STUDIES ON THE PHYSIOLOGY OF PLAIN MUSCLE.

I. The effect of alteration of hydrogen-ion concentration on the tone and contractions of plain muscle.

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ALTHOUGH many investigators, *e.g.* Dixon, have recorded the effects of acid or alkali on plain muscle from various sources, yet in no published work which we have been able to trace has the effect of alteration of the hydrogen-ion concentration of the environment been studied from the general standpoint of the physiology of plain muscle. In most of the published work, the effects of change of reaction on the tonus, or on the spontaneous rhythmic contractions, of some particular preparation, have been studied. Even here, however, there is a lack of unanimity as regards the effects; the musculature of the blood vessels, for instance, is generally considered to be relaxed by increase, and constricted by decrease of hydrogen-ion concentration, and though there cannot be the slightest doubt that this is generally true, several investigators have obtained the opposite result. The explanation of this has been supplied by the careful researches of Fleisch, who has shown that in many of the perfused preparations employed for such experiments, the hydrogen-ion concentration and oxygen supply of the fluid used for perfusion were very abnormal, with the consequence that the vessels started in a state of maximal dilatation, which was not further influenced by small amounts of acid, but was replaced by constriction when an extremely acid reaction, far beyond any obtainable *in vivo*, was reached. Somewhat similar conclusions were reached by Atzler and Lehmann by the use of buffered perfusion fluids of known hydrogen-ion concentration. Whether the action is on the muscle tissue or on the nerves has not been finally decided.

We have therefore studied the question rather more fully, using various types of plain muscle, in order to ascertain how far, if at all, there was a general resemblance between plain and striated muscle in their behaviour towards change of reaction. The theoretical importance of such an investigation is obvious; if the effects of acid on plain and striated muscle are similar, it might be reasonable to suppose that the mechanism of contraction is essentially the same in both types; but if the effects are fundamentally different then it must be inferred that acid production has, in plain muscle, no causal connection with contraction.

In carrying out these experiments, we have avoided very great changes in hydrogen-ion concentration, which can have little physiological significance. This probably explains why some of our results differ from those of previous investigators. There can be no doubt that contraction of many forms of plain muscle can be obtained by very great and sudden change of reaction, as by almost any other sudden change, chemical or physical. As instances of the effects of such sudden changes of reaction may be quoted some of the experiments on the uterus by Farndon and of one of us (C. L. E.) who found that the plain muscle of the heart of *Helix pomatia* went into tonus when treated with carbon dioxide.

According to Clark, living animal cells are not permeable to hydroxyl ions; if this quite probable view be accepted, then the effects which we have found must either be due to the hydrogen ion alone, or must be confined to alterations at the surface of the cells.

Methods. A few experiments on perfused blood vessels served to confirm the correctness of the conclusions drawn by Fleisch, and these need not be further referred to here. The tissues employed were the uterus and intestine of the cat, guinea-pig and rabbit, the sphincter of the iris of the cat, and the retractor penis of the dog. The method adopted was to suspend the portions of surviving plain muscle in a bath of some suitable saline solution at 38° C. in the manner described by Burn and Dale for the guinea-pig uterus. Except where otherwise stated, oxygen was bubbled at a constant rate through the saline solution in which the preparation was suspended. The record of the state of contraction of the tissue was made in most cases by the use of the frontal lever described by one of us (C. L. E.), but in some instances an ordinary tangentially writing lever was employed.

The reaction of the saline solution in the bath was determined at intervals by the use of the indicator method described by Dale and Evans, the procedure of dialysis being omitted. The reaction of the bath was altered, when required, by the addition of acid or alkali of such concentration (usually $n/10$) that the volume added was only 1–2 p.c. of the volume of saline already in the bath: it was found that unde these conditions it was immaterial whether the acid or alkali was mad up with water or saline solution, or whether it was warmed or cold when added. The acids used were hydrochloric, phosphoric, sarcolactic, fermentation lactic, and carbonic. The last named was added either in the form of a saturated solution in saline solution similar to that in the bath, or by a carefully regulated stream of fine bubbles of the gas led into the bath at a slow and constant rate from a capillary delivery tube; regulation of the rate of flow was effected by a mercury column valve. As there was no essential difference between the effects of these different acids, we do not propose to describe the results obtained with each one; unless otherwise stated, it will be understood that the effects described apply equally to similar changes of hydrogen-ion concentration effected by any of these acids. Alteration in the direction of reduced hydrogen-ion concentration was brought about by addition of $n/10$ sodium hydroxide, or, in a few instances by sodium bicarbonate solution.

In some of the earlier experiments Ringer's solution of the composition given by Burn and Dale was used in the bath; it soon became apparent, however, that a stable reaction could not be maintained with this solution when oxygen was constantly passed through it, so a saline solution free from bicarbonate and lightly buffered with phosphate was subsequently used. This had the following composition: NaCl 8·5 grms., KCl ·42 grm., $CaCl_2$ ·24 grm., Na_2HPO_4 ·6 grm., molar H_3PO_4 ·2 to ·6 c.c. Distilled water, 1 litre (with or without addition of 1 grm. glucose). The distilled water was nearly always glass-distilled and free from carbon dioxide, but in a few of the experiments on the intestine ordinary distilled (condensed steam) water was used with the same results.

The reaction of the solution, as usually made up was about pH 7 to 7·3. This is much less alkaline than the usual Ringer bath after prolonged passage of oxygen bubbles. The phosphate solution cannot safely be made strongly alkaline, because of the ease with which calcium phosphate precipitates out from it, and this constitutes its chief drawback: solutions containing borate instead of phosphate were tried on this account, but were not found at all suitable.

The effect of mechanical conditions on plain muscle. It is well known that plain muscle is extremely sensitive to mechanical stimuli (Grützner); and Gôhara has found that the effect of mechanical stimulation plays an important part in experiments on the surviving vas deferens. In our opinion, the effects of mechanical stimulation are not sufficiently taken into account in using surviving preparations; some forms of plain muscle show contraction and others show relaxation as a result of mechaniacl stimuli. A most important factor is the mechanical effect produced by the passage of the stream of oxygen bubbles through the bath in which the preparation is suspended. The guinea-pig uterus, which normally shows but little tone, responds by a prolonged contraction when the stream of oxygen bubbles is stopped. It might be supposed that this is due to oxygen lack, but such an explanation is not adequate, because if a stream of nitrogen or hydrogen be substituted for one of oxygen (which change itself causes some increase of tone), exactly the same thing occurs when the current of bubbles is interrupted (Fig. 1); the relaxed condition is resumed when the bubbling of gas is recommenced, or if the liquid is agitated by stirring or if the surface of the tissue is gently stroked by a camel hair brush. Sometimes even briskly tapping the sides of the vessel with a glass rod is enough to produce the relaxation. It is curious that, when the tissue is in a contracted state, as in the presence of histamine or acetyl choline, the effect of the mechanical stimulation is reversed, since it now gives rise to the maximal state of tone, which becomes decidedly reduced when the stimulus due to the bubbling of oxygen, nitrogen or hydrogen is stopped (Fig. 1).

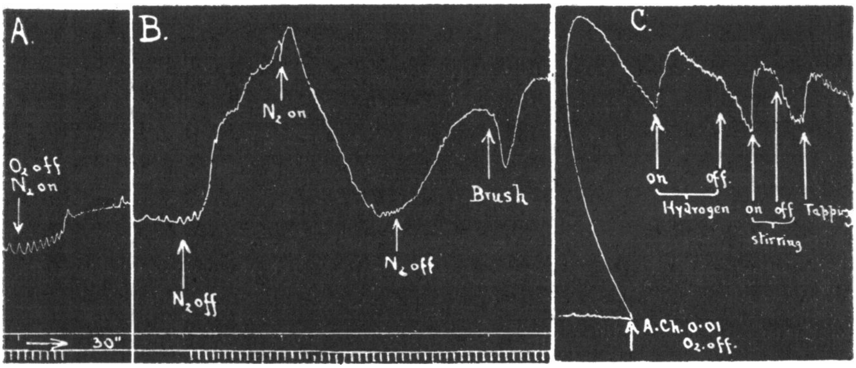

Fig. 1. Guinea-pig uterus—effect of agitation of the solution in the bath. *A*. Oxygen replaced by nitrogen bubbles. *B*. 35 minutes after *A*, the nitrogen was turned off. *C*. Mechanical agitation on guinea-pig uterus contracted by acetyl choline.

A similar phenomenon was shown by the guinea-pig intestine before and after the addition of acetyl choline. The cat's uterus, which is normally tonic, behaved, without addition of acetyl choline or histamine, in the same way as the tonically contracted guinea-pig uterus, *i.e.* mechanical stimuli brought about or maintained the contraction, but it was impossible to use nitrogen for this purpose, because oxygen lack speedily led to a relaxed state of the tissue, which then showed little further change on stoppage of the current of gas; such change as there was, however, was in the direction of a further slight relaxation, and not contraction. We mention these phenomena to indicate that they play an important part in the experimental conditions, and further because we wish to anticipate false arguments causally connecting oxygen lack, and the lactic acid formation, which we believe to be consequent on it in this tissue, with the state of contraction.

Our opinion is, rather, that contraction and tonus, and perhaps also relaxation, of plain muscle can be caused by the production of some metabolites. The effect of the stoppage of the stream of gas bubbles we imagine to be largely due to the consequent accumulation of such metabolites in the tissue, and it is significant that the guinea-pig uterus, when suspended in warm moist oxygen gas soon passes into a state of tonus, which we believe to have a similar origin. Not all forms of plain muscle behave in the same way, but a similar effect has been described by Mangold in stomach muscle, under the name of rigor mortis.

The small intestine. Our results in a long series of experiments on the ileum of the rabbit and cat have been generally concordant, though the magnitude of the effects obtained has been found to vary according to the physiological condition of the bowel, the extent and rapidity of the change in hydrogen-ion concentration, and the hydrogen-ion concentration existing at the moment the alteration was made. The cat's intestine was generally less sensitive to change of reaction than the rabbit's.

In the rabbit, starting from about pH 7·3, a sudden change to pH 6·5, or sometimes even to 6·8, stops the contractions and causes relaxation: the contractions may start again later, but the tonus is not usually restored. In the cat, a change to pH 6·5 causes slowing of the rhythmic contractions, and some loss of tonus, but a reaction of pH 6 or less is usually necessary to stop the rhythmic contractions (Fig. 2). In both cases, a change to the acid side of neutrality causes a decrease in the amplitude of the rhythmic contractions previous to their disappearance. A sudden change in the opposite direction, *e.g.* to pH 8, produces an increase of tonus, and again a reduction, though a less conspicuous one, in the amplitude of the rhythmic movements. Sometimes, especially when the tonus change is a steep one, there is a tendency for the effect to be only transitory, and partly to pass off afterwards. If the change in reaction is made quite slowly, *e.g.* over an interval of one or more minutes, the alteration of tone is either absent or is much less pronounced, and the effects on the rhythmic contractions are then more clearly seen. These appear at their best in a medium of which the reaction is neutral, or very slightly on the acid side: a definite depression is seen at *p*H 6·5 and again at about *p*H 8; there is also commonly a slight alteration in rate in the direction of a slowing in an acid and a quickening in an alkaline medium (Fig. 3); in one experiment, for instance, a piece of rabbit's ileum showed 10 beats per minute at *p*H 6·58 and 13 at 7·74. So far as we could ascertain, the effects on the circular and longitudinal muscular coats were similar in all respects.

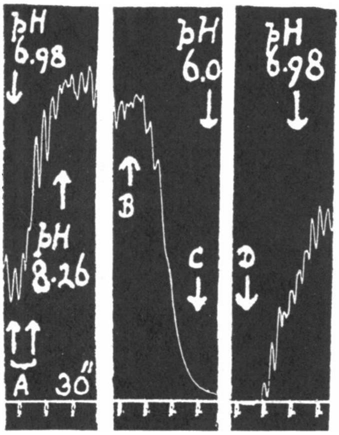

Fig. 2.

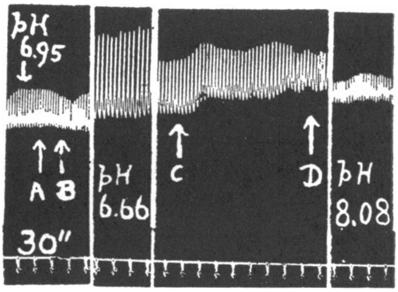

Fig. 3.

Fig. 2. Small intestine of cat. At *A*, 1·5 c.c. warm ·1 *n* NaOH. At *B*, carbon dioxide bubbled into bath; at *C*, CO_2 off; at *D*, contents of bath changed for fresh phosphate solution. Interval between portions of tracing, 3 minutes.

Fig. 3. Ileum of rabbit. Between *A* and *B* 4 c.c. of warm saline solution containing ·01 *n* HCl added. Between *C* and *D*, 7 c.c. dilute NaOH added gradually. Intervals between portions of tracing, from left to right = 17 mins., 8 mins., 9 mins.

In one particular, we found a departure from the description given above, and this was with a rabbit's intestine in a bath the alkalinity of which exceeded *p*H 7·6, when a slightly relaxed state set in. When a sudden addition of acid was made, an increase of tonus appeared (Fig. 4). The same phenomenon was less clearly seen in some other instances when the *p*H exceeded 8. We regard this effect as due, not directly to the alteration of hydrogen-ion concentration, but to change in the ionic calcium content of the phosphate solution. At about *p*H 8 the liquid begins to show signs of turbidity, which rapidly increases as the alkalinity rises; this fine precipitate of calcium phosphate, which is especially liable to be produced when NaOH is suddenly added, leads to a reduction in the calcium-ion concentration of the bath; indeed, it is not unlikely that, owing to the presence of small amounts of protein washed out from the tissue, which act as a protective, colloidal calcium phosphate is often present even before *p*H 8 is reached. We have not studied the effect of the calcium ion on plain muscle in any detail, but have observed that lack of calcium produces relaxation in the guinea-pig uterus; both Stiles and Fienga have observed that calcium in excess leads to a tonic contraction of the frog's and hen's œsophagus. The effect of acid in producing contraction when added to a bath of *p*H 8 or more does not seem to be at all surprising, since it would raise the calcium concentration.

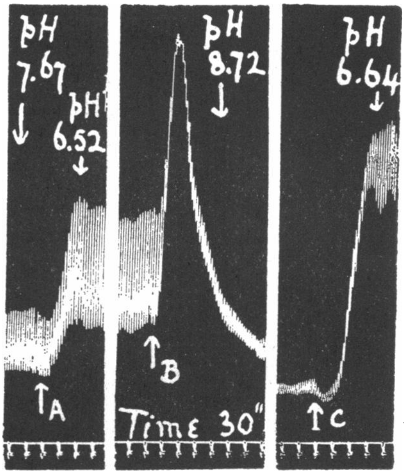

Fig. 4. Small intestine of rabbit. At *A*, ·85 c.c. *n*/10 HCl. At *B*, 1·5 c.c. *n*/10 NaOH. At *C*, 1·5 c.c. *n*/10 HCl. Interval between portions of tracing, from left to right = 8 mins., 7 mins.

The response of the intestine to pilocarpine was tested at various reactions: in neutral and alkaline fluids there seems to be little difference, but the response is definitely reduced in a bath of an acid reaction, and is abolished when the *p*H reaches 6. Extremes of reaction, *i.e.* beyond *p*H 8 and 6, rapidly kill the tissue, and it is interesting to note that when death is produced by the addition of acid, it happens with the tissue in the fully relaxed state.

Our results with the intestine agree generally with those of Hammett and of Bottazzi. Mansfeld and Hooker state that under certain conditions carbon dioxide can act as a stimulus to the intestinal muscle; in some of our experiments too, carbon dioxide has caused an increase of tonus, but we are inclined to attribute this exceptional result to change of calcium concentration, as explained above.

The guinea-pig uterus. The uterus of the non-pregnant guinea-pig proved to be much more sensitive to change of hydrogen-ion concentration than the small intestine, and owing to its greater instability, to give less concordant results. When the bath was definitely alkaline to begin with (*p*H 8) small additions of acid almost invariably produced a large contraction, which slowly declined and gave place to slow large rhythmic movements (Fig. 5). As the reaction of the original bath was made less alkaline, this response became less pronounced, and when the initial reaction had reached pH 6 or 5, further acidification usually led to a definite and rapid relaxation, with disappearance of the spontaneous rhythmic contractions (Fig. 5). In this relaxed state, the tissue is quite insensitive to even large doses of oxytocic drugs such as histamine; the tissue is not dead, however, as can be demonstrated by changing the contents of the bath back again to a solution of about neutral reaction, when the response to the drug returns in a short time (Fig. 6).

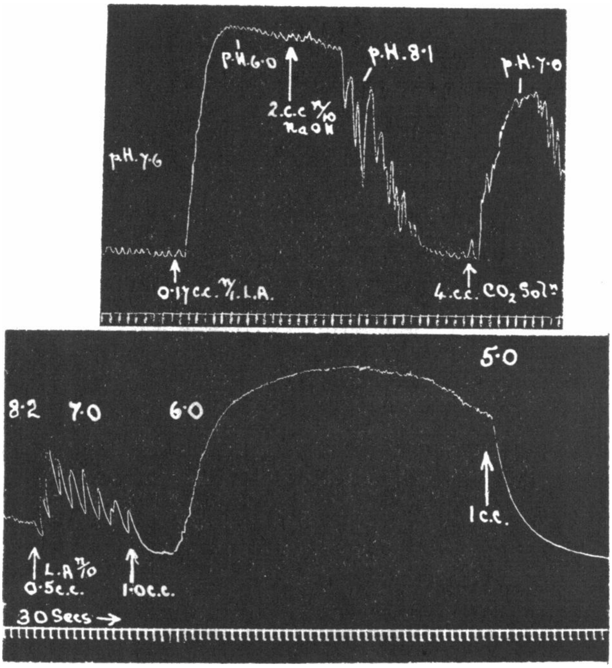

Fig. 5. Different effects of change of reaction on the guinea-pig uterus.

After a change of reaction (apart from excessive ones leading to temporary paralysis, such as that described above), the tissue slowly settles down again and becomes more or less quiescent under the changed conditions at an approximately normal extended length. When this has happened (which often takes an hour or so), it is found that the response to drugs is unchanged. Fig. 7 illustrates the constancy of response to ·01 mg. of histamine at pH values from 6·5 to 9·2 (bicarbonate Ringer). The effect of alkalies is the reverse of that of acids (Fig. 5).

The general effect of alteration of reaction is similar to that seen in the experiments on the intestine, but the contraction seen on addition of acid to a preparation in an alkaline bath is much more pronounced with the uterus. We believe the explanation to be similar, and the effects to be more conspicuous because this tissue is more sensitive to an alteration in the ionic calcium content of the fluid in which it is immersed.

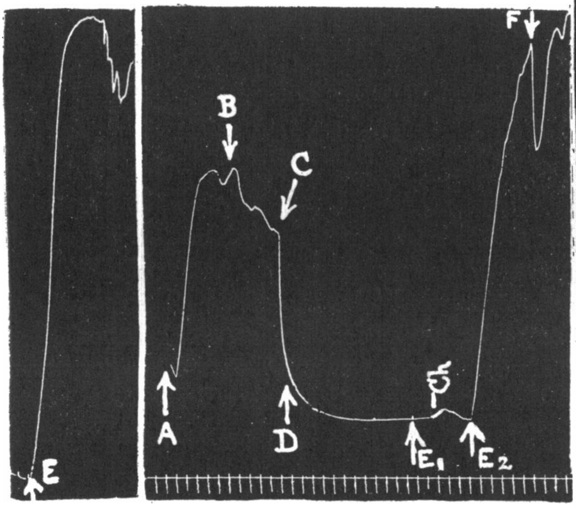

Fig. 6.

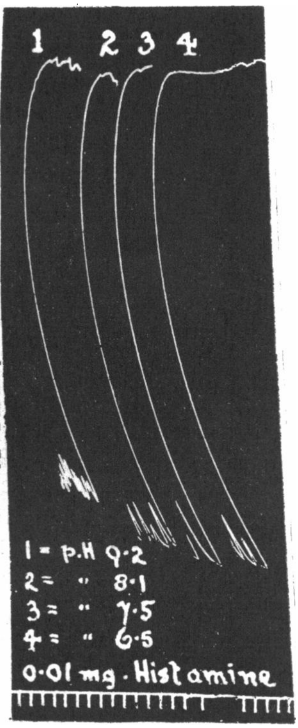

Fig. 7.

Fig. 6. Guinea-pig uterus. First portion shows the normal response to ·1 mg. histamine. At *A*, ·25 c.c. 5 p.c. lactic acid; between *B* and *C*, 15 c.c. saturated CO_2 solution, and at *D*, ·5 c.c. 5 p.c. lactic acid. The reaction was now about *p*H 5. At E_1, ·1 mg. histamine produced no effect. The contents of the bath were then changed, and at E_2, ·1 mg. of histamine produced the usual effect. At *F*, ·5 c.c. saturated CO_2 added produced a transitory intermission of the contraction.

Fig. 7. Response of guinea-pig uterus to ·01 mg. histamine at different reactions.

The uterus of the rabbit and cat. Our results on these tissues agree with those of Farndon on the cat's uterus. The plain muscle of these organs and particularly that of the cat, differs from that of the guinea-pig uterus in exhibiting a more definite tonus when placed in baths at ordinary reactions. The response to acidification was in every case a relaxation of tonus, and usually at first a slowing and amplification of the rhythmic contractions: with further increase of hydrogen-ion concentration the rhythmic movements slowly faded away (Fig. 8).

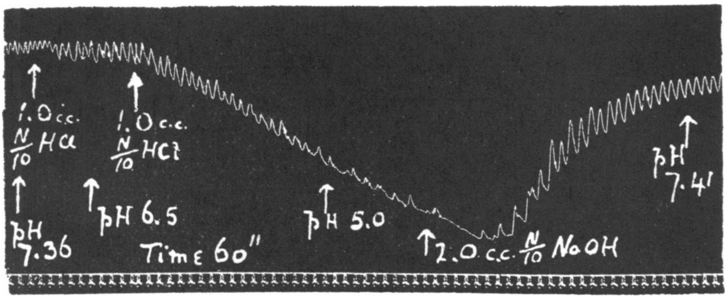

Fig. 8. Effect of change of reaction on the cat's uterus.

The retractor penis and iris. Mosso has stated that pure carbon dioxide causes contraction of the retractor penis of the bullock, while Bottazzi states that carbon dioxide diminishes the tonus of the dog's retractor. Our results are at variance with those of both observers, since we have never observed any effect on moderate change of reaction. We should not have expected to see any relaxation, because the retractor penis, in our experience, does not normally display tonus. The same remarks apply to the isolated iris of the cat, which we tried on two occasions, with negative results.

The influence of local nervous mechanisms. The old and obvious objection that different responses in plain muscle may be attributed to varying effects on the nervous elements present in the preparation leaves us in somewhat of a quandary in interpreting our results; we have tried by various methods to circumvent these objections, in order to be able to relate the results to effects upon the muscle alone. It is difficult, however, or even impossible, entirely to dissociate the two, as a consideration of the state of affairs in the cat's uterus will show. According to Langley and Anderson the nerve supply to this organ is a sympathetic one, and their experiments also give evidence for the existence of some terminal cell stations in the organ itself. Dale and Cushny found that the principal effect of hypogastric stimulation on the virgin uterus was inhibitory, but that in the pregnant uterus the principal effect was motor, due, according to Cushny, to the predominating effect of motor fibres in the normal nerve supply. Both the motor and the inhibitory effects of hypogastric stimulation were abolished by nicotine in large doses, but were unaffected by atropine. If we assume that the normal tonus of the cat's uterus is of nervous origin, it would seem not unreasonable to suppose that the action of increased hydrogen-ion concentration is akin to that of adrenaline, and stimulates the inhibitory nervous structures; in any case, acid would presumably show no effect at all in the absence of the initial tonus, which it is able to relax. So that the nervous mechanism in the tissue would on this assumption, be at least indirectly responsible for the effect produced by the addition of acid, or even directly responsible for it, if we suppose it to act by exciting the inhibitory nerves.

We have studied the effect of alteration of reaction upon cats' uteri previously treated with nicotine or atropine; we used the latter because of the statement made by Schultz and other authors that atropine paralyses all the nerve endings in plain muscle (Grützner, p. 59). It was found that the effect of change of reaction in presence of ·1 p.c. nicotine or ·01 p.c. atropine was essentially the same, as regards the effect on the tonus, as in the absence of these substances, which would lead us to infer that the relaxation is a real effect on the muscular tissue. Further, we have observed the responses of cats' uteri preserved at 0° C., from day to day, until the tissue was dead. The response was never altered in direction, but merely in magnitude, as the vitality and normal tonus of the tissue slowly fell off as a result of keeping, until finally the tissue ceased to react to all stimuli. It would seem very unlikely that nerve cells and plain muscle tissue should exhibit exactly the same degree of resistance to cold, and perish simultaneously, so that we are probably justified in regarding the response throughout as due to effects on the muscle itself.

Similar experiments were carried out on the isolated small intestine: the response to change of reaction is unaltered by nicotine, or by preservation at 0° C., which in one case extended up to 8 days. Although Cannon and Burkett have shown that the myenteric nerve plexus is much more resistant to anæmia and low temperatures than might have been imagined by analogy with other nerve cells, the same argument applies here as to the cat's uterus. Hammett has found that the contraction of the rat's intestine produced by sodium carbonate is abolished by cocaine, but not by nicotine; he concludes that the action is on the nerves.

An attempt was also made to decide the question by reference to strips of the inner part of the circular muscle coat of the intestine prepared by the method of Gunn and Underhill. Although nerve cells are not considered to penetrate the circular muscle coat, the strips of muscle after use were fixed, serially sectioned, stained with Pappenheim's stain, and examined for the presence of nerve cells. In some preparations one or two nerve cells were seen, but, so far as our examinations went, these were not regularly present. The isolated rings or strips of intestinal muscle are very sensitive to change of reaction, and rarely recovered from any considerable alteration, so that the results are somewhat ambiguous: also, we have never seen any alterations of tone at all in these preparations, but only in the rate and amplitude of the rhythmic contractions. The handling of the tissue involved in the removal of the rings of muscle is rather severe, and it may be that the natural tonus of the tissue has suffered in consequence, so that the effect of acid in causing relaxation was not seen as with the intact intestine, which had been less roughly treated. When there is no natural tonus in a preparation it is clear that a relaxation effect cannot be demonstrated: for this reason, the retractor penis showed no effect with the moderate changes of reaction which we have used. Incidentally, this form of plain muscle is, according to Fletcher, devoid of nerve cells. The same is true of the cat's iris, in which also acids produced no effect.

Comparison of effects of change of reaction on plain with those on striated muscle. As compared with plain muscle, striated muscle appears to be much more indifferent to small changes of hydrogen-ion concentration: when the acidity passes a certain limit, however, rigor rapidly sets in, and the muscle shortens. Plain muscle behaves in an entirely different way: any tonus it may have is, with certain exceptions, explicable in other ways, reduced by addition of acid, so that ultimately

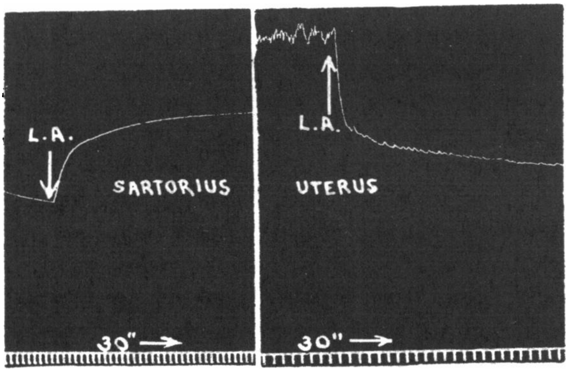

Fig. 9. Death of striated and plain muscle by acidification compared. In each case the bath was made up to a lactic acid content of ·27 p.c.

in all cases, a fully relaxed state is reached, in which all spontaneous movement is abolished, and in which the tissue is unresponsive to drugs normally producing contraction; further addition of acid soon leads to death, but this occurs in the relaxed state, and is not followed by any shortening. This result is clearly shown in Fig. 9, where the effect of equal acidification of striated and unstriated muscle from the same animal is shown. In both cases 3 c.c. of normal sarcolactic acid was added to the bath of 100 c.c. of Ringer's solution containing the tissue, so that the ultimate concentration of lactic acid in the bath was ·27 p.c. Death of the plain muscle was confirmed by changing the fluid for the usual saline solution, and showing that addition of ·25 mg. of acetyl choline was without effect.

CONCLUSIONS.

1. The effect of increase of hydrogen-ion concentration, within limits compatible with life, is to cause relaxation of the tone of plain muscle. Rhythmic contractions, such as those of the small intestine, are slowed and depressed in extent on the acid side of neutrality, and quickened and depressed when the reaction becomes decidedly alkaline. Excessive acidification leads to death in a relaxed state.
2. In muscle preparations devoid of tonus, increase of hydrogen-ion concentration has no effect.
3. These effects are independent of action on local nervous mechanisms.
4. When the preparation is immersed in a definitely alkaline bath of phosphate solution (*p*H 8 or more), addition of acid often produces an increase of tonus, or a large contraction. This effect, well seen with the guinea-pig uterus, is believed to be due to alteration in the ionic calcium content of the fluid, and not directly to change in the hydrogen-ion concentration.
5. The mechanism of contraction and tonus in plain muscle is different from that in striated muscle.

The expenses of the investigation were in part defrayed out of a grant from the Government Grants Committee of the Royal Society to one of us (C. L. E.).

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