodium chloride from 0.9 to 0.8 per cent, and that of the other constituents in proportion, is illustrated in figure 5. In experiments on the whole guinea-pig some observers have found that a minute reinjection of antigen, sufficient to produce only a small rise of temperature when given in a small volume of saline, will cause death if given in a large volume of distilled water—Thiele and Embleton (10). The observation involves the response of other cells, as well as those of plain muscle, but the parallellism with the effect on isolated plain muscle is suggestive.

MODIFICATION OF THE RESPONSE OF NORMAL PLAIN MUSCLE

It was shown above that the modification of the anaphylactic reaction by altered concentration of the Ringer's solution is apparently due to the altered responsiveness of the muscle fibers, and not to inhibition or facilitation of the antigen-antibody reaction which furnishes the stimulus. This is confirmed when the effect of similar changes on the action of stimulant drugs, such as β-iminazolylethylamine or pituitary extract, is studied. With these we can study the phenomenon to much better advantage, since, as Laidlaw and I have shown, the plain muscle of the guinea-pig's uterus gives a very uniform series of responses to a succession of equal doses of these substances, provided that each dose is thoroughly washed away, and the normal condition of minimum tonus regained, before the next dose is added. I have used β-iminazolylethylamine for these experiments, as eliciting the more rapid and, on the whole, more uniform response: but pituitary extract, or pilocarpine, or, indeed, any stimulant drug, to which the muscle does not rapidly acquire tolerance with successive doses, will show the effect. It is necessary to emphasise this, since the resemblance between the action of iminazolylethylamine and certain features of the anaphylactic response might give the impression that the phenomenon with which we are dealing was peculiar to effects of this type.

Figure 6 shows the modification of the effect of a just maximal dose of iminazolylethylamine, produced by adding increasing

doses of sodium chloride to the 250 cc. of Ringer's solution (II) with which the bath was uniformly filled. It will be seen that the change from 1.3 to 1 per cent NaCl restores the activity almost to the original level, and that the restoration is practically complete on subsequently changing to solution of the original concentration, with 0.9 per cent sodium chloride.

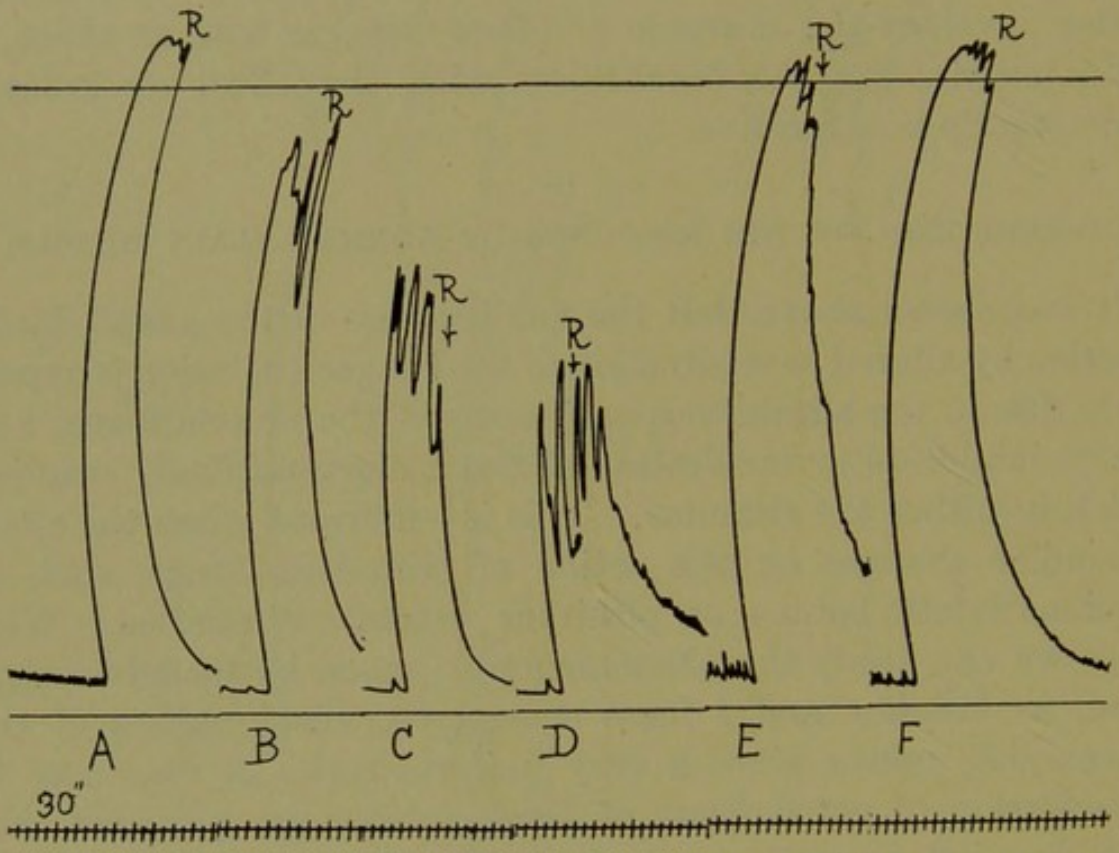

Fig. 6. Horn of Uterus of Normal Virgin Guinea-Pig (also in Figs. 7–11)

Dose in each case 0.05 mgm. β-iminazolylethylamine. At *A* uterus is in Ringer II with 0.9 per cent NaCl (normal), *B* 1.1 per cent, *C* 1.2 per cent, *D* 1.3 per cent, *E* 1 per cent, *F* 0.9 per cent (normal). At *R*, *R* (in this and subsequent figures), change of Ringer's solution.

In the previous section it was noted that the depressant action is not due to increasing the proportion of sodium ions. It has now to be added that increase of the osmotic pressure of the solution by adding another electrolyte, such as sodium sulphate, or a non-electrolyte, such as cane-sugar, is equally effective in depressing the response of the plain muscle. No difference could be detected between the effects of sodium chloride and sodium

sulphate added in equimolecular proportions. The effect of adding 2 per cent of cane-sugar is illustrated in figure 7.

It was found, as expected, that the sensitiveness of the plain

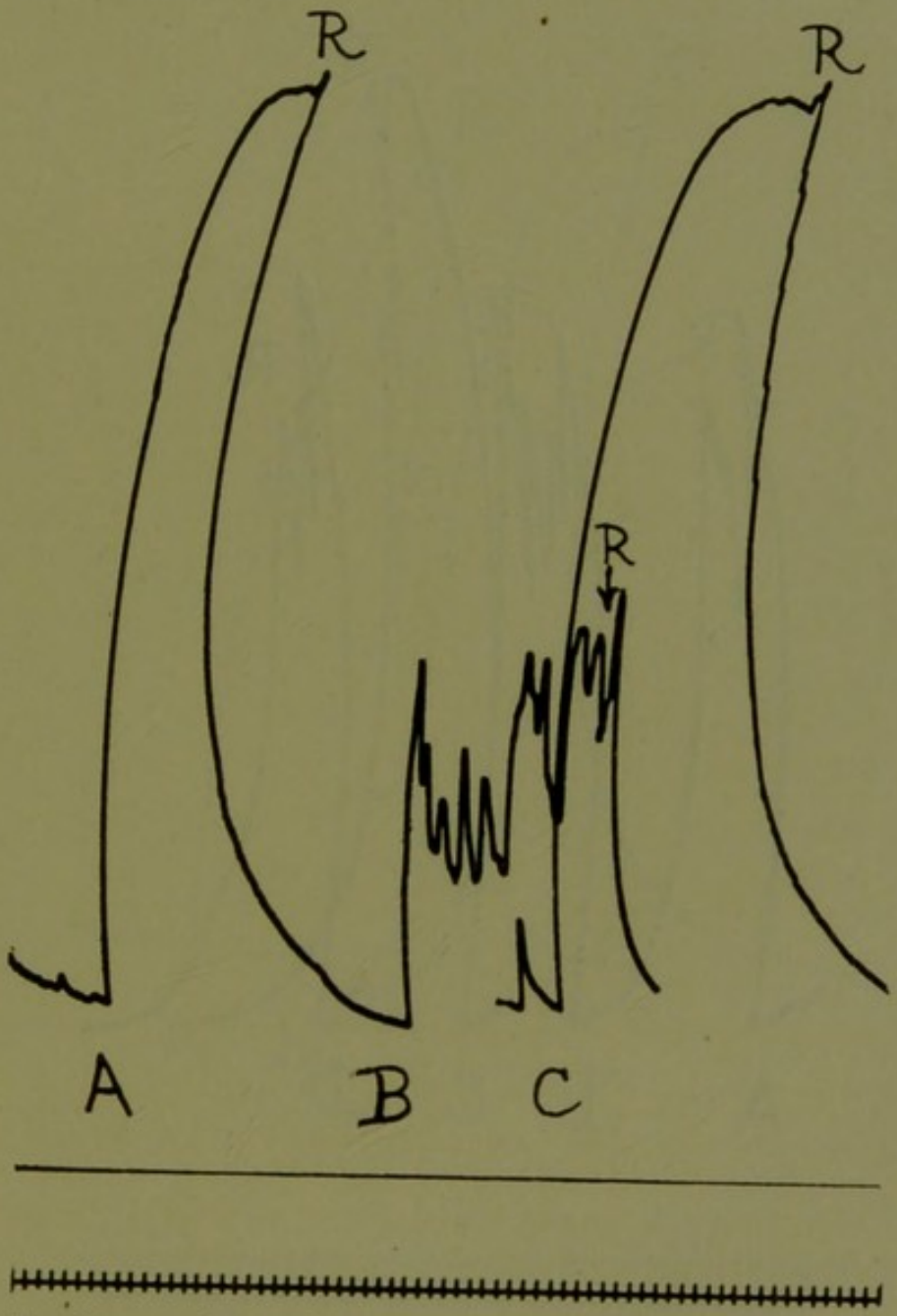

FIG. 7. DOSE IN EACH CASE 0.05 MGM. β-IMINAZOLYLETHYLAMINE

At *A* and *C* uterus is in normal Ringer II, at *B* in the same with the addition of 2 per cent cane sugar.

muscle to iminazolylethylamine was increased when the concentration of the Ringer's solution was lowered by addition of distilled water. A very small change of concentration produced a well-marked change of excitability. Figure 8 shows the effect

of four successive, equal, submaximal doses of iminazolylethylamine. The first two contractions were produced with the uterus in the ordinary undiluted Ringer's solution (II). The

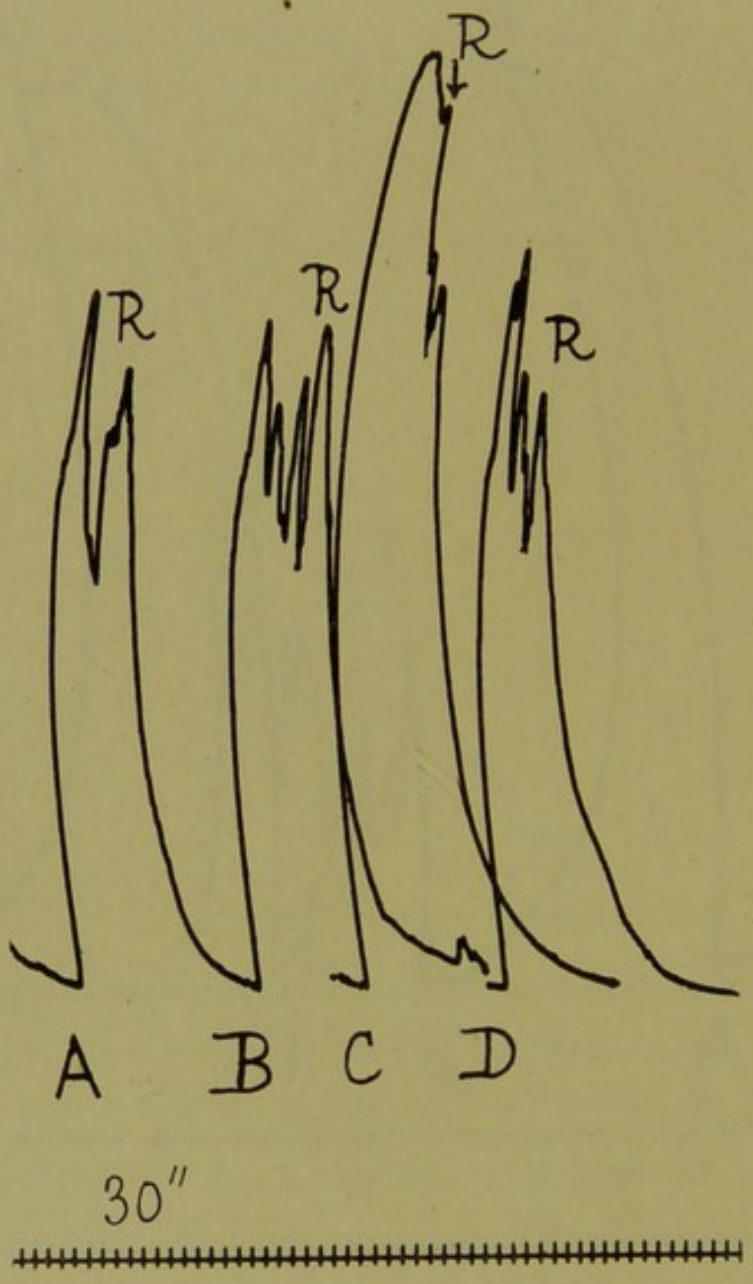

FIG. 8. DOSE IN EACH CASE 0.01 MGM. β-IMINAZOLYLETHYLAMINE

At *A*, *B*, and *D*, uterus is in normal Ringer II (with 0.9 per cent NaCl), at *C* in the same diluted with one-seventeenth volume of distilled water (reducing NaCl to 0.85 per cent).

dose producing the effect at *B* was washed away as usual, and the standard volume of 250 cc. fresh solution run in. To this 15 cc. of distilled water were added, bringing the concentration

of NaCl to about 0.85 per cent and lowering that of the other constituents in proportion. The volume in the bath was then again brought to 250 cc., and, when the muscle had returned

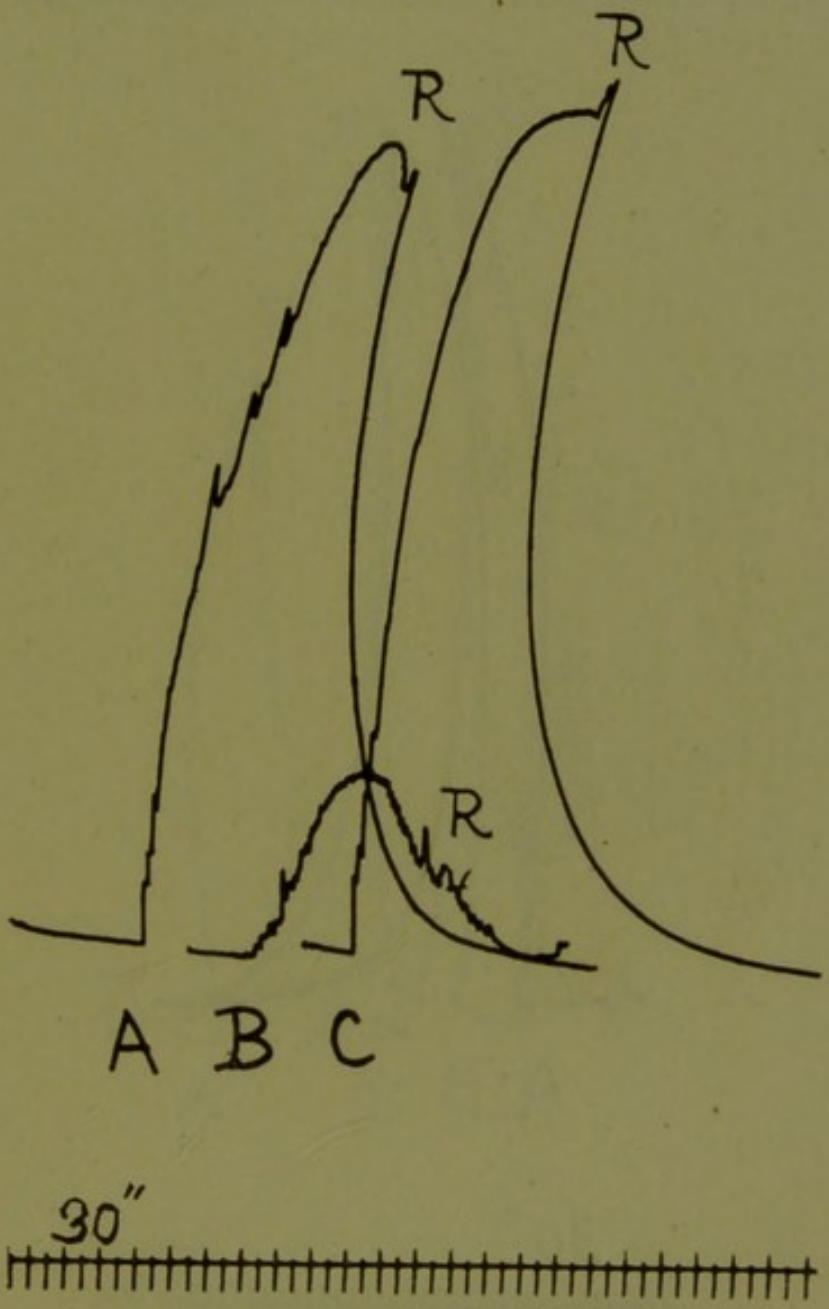

FIG. 9. DOSE IN EACH CASE 0.002 MGM. β-IMINAZOLYLETHYLAMINE

At *A* and *C* uterus is in Ringer II, reduced by dilution to 0.75 per cent NaCl: at *B* in the same diluted to 0.8 per cent NaCl.

to the condition of minimum tonus, the third dose was given at *C*, producing a much larger effect. A change back to the ordinary concentration then restored the excitability to the original level, as shown at *D*.

Figure 9 shows the effect of a similar change, made at a lower level of concentration; it will be observed that in this case an extremely small dose (0.002 mgm.) has to be used in order to avoid producing a supramaximal effect in the solution containing 0.75 per cent NaCl.

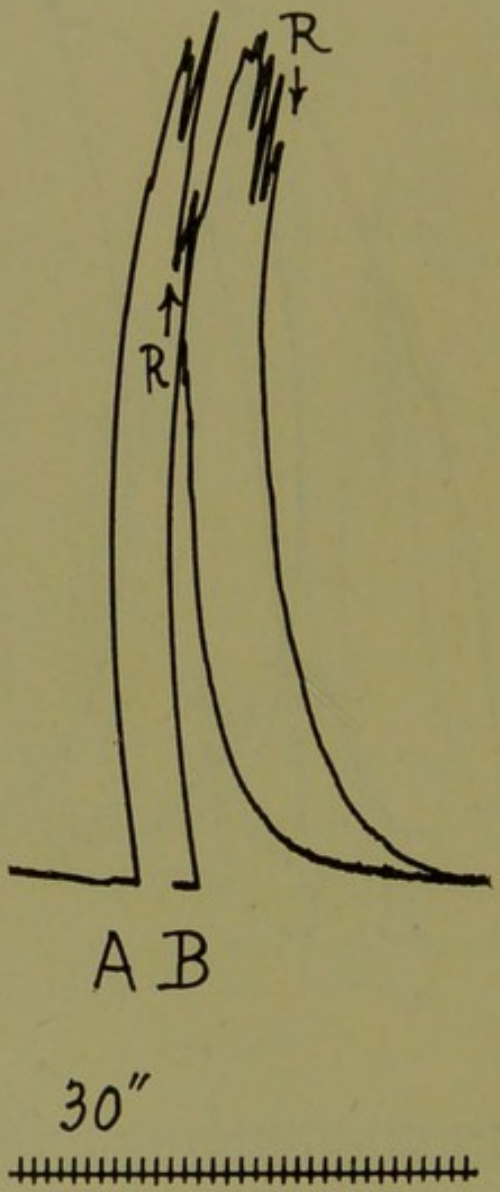

Fig. 10. Dose in Each Case 0.01 mgm. β-Iminazolylethylamine

At *A* uterus is in normal Ringer II, at *B* in the same diluted with one-eighth volume of 12 per cent cane sugar.

The effect of dilution is due to the lowering of the tonicity of the solution. A diminution of the concentration in the electrolytes, within the limited range with which we are dealing, has no effect, provided that the osmotic pressure of the solution is

maintained by adding an equivalent amount of a non-electrolyte, such as cane-sugar. Figure 10 shows the equal effects of two doses of iminazolylethylamine (0.01 mgm.), given when the bath was filled respectively with ordinary Ringer's solution, and with

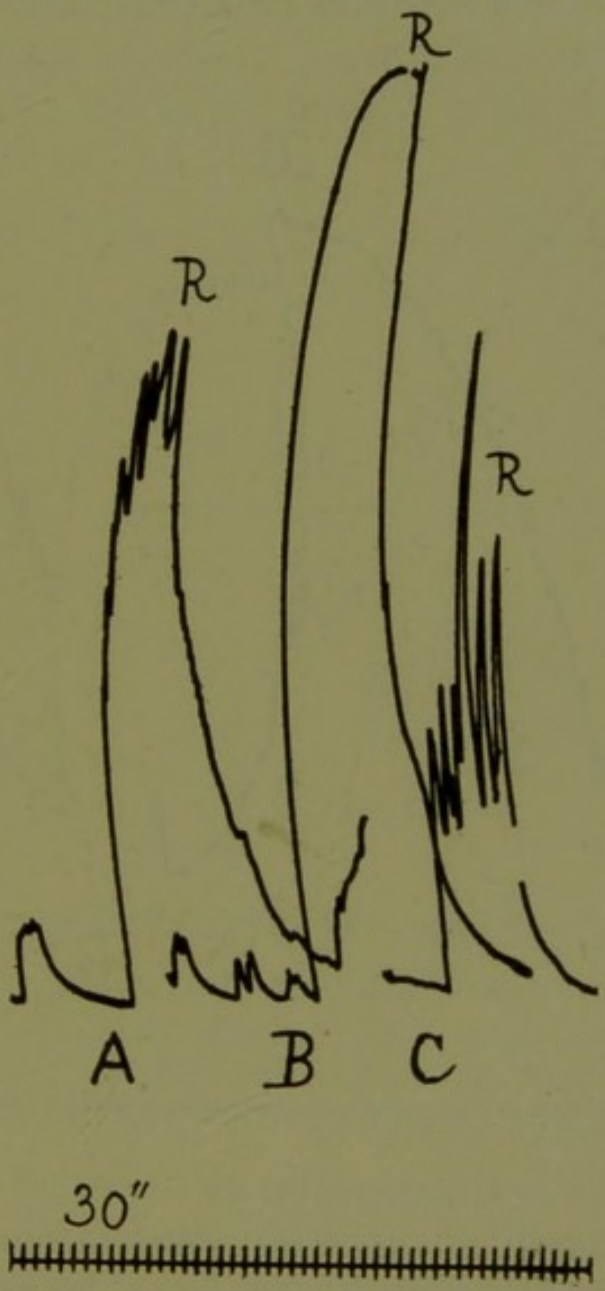

FIG. 11. DOSE IN EACH CASE 0.005 MGM. β-IMINAZOLYLETHYLAMINE
At *A* and *C* uterus is in normal Ringer II, at *B* in the same diluted with one-eighth volume of 2 per cent urea.

Ringer's solution of which the saline content had been reduced from 0.9 to 0.8 per cent NaCl by adding one-eighth of its volume of a 12 per cent (isotonic) solution of cane-sugar in distilled water. On the other hand, a 2 per cent solution of urea, which has about

the same osmotic pressure as Ringer's solution, acts as a diluent like distilled water (fig. 11). This, again, is in accordance with the supposition that the effect is due to change in the tonicity of the solution, since urea, to which the cell-membranes are perfectly permeable, is osmotically indifferent where living cells are concerned.

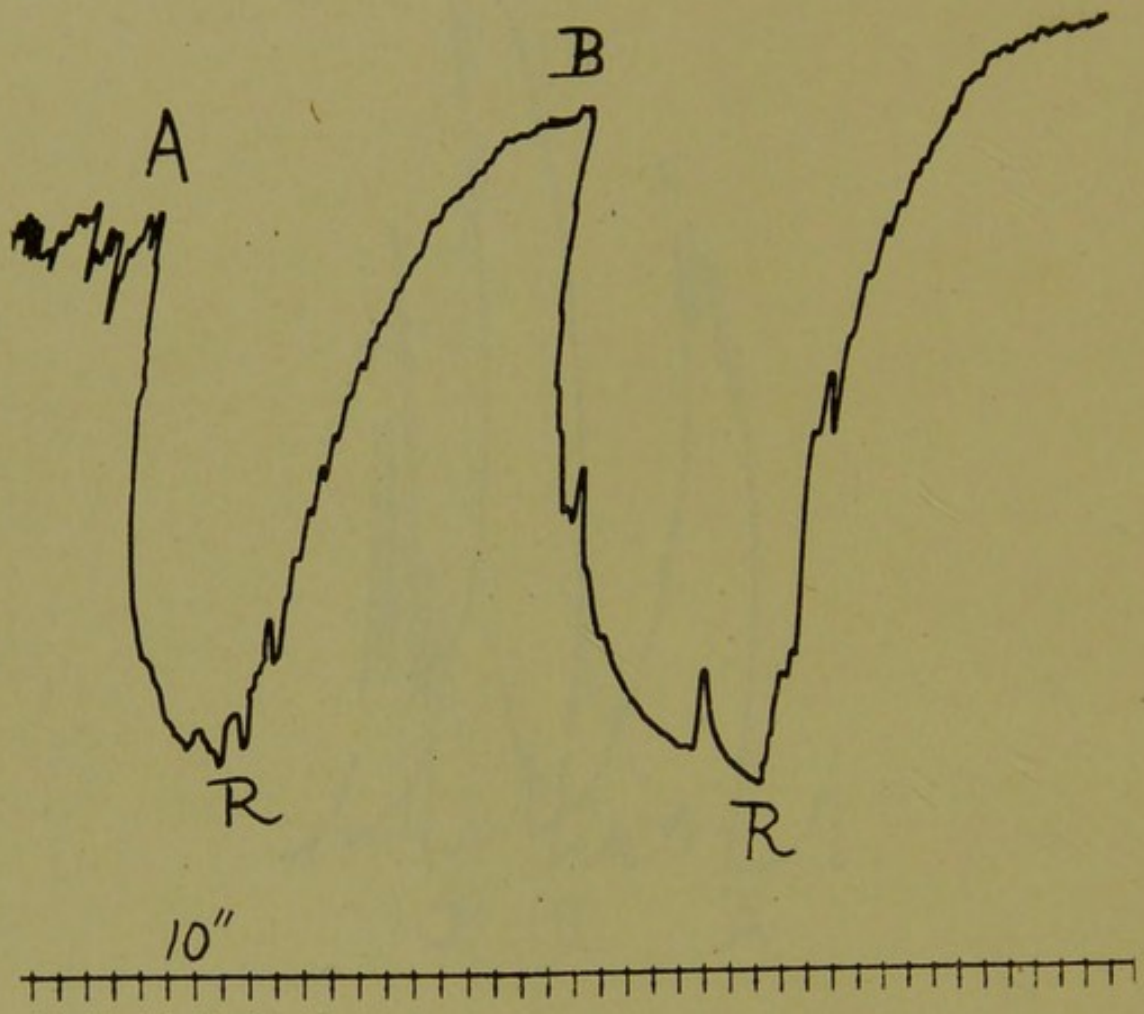

Fig. 12. Horn of Uterus of Virgin Cat in 250 cc. Ringer II

At *A*, 0.002 mgm. adrenine. *R*, change to fresh Ringer. At *B* – 1 cc. 25 per cent NaCl (raising NaCl concentration from 0.9 to 1 per cent).

A fall in the tonicity of the bathing-fluid, if sufficiently large and sudden, is itself an efficient stimulus. Thus the addition of 30 cc. of distilled water to a bath containing 250 cc. of Ringer's solution usually provokes a contraction of the plain muscle suspended therein. A more marked contraction is produced by adding 50 cc. of water, which reduces the saline concentration

from 0.9 to 0.75 per cent NaCl. Indeed, the effect of a stimulant drug, such as iminazolylethylamine, may be closely simulated by thus suddenly reducing the concentration of the fluid; when the requisite amount of salt is added to restore the original concentration, relaxation occurs, as when the stimulant drug is washed away by fresh solution. It may be stated, therefore, in general terms, that a fall in the tonicity of the bath excites tonic

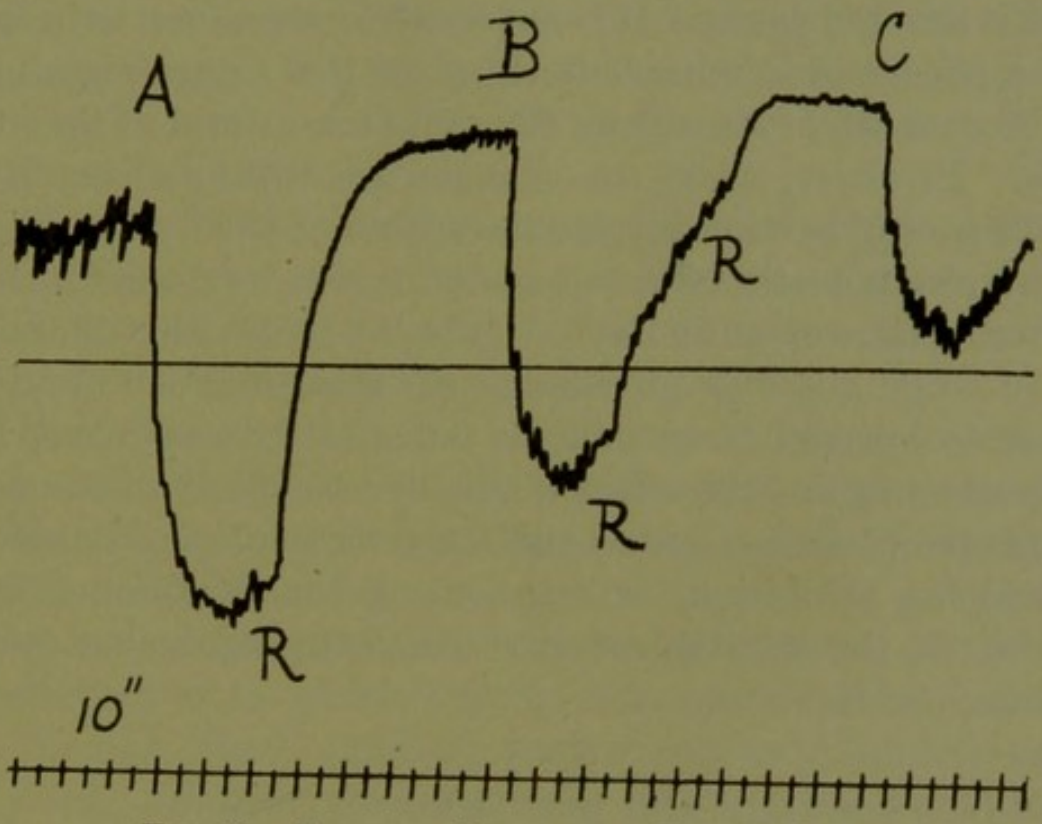

FIG. 13. HORN OF UTERUS OF VIRGIN CAT

Dose in each case 0.002 mgm. adrenine. At *A* in Ringer II + 0.05 per cent NaCl (0.95 per cent in all). At *B* in normal Ringer II (0.9 per cent NaCl). At *C* in Ringer II diluted with one-seventeenth volume of distilled water (0.85 per cent NaCl).

contraction, a rise in osmotic pressure inhibits existing tonus in isolated plain muscle.

The directly inhibitor effect of a rise in concentration is much better seen in the isolated uterus of the non-pregnant cat, which normally maintains a high tonus in Ringer's solution. Figure 12 shows the inhibitor effect on such a uterus of adding 1 cc. of 25 per cent NaCl to the bath, thus raising the concentration from 0.9 to 1 per cent NaCl. The effect of a small dose of adrenine is shown for comparison.

This suggests the enquiry, how the effect of an inhibitor drug will be changed by small changes in tonicity of the fluid in which the organ is suspended. The inhibitor effect of adrenine on the cat's non-pregnant uterus can be repeated indefinitely with fairly uniform result and is, therefore, suited to the experiment. The only difficulty is occasioned by the fact that the inhibitor action produced by increasing concentration persists till the concentration is lowered again. It is not possible, therefore, as in recording the effects of a stimulant drug on the guinea-pig's uterus, to make each effect start from the same tonus-level of the uterine muscle. However, when the changes are within a very limited range, it is easy to demonstrate the adjuvant effect of an increase, and the antagonistic effect of a diminution of tonicity, to the inhibitor action of adrenine. Figure 13 shows the diminishing effect of equal doses of adrenine as the concentration of Ringer's solution is lowered from 0.95 to 0.9 and 0.85 per cent NaCl. In this case, again, the effect is not due to specific ionic action; the addition of cane-sugar to the Ringer's solution is as effective in producing inhibition, or augmenting the inhibitor action of adrenine, as the addition of an osmotically equivalent amount of sodium chloride.

SUMMARY AND DISCUSSION OF RESULTS

These experiments, begun with the object of investigating the influence of strong saline in protecting the anaphylactic guinea-pig from the fatal effects of reinjection, have led to conclusions of more general application to the activity of plain muscle. It should be noted that it is with the tonus rather than with the rhythmic activity of plain muscle that the effects described in this paper are concerned; though the probability of their applicability to rhythm also is indicated by a few experiments which I have made on isolated intestinal muscle, of which rhythm is the predominant characteristic, as tone is that of the isolated non-pregnant uterus. The abolition of the rhythm of isolated intestinal muscle, by addition to the Ringer's solution of 1 per cent of dextrose, has been previously described by Cohnheim (11).

But, dealing only with the results described in detail in previous sections, they may be summarised as follows:

1. Any increase in the tonicity of the Ringer's solution, in which isolated plain muscle is suspended, has an inhibitor effect on tonus; any decrease in tonicity acts as a stimulus.

2. When the change in either direction is too small to cause a rise or fall of tonus by itself, or when the plain muscle has adjusted itself to the new conditions, the subsequent effect of a stimulant drug is decreased, that of an inhibitor drug increased, by a rise in tonicity, while a fall in tonicity produces the opposite effects in both cases.

3. The protective effect of strong saline injections in the anaphylactic shock of the guinea-pig is readily explicable, as a particular case of the depressant effect of raised tonicity on the response of plain muscle to stimulant drugs.

It does not seem desirable at the present stage to attempt to base on these results any positive conclusions as to the mechanism of plain muscle contraction. At first sight, indeed, the apparent dependence of the effect on the tonicity of the solution only, and its independence within limits of the nature of the salt or non-electrolyte to which this is due, seems to be inconsistent with theories which attribute contraction to imbibition of water (Quellung) by the muscle-colloids. It must be borne in mind, however, that we are dealing with living cells, freely permeable to water, but relatively impermeable to sodium ions or cane-sugar. The immediate effect of changes in the osmotic pressure of the bathing-fluid, whatever the substance to which the osmotic pressure is due, will be to withdraw water from, or add water to the constituents of the muscle fibers, thereby raising or lowering the concentration of their intrinsic salts. Since the interchange in contraction is regarded as taking place between fibrils and interfibrillar substance, and not between the whole organ and the fluid bathing its surface, there is no necessary inconsistency between the facts here presented and an imbibition theory of contraction. With one particular view of the nature of plain-muscle contraction, on the other hand, they seem to be definitely discordant. This is the theory of Meigs, according to which the

fibers of plain muscle undergo, in contraction, a shrinkage in both dimensions, owing to loss of water to the intervening ground-substance. Meigs (12) bases his theory on histological observations, on which my results have no bearing, and on the behaviour of rings of plain muscle from the frog's stomach extended by a weight, when plunged into distilled water and saturated sodium chloride respectively. In distilled water the ring, after a small preliminary contraction, slowly gives way and lengthens: in saturated salt a small preliminary relaxation, followed by a steady shrinkage, occurs. I would suggest that, if these observations have any bearing on the physiological process of contraction, it is only to the brief initial reaction—the last feeble response of the dying cells—that significance can be attached. The latter portions of Meigs' curves seem to be adequately accounted for by supposing that the dead tissue as a whole shows a diminishing resistance to extension as its cells become disintegrated by distilled water, and that it shrinks as a whole under the dehydrating influence of strong saline. When the changes of tonicity are kept within such limits that they are consistent with the continued vitality of the muscle fibres, as in the experiments with which this paper deals, the effects correspond with the small initial effects mentioned by Meigs, but regarded by him as negligible, in comparison with the secondary and more prolonged effects seen in his experiments

My object is to place on record observations, made incidentally to another investigation, which may find their place in the development of a theory of plain muscle activity, when further light has been thrown on the subject from other aspects. The results further seem worthy of attention from the purely practical point of view, that they indicate the necessity of precaution against even small alterations in the concentration of the saline bath, when long experiments are conducted on isolated plain-muscular organs, especially when these experiments involve quantitative observations on the degree of response of the plain-muscle to various stimuli. With a shallow layer of warm fluid, and a vigorous stream of oxygen bubbling through it, concentration to a degree sufficient to modify the response in an important degree

might easily occur; and the error would be accentuated, if an effect, in saline thus concentrated, were compared with one produced soon after a change to fluid of the original tonicity.

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