ADAPTATION OR ACCOMMODATION IN UNSTRIATED MUSCLE

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Irritable tissues are known to adapt or accommodate themselves to the continued presence of a stimulus by becoming less responsive. This adaptation depends upon the particular kind of tissue, and upon the nature of the stimulus. In unstriated muscle, adaptation is very conspicuous, but it depends upon the nature of stimulation (Singh, 1936; 1937; 1938*a*, *b*, *c*, *d*, *e*, *f*; 1939*a*, *b*; 1943*a*, *b*, *c*, *d*, *e*, *f*, *g*, *h*, *i*; Rao and Singh, 1940; Singh and Mrs. Singh, 1943; Gokhale and Singh). It adapts rapidly to alternating current, but very slowly to some chemical substances. Not only it adapts to excitation (Singh, 1938*c*) but also to inhibition (Singh, 1942*b*), the tendency for the muscle being to produce an opposite change. In this paper various factors which influence adaptation in unstriated muscle are described.

Results

When *Mytilus* muscle is stimulated with alternating current (A.C.), the tension may subside while the current is passing through the muscle. Two classes occur; in potassium sensitive muscles, when the tension has declined, the sensitivity to potassium increases or at first decreases and then increases, while in comparatively insensitive muscles, the sensitivity decreases or shows no increase. The same effects are noted in frog muscle; here the sensitivity at first decreases and then increases.

On cessation of the current also, two phenomena are noted; the sensitivity to potassium either increases or decreases or shows no increase. When *Mytilus* muscle is stimulated intermittently (A.C. 8 V/10 sec. per min.), fatigue occurs. Again two phenomena are noted, either increase or decrease of sensitivity. This is beautifully shown by the A.C. off-contracture (Figs. 1, 2). It increases with fatigue in potassium sensitive muscle and decreases in comparatively insensitive muscle, if it occurs in the latter.

Reactions of potassium insensitive Mytilus muscles.—When these muscles are stimulated with A.C. the tension subsides to zero. This adaptation appears to be analogous to the "accommodation" in nerve described by Hill (1936). The tension subsides when "U" rises higher than "V" (Singh, 1943 *d*).

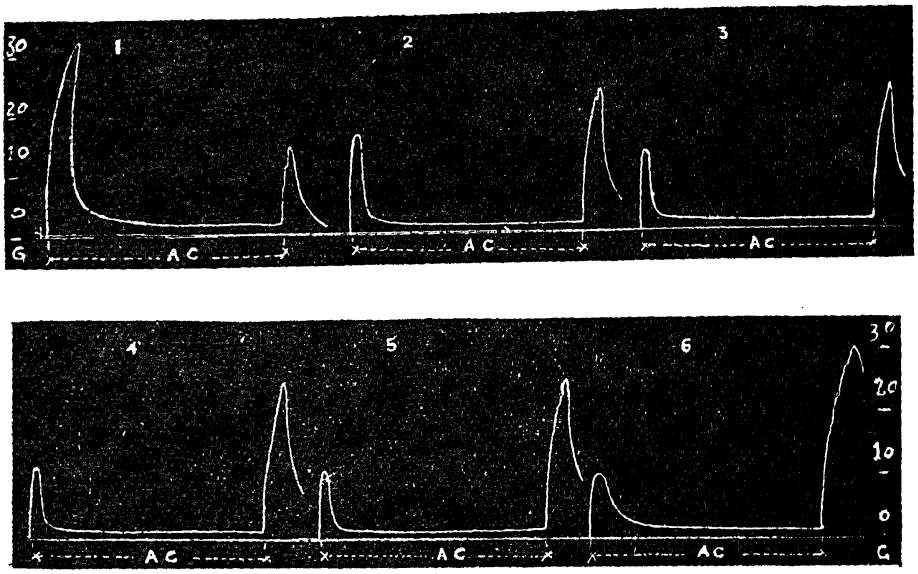

FIG. 1. *Mytilus* muscle. Stimulation with A.C. 10 V/5 min. per 10 min.

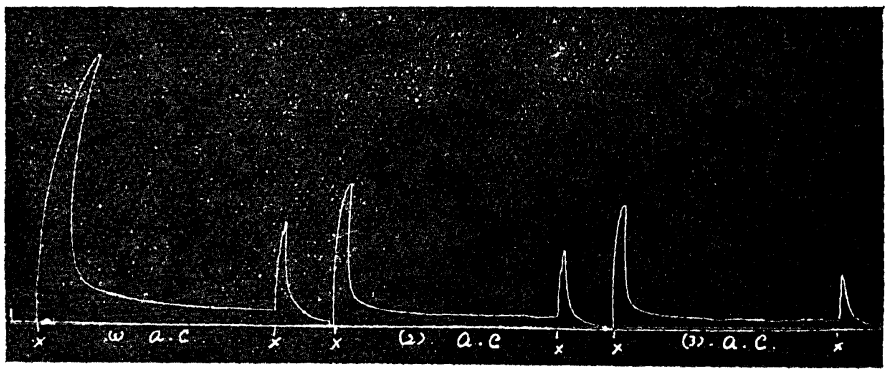

FIG. 2. Same as in Fig. 1

The above view is supported by the fact that calcium increases adaptation (Singh, 1938*d*). Further when the current is stopped, the sensitivity to A.C. must return to its original value. Calcium retards this recovery (6 experiments; Fig. 3). When the muscle is stimulated with A.C. intermittently calcium increases fatigue (6 experiments; Fig. 4). Such summer *Mytilus* muscles, when placed in *Mytilus* saline lose weight (Singh, 1938 *a*, *d*), behaving as if they contain excess of calcium; in calcium the muscle loses weight.

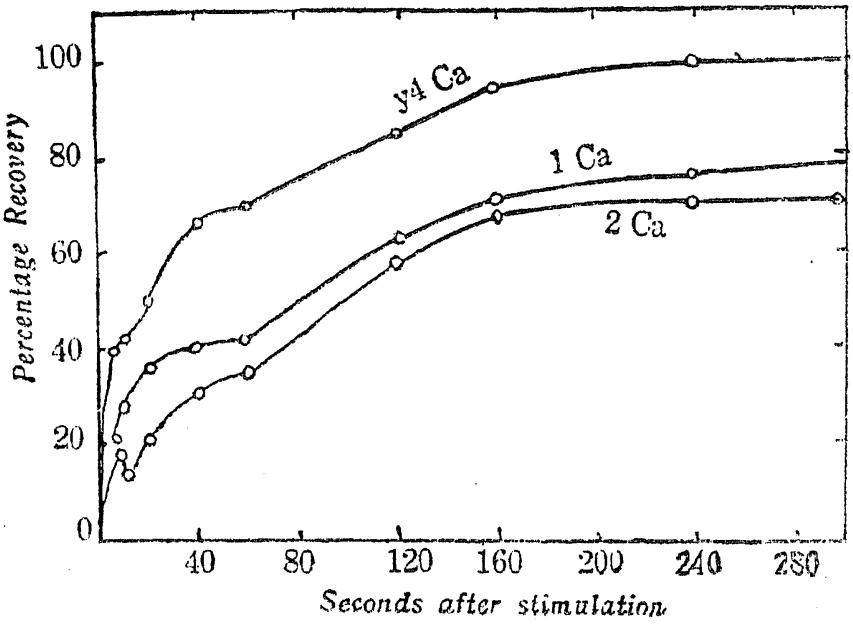

FIG. 3

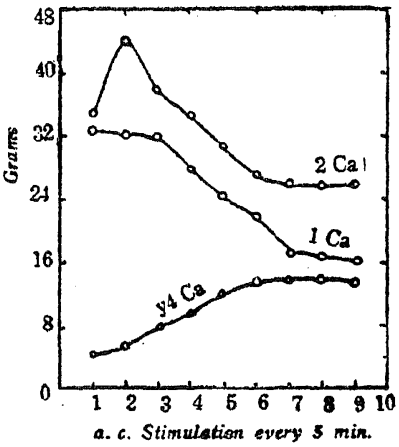

FIG. 4

FIG. 3. *Mytilus* muscle. Effect of calcium on recovery of excitability in potassium insensitive muscle after stimulation with A.C. 10 V/5 min.

FIG. 4. *Mytilus* muscle. Effect of calcium on fatigue to A.C. 10 V/10 sec. in potassium insensitive muscle.

The above adaptation appears to be brought about by liberation of calcium. Liberation of calcium by muscular activity has been shown by Zagami (1928), Wacker (1929); Dill, Talbot and Edwards (1930); Beznak (1931); Goldberg *et al.* (1931); Cloetta, *et al.* (1934); Weise, (1934); Coombs *et al.* (1934): Miko and Pala (1927): Bachromojew and Pawlowa (1935).

In *Mytilus* muscle excess of magnesium increases the latent period of the A.C. contraction. In some muscles, in 0·1 M $MgCl_2$, current was passed for 10 sec. and latent period was about 4–5 sec. after which the muscle contracted abruptly (6 experiments). Potassium (0·01–0·02 M KCl) similarly increased the latent period of the contraction produced by stretch and release (6 experiments) and that of the barium contraction (3 experiments) potassium and magnesium increase accommodation in nerve (Solandt, 1936), so that the latent period is probably the time required by " V " to catch " U ".

When A.C. is passed for 5 min. the muscle may remain contracted during the period (adaptation is slow). If contracture is induced by the exclusion of calcium from the saline, the current merely causes relaxation; the rise of " V " in the former case produced contraction, and in the latter case, relaxation. On cessation of the current, the contracture recurs abruptly; this shows that the rise of " V " practically lasts during the passage of the current only. If the muscle is stimulated for a short duration (A.C. 10 V/10

sec.) then, when the stimulus is over, fatigue persists to A.C. and potassium and tone is neutralised for 3–4 sec. As this fatigue is due to adaptation, it suggests that "U" returns to its normal value more slowly than "V", because the rise of "V" lasts during the passage of the current, while the rise of "U" persists after the cessation of the current.

Reactions of potassium sensitive muscles.—Two factors are antagonistic to A.C.; one is adaptation, and the other contracture producing substances. The latter effects the two excitabilities oppositely (Singh, 1938*b*; 1939*b*). The decline of tension with continuous stimulation with A.C. can, then, be due to two possible factors: (1) adaptation, as has been described above; (2) sensitisation to ions outside that is, sodium chloride, which increase the excitability to potassium, and decreases that to A.C.

That the second factor also occurs, is shown by the following experiments :—(1) When the excitability to A.C. declines, that to potassium increases during the passage of A.C. (6 experiments); (2) the decline of tension may be diminished by decreasing the concentration of sodium chloride in the saline (Fig. 5) or increased by contracture producing substances. (3) When the muscle is stimulated continuously with A.C., the tension after

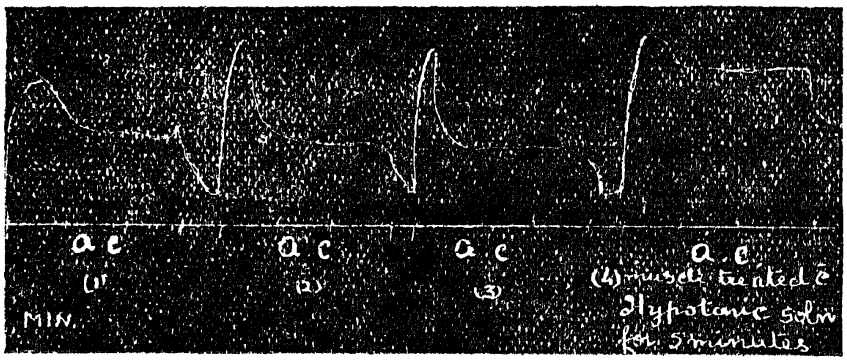

FIG. 5. *Mytilus* muscle. Stimulation with A.C. 10 V/3 min. 1st three in normal saline. 4th in Hypotonic solution containing sodium chloride, 80% of normal for 5 min.

having declined, begins to rise again. This secondary rise is due to the action of ions outside, as shown by the fact that (*a*) its relaxation is slow; (*b*) it is increased by thiocyanate and cyanide which increase the excitability to potassium and decrease that to A.C.; (*c*) it is abolished by veratrine, adrenaline which decrease tone, presumably caused by sodium chloride. (4) It is never found in frog or mammalian muscles, in which the action of sodium chloride is inhibitory. This suggests that adaptation to A.C. in these muscles is due to inhibitory action of ions in the solution, just as it is due to the antagonistic excitatory ions in *Mytilus* muscle. Further in frog muscle the excitability to potassium decreases during passage of A.C., as inhibition depresses both the excitabilities in frog muscle (Singh, 1939*b*).

The secondary rise of tension during passage of A.C. is due either (1) to direct stimulating action of ions outside or (2) to neutralisation of the factor of adaptation, producing " adaptation to adaptation " or " accommodation to accommodation " (Singh, 1943*e*). In the former case the properties of the contraction should be that of the potassium contraction and in the latter case that of the A.C. contraction. Both these effects are found, as the tension may be increased by cyanide, thiocyanate, iodide, which increase the excitability to potassium and diminish that to A.C. or decreased by veratrine, adrenaline which decrease the excitability to A.C. and increase that to potassium.

It is probable that the first effect is to diminish adaptation, and if the action of ions is potent enough it is carried to a further stage, a tonic contraction being produced (Singh, 1942*b*).

At the end of the period of stimulation, the disappearance of the factor producing A.C. tension, should result in the A.C. off-contracture, as is experimentally found.

Adaptation due to leakage of ions.—If excitation is due to difference in concentration of ions within and without the muscle fibres (Singh, 1940) then it is to be expected that equalisation of these concentrations by diffusion of ions would produce adaptation (Singh, 1943*f*). That ions do diffuse into and out of muscle is well known. Such a possibility is suggested by the fact that calcium, the absence of which favours leakage of potassium from the fibres, may also increase the subsidence of tension during passage of A.C.; calcium thus affects the two kinds of adaptation oppositely. In the absence of calcium, the tension produced by potassium also subsides quickly; absence of calcium favours the entrance of potassium (Singh, 1938*a*). In frog muscle adaptation to ammonium is rapid compared with that to the quarternary ammonium salts, which diffuse less rapidly than ammonium (Gokhale and Singh).

The A.C. off contracture.—This has properties of the potassium contraction, so that it is produced by ions without the muscle fibres. This suggests that during stimulation ions leak out of the muscle.

The magnitude of the contracture increased exponentially with time of passage of the current (Fig. 6) and reaches its maximum when the current is passed for 20 min. and thereafter it declines; this suggests that the ions which leak out of muscle, diffuse away from the seat of action, probably into the solution. If this is so, then movement of solution in the muscle chamber might accelerate this diffusion. Out of 6 experiments, in every

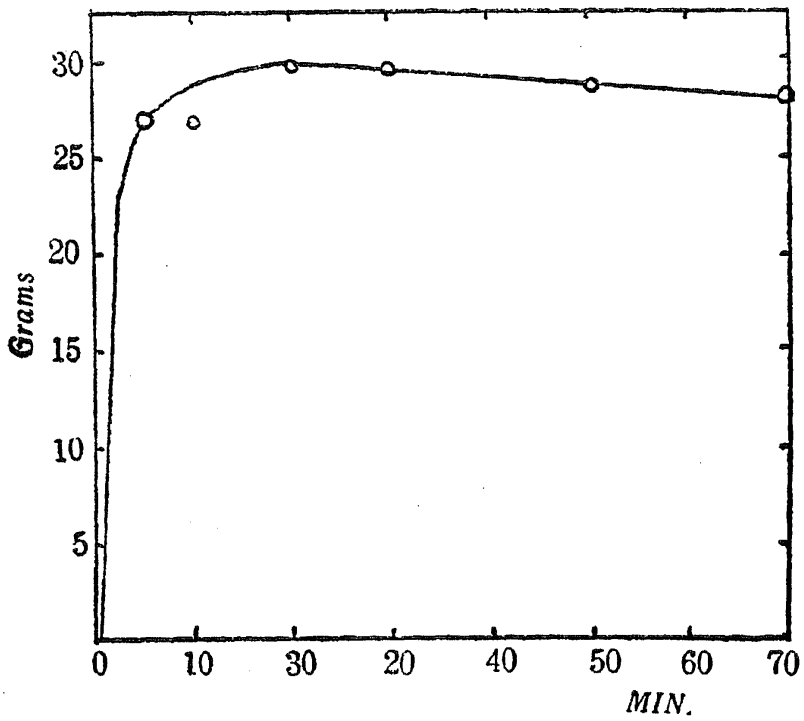

Fig. 6. *Mytilus* muscle. Effect of duration of flow of A.C. 12 V on the off-contracture

instance the magnitude of the contracture decreased if saline was made to flow at the rate of 15 to 40 c.c. per min. (Fig. 7). A drop in temperature

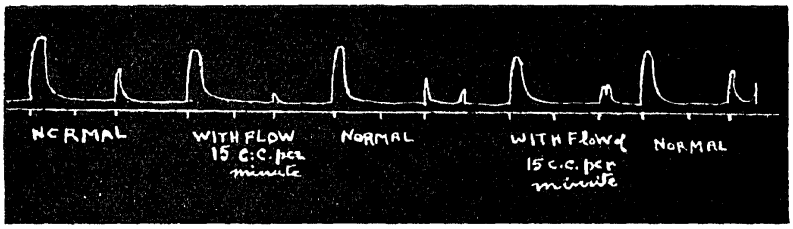

Fig. 7. *Mytilus* muscle. Effect of flow of saline upon the off-contracture

would produce the same result; the rise in temperature produced by passage of the current when there was no flow was less than a tenth of a degree as measured by inserting of thermo-couple of copper and constantan and this fluctuation is too small to affect the result. Mechanical effect of the passage of the solution should produce an opposite effect.

Equilibrium between excitation and adaptation.—In unstriated muscle " U " and " V " appear to be equilibrium. Thus if the muscle is stimulated with A.C. 8V/5 min., the tension may soon subside, showing that " U " has risen, as a consequence of the rise of " V ". If the muscle is stimulated again with 16 V, then the tension again rises only to decline to zero again. If the voltage is decreased to 8 V, then the muscle again can be stimulated with 16 V, showing that " U " had decreased as a consequence of

decrease of " V ". Ultimately, however, " V " rises higher than " U ", as shown by the fact that if continuous tension is not obtained with low voltages such as 8 V, 16 V, it may be obtained with 24 V. This shows that " U " and "V " are produced by independent agencies.

The equilibrium between " U " and " V " has a bearing on excitation at the cathode and the anode.

The fact, that on closing a current through a nerve, the excitation wave starts from the cathode, shows that cations are important agents. It is pointed out by Kieth Lucas (1912), that " the one feature which is common to the cathode when the current is made and the anode when the current has just ceased to flow, is an increase in concentration of cations above the value which occurred at each of these points immediately before ". At the anode, the concentration of cations only rises to its normal value by diffusion, after having been decreased.

The decrease in concentration of cations at the anode would diminish the adaptation factor, so that when the concentration of cations rises to its normal level, excitation takes place, as the increase in the adaptation factor is slower than increase in cation concentration.

The above view is direclty born out by experiment on the frog stomach and the guinea pig uterus. Normally the frog stomach is adapted to the ions in the saline. If the muscle is deprived of sodium by immersion in a sodium deficient solution, re-immersion in the original sodium chloride concentration causes a contraction. Thus contraction is caused when the ions, which previously were inert, were restored to their original concentration. In the guinea pig uterus, cations cause inhibition instead of contraction. The potassium normally present in the saline has no action, or the muscle slightly relaxes when it is withdrawn. If the muscle is deprived of potassium for ten minutes, restoration of the normal concentration of potassium causes marked inhibition (Singh, 1942*b*). Thus " U " and " V " are in equilibrium.

The adaptation to normal concentration of ions described above is the same as the " paradoxes " described by various authors (Libbrecht, 1920, 1921; Guerra, 1924; Witanowski, 1926; Kisch, 1927, 1930; Wells, 1928; Chao, 1934).

Effect of initial length on adaptation.—For inhibition in unstriated muscle, an optimum length is necessary (Singh, 1942*b*). As adaptation is a kind of inhibition, it would be expected that adaptation would be affected by length of the muscle. This is shown by the following experiment,

Ordinarily the isometric contraction produced by 0·1 M KCl in *Mytilus* muscle subsides in 10 to 60 min. If the muscle is allowed to shorten, the contractile process does not completely subside in 12 hours, as shown by periodically recording a stretch curve; this should not be confused with the known fact that the isometric contraction lasts longer than the isotonic one. The effect of initial length on adaptation would have a bearing on the rate of extension of unstriated muscle (Singh, 1943*b*). It would diminish tone with extension and so account for the diminished oxygen consumption if the muscle is stretched (Rao and Singh, 1940).

Another interesting feature is that if contraction is not produced with greater length, it may be produced, if the muscle is allowed to shorten

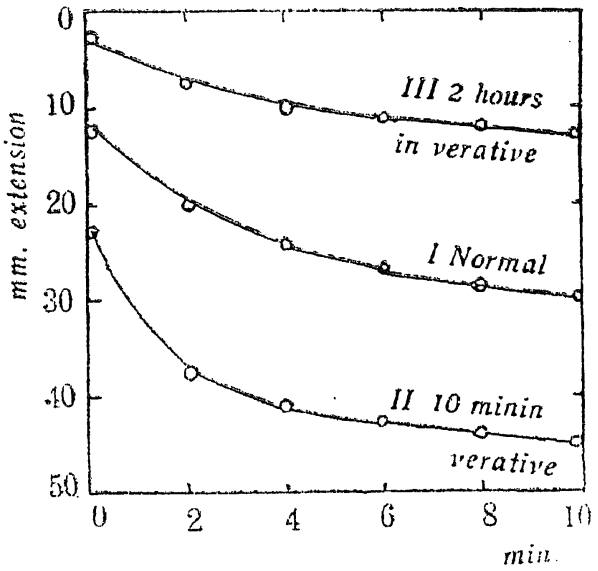

FIG. 8. *Mytilus* muscle. Stretch curves. Effect of varatrine (1 in 1000)

(Fig. 8). For this, however, the muscle is to be soaked for some time in the solution. Thus veratrine in *Mytilus* muscle and ammonium in frog muscle may produce inhibition only but if the muscle is left in the solutions for a couple of hours and allowed to shorten, a contraction results as shown by stretch curves. This phenomenon resembles that described by Parnas (1910), wherein unstriated muscle is unable to lift a weight, but if the muscle is allowed to shorten, it is then able to keep up the weight, which it was unable to lift.

In the above experiment, not only is the factor of length, but also that of time. It appears that either the factor causing adaptation leaks out of the muscle, or antagonistic substances diffuse into the fibres; ammonium and potassium are antagonistic to calcium. Small concentrations of potassium (0·02–0·05 M KCl), which are unable to evoke an isometric contraction

in *Mytilus* muscle do so if the muscle is allowed to shorten. This may occur apart from soaking. If *Mytilus* muscle is kept at rest, and allowed to shorten tone may increase together with its sodium content.

It appears that adaptation may diminish; (1) owing to decrease in initial length, (2) diffusion into the fibres of ions that are antagonistic to the factor causing adaptation, which is probably calcium. This second factor is of importance in considering diminution of adaptation to potassium and A.C.

When frog muscle is stimulated with potassium, a twitch is produced to which the muscle rapidly adapts. This twitch can be differentiated from that produced by A.C. by changes in osmotic pressure (Fig. 9). (Such a twitch

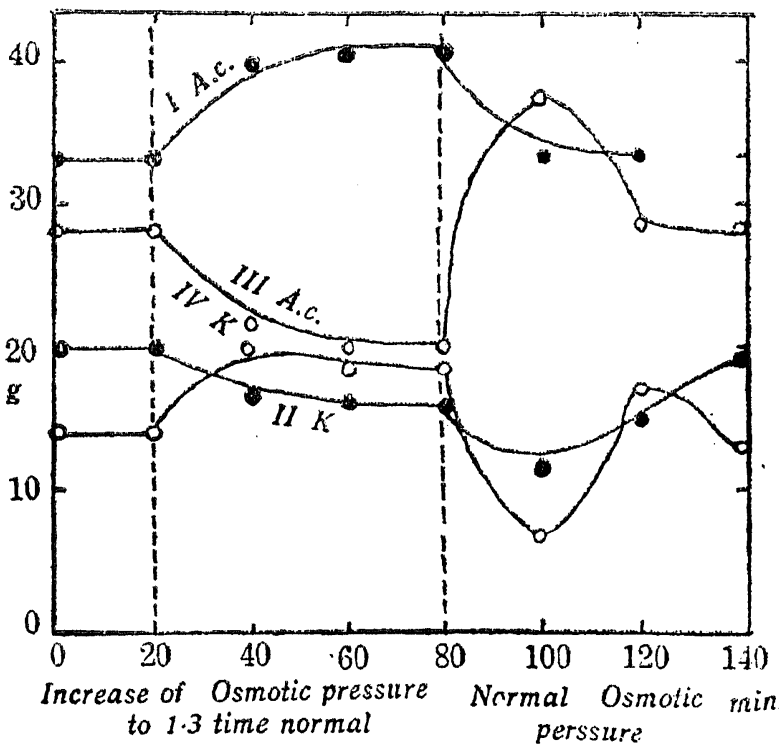

FIG. 9. Frog stomach. Effect of osmotic pressure (increased by adding sodium chloride at pH 8) on the response to A.C. 8 V/10 sec. and potassium (0·22 M).

has only been produced by A.C., potassium or ammonium and is interesting in view of the suggestion that A.C. twitch is produced by potassium within the fibres.) After the twitch, adaptation diminishes and a tonic contraction develops. With ammonium such a phenomenon is produced if preliminarily a contracture is induced by thiocyanate or iodide.

It appears that with time, the factor of adaptation has been neutralised, and as potassium and ammonium are antagonistic to the action of calcium, they probably diffuse into the muscle, and antagonise the action of the latter.

The diminution in adaptation to A.C. mentioned above is presumably due to the ions being carried into the muscle fibres by the current instead of by diffusion. The phenomenon is well marked in thiocyanate or cyanide and these are antagonistic to the action of calcium. If diminution in adaptation to A.C. is not produced with small voltages (8–16 V), it is invariably produced by higher voltages (20–40 V) the number of ions entering the muscle fibres, presumably increasing as the voltage increases; hence the rate of relaxation also diminishes.

The decline in excitability at temperature above 30° C. is due to adaptation (Rao and Singh, 1940), which is probably due to liberation of calcium. The liberation of calcium from protoplasm at high temperatures has been shown by Mazia and Clark (1938). The release of calcium would also diminish the viscosity. At higher temperatures the excitability to potassium and tone again increases. This again is probably due to neutralisation of adaptation by ions outside. The increased activity by ions outside is shown in *Mytilus* muscle by (1) increase of the A.C. off contracture; (2) increased tone; (3) increased excitability to potassium. At temperature above 37° C., the contractile mechanism is directly affected, as isolated myosin also contracts (Astbury and Dickinson, 1942).

Adaptation to direct current.—In *Mytilus* muscle, adaptation to direct current is slower than to alternating current, but in frog stomach it is more rapid. The properties of the contraction on cessation of D.C., as expected from the reasoning above, are those of the initial A.C. contraction. No contraction having the properties of the A.C. off contracture has been observed on the cessation of D.C.; this precludes the possibility of electrolysis as being the causation of the former. It therefore appears that the off contracture is associated with the reversal of the current in each wave, the ions being carried without the fibres by the reversal of the direction of the current.

The slow relaxation and the high viscosity of the D.C. contraction suggests that ions are carried with the current into the muscle fibres from without. The fact that a greater intensity of alternating current is required to produce the same effect as D.C. of lesser intensity, suggests that the only one direction of the alternating current is effective, the accumulation of ions at some place being due to rectification. The A.C. off contracture would be due to accumulation of ions external to muscle fibres, due to rectification. Delayed excitation produced by alternating current has been ascribed to rectification (Cole, 1941).

In frog stomach the inhibitory action of ions outside increases adaptation to D.C., and so decreases the primary tension. For the same reason, in the guinea pig uterus D.C. is more inhibitory than A.C.

Mytilus muscle is less excitable to D.C. than A.C. owing to the action of ions outside; an increase in calcium ion concentration may reverse this effect (Fig. 10).

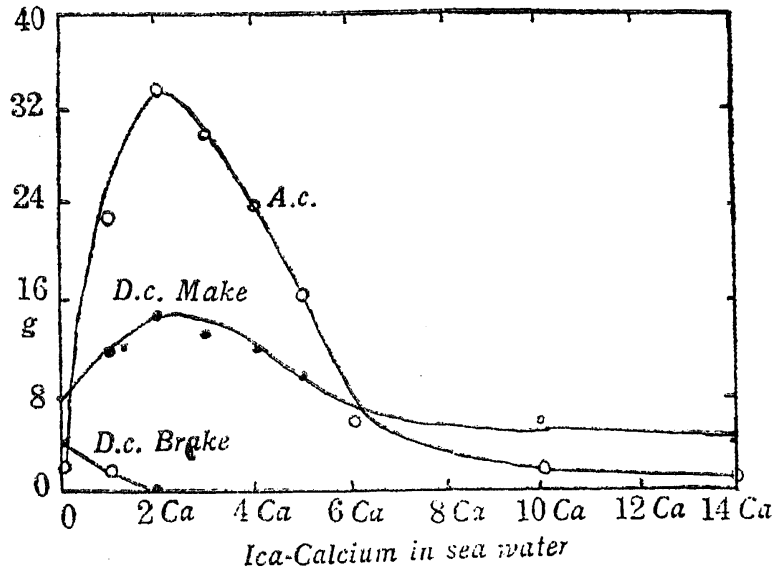

FIG. 10. *Mytilus* muscle. Effect of calcium on the response to A.C. and D.C.

In the guinea pig uterus, adaptation to A.C. inhibition is more rapid than D.C. inhibition.

Adaptation to inhibition.—There are two kinds of adaptations to inhibition; in one excitation also decreases, and in the other it increases. Adaptation to inhibition may be produced by three possible methods.

(1) Liberation of calcium. As calcium antagonises the inhibitory effect of sodium, liberation of calcium might occur. Excitatory action of ions would then also decrease. This probably occurs at high temperatures.

(2) Inhibition of adaptation. If increase in excitatory fact or "V" increases "U", then diminution of "V" or inhibition would diminish "U"; inhibition of adaptation is shown by increase of tone. This method of adaptation to inhibition appears to be produced in majority of instances.

There is experimental support for the suggestion, that ions may suppress the adaptation factor. Mazia (1933) has found that when eggs of the sea urchin Arbacia are immersed in pure sodium chloride solutions, there is actually an increase in the free calcium concentration of the cell interior. Presumably calcium is set free from combination in the cell cortex; this effect is prevented if a trace of calcium is added to the sodium chloride solution.

(3) Penetration of ions into the interior. Just as one kind of adaptation to excitation has been presumed to take place by penetration of the ions

into the interior, a similar action is possible. The reversal of the inhibitory effect of ammonium with time may be due to penetration (*c.f.* Ing and Wright, 1931; Straub, 1903, 1907).

Inhibition of adaptation and withdrawal contractions.—The suppression of adaptation by inhibitory substances offers an explanation of the withdrawal contractions. If increase in "V" over "U" causes contraction, then excitation will also occur if "U" decreases below "V", the latter remaining constant. Thus most of the substances that produce withdrawal contractions, produced a preliminary inhibition.

The simplest explanation of a withdrawal contraction (as advanced by Straub) would be that the contraction is due to difference in concentration of ions without and within the fibres. The initial contraction would be due to the presence of ions without the fibres, and the withdrawal contraction, to their presence within the fibres. If this explanation is correct, then the initial tension should subside completely in the continued presence of the drug, and a contraction occur when the latter is withdrawn. The majority of the withdrawal contractions do not show such a phenomenon, and so the above explanation is not tenable; these contractions are tonic, and so due to ions without the fibres.

Contraction produced by withdrawal of calcium.—This contraction is clearly due to decrease of the adaptation factor, that is, calcium. In *Mytilus* muscle a tonic contraction is produced on withdrawal of calcium or potassium (0·01-0·02M). If larger concentrations, which cause contraction are used, then a withdrawal contraction is not produced; this is therefore associated with the inhibitory property of these ions. In skeletal muscle Gelhorn (1931) found that only those concentrations of thiocyanate produce a withdrawal effect which do not produce a contraction.

That the contraction produced on withdrawal of calcium is due to sodium chloride ions without the fibres is shown by the fact that in frog and mammalian muscles, the action of sodium chloride is inhibitory, and so a withdrawal inhibition is produced. Calcium thus antagonises the action of sodium or chloride. In the guinea pig uterus, a withdrawal contraction or inhibition is produced depending upon the action of sodium chloride (Singh, 1942*b*, 1943*g*).

In the guinea pig uterus, though withdrawal of calcium, normally present in the saline does not produce contraction, large concentrations that produce inhibition produce a contraction on withdrawal (Fig. 11). This suggests that excess of calcium suppresses the liberation of calcium. It appears that calcium exists in some combination with protoplasm; in this

combination it is inactive. There further appears to be some relation between the combined and uncombined calcium; if there is too much of

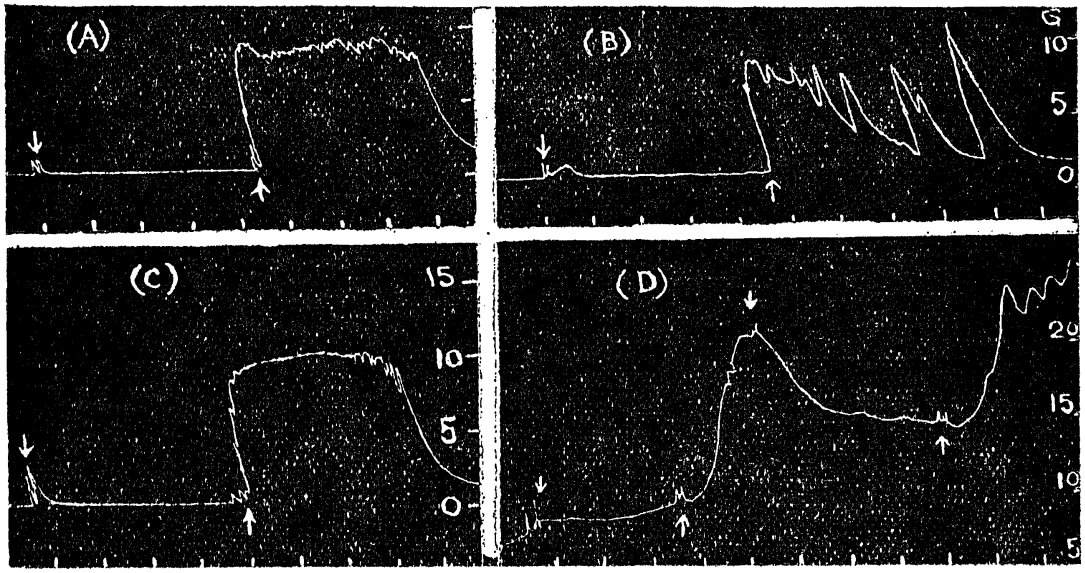

FIG. 11. Guinea pig uterus. Withdrawal contractions: (A) With drawal of 0·03 M $CaCl_2$ added at ↓ and withdrawn at ↑; (B) Withdrawal of 0·05 M $CaCl_2$; (C) Withdrawal of 0·05 M $SrCl_2$; (D) Withdrawal of adrenaline, 1 in 10^5 (dog stomach).

the latter it goes into combination, a relation similar to that which would be produced by the law of mass action.

At the equilibrium point there is a definite relation between the concentrations of ethyl alcohol, acetic acid and etyhl acetate. If more ethyl alcohol is added then more ethyl acetate will be formed. If now by some process we are suddenly able to withdraw the ethyl alcohol that was secondarily added, there would be left behind a disproportionately less amount of ethyl alcohol than was present in the beginning.

Similarly, if a compound between calcium and protoplasm is governed by similar relations, then if the concentration of calcium is increased more of it would go into combination, and if the excess of calcium is withdrawn, there would be a deficiency of ionised calcium and so a tonic contraction produced.

Liberation of calcium from protoplasm is known to occur in a variety of conditions (Heilbrunn, 1937, for reference). From the above process of reasoning, withdrawal of calcium should result in liberation of calcium, as has been shown by Mazia (1933).

The recombination of calcium appears to be produced not only by calcium, but also by allied ions such as strontium, the withdrawal of which causes a contraction (Fig. 11).

Contraction produced on withdrawal of ammonium.—(Singh, 1939*b*). Ammonium produces the following kinds of withdrawal contraction :— (1) A contraction which completely subsides, occurs on addition of ammonium; on withdrawal a further contraction occurs (Fig. 12). As there is

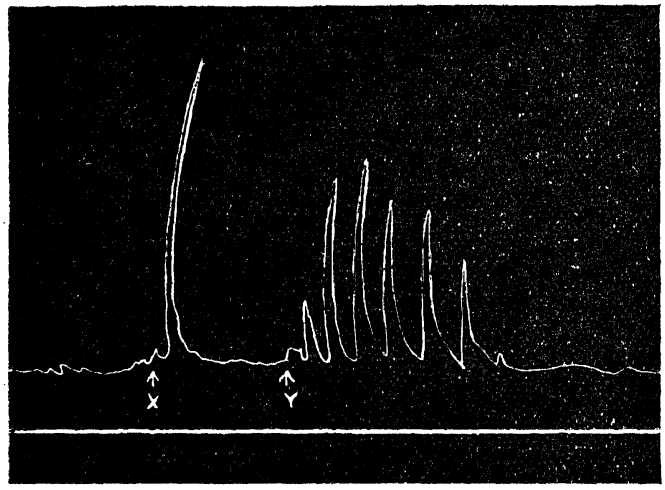

FIG. 12. Frog stomach. Contraction produced by addition (X) and withdrawal (Y) of 0·02 M NH_4Cl.

no preliminary inhibition, the withdrawal contraction could not be due to suppression of the adaptation factor. It can be explained on the basis of penetration, of ammonium ions into the muscle fibres. As the initial contraction subsides in a minute or two, the response must be of the surface fibres only (Singh, 1943*f*), and equilibrium must be rapid, the ions probably just crossing the membrane; the rapid subsidence may also be due to the liberation of the adaptation factor, so that in this case, there is probably excess of the latter.

The above view is supported by chemical analysis. In frog stomach, ammonium in concentration used to produce the withdrawal contraction replaces potassium within the fibres (Gokhale and Singh); besides the properties of the contraction are that of the A.C. contraction. The muscle is less permeable to quarternary ammonium salts, and they do not produce such a contraction. In dog stomach, there is no appreciable difference between the potassium contents of muscles, soaked in ordinary saline, and saline containing ammonium in concentration, used for production of the withdrawal contraction, and such a contraction has not been observed (Gokhale and Singh).

(2) The initial contraction on addition of ammonium may be absent, or only a preliminary inhibition is produced. In frog stomach this preliminary inhibition is increased if the sodium chloride of the saline is decreased by 20 p.c.

(3) A preliminary inhibition occurs, but the muscle recovers from the inhibition, and may even contract. On withdrawal there is a tonic contraction (Fig. 13).

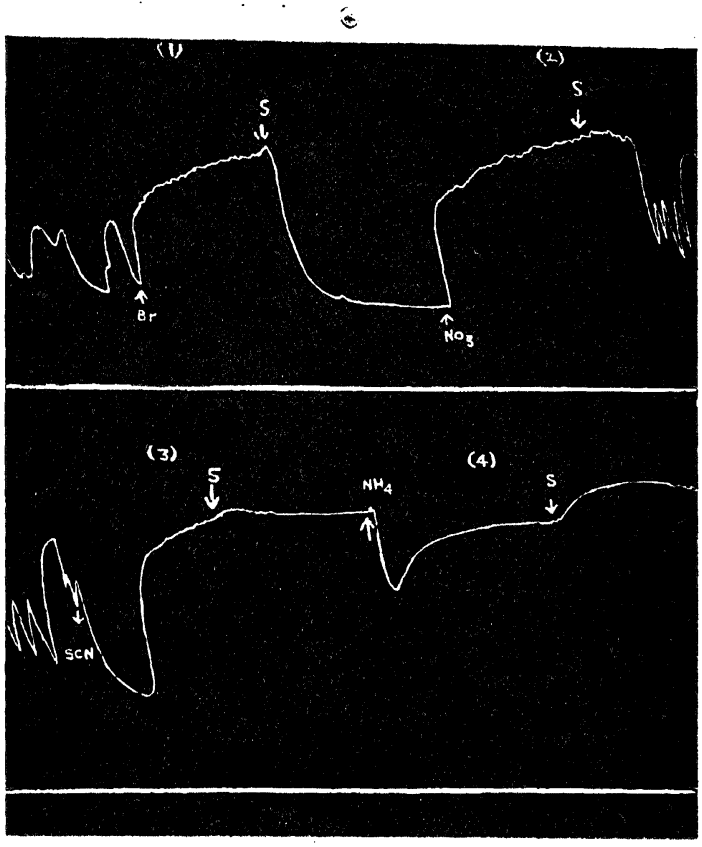

FIG. 13. Guinea pig uterus. Chloride of the saline replaced by bromide at Br, withdrawn at S. Nitrate added at NO_3, thiocyanate at SCN, and ammonium at NH_4 and respectively withdrawn at S.

The recovery from inhibition, and the subsequent contraction, shows that the adaptation factor has been suppressed as mentioned above. The phenomenon is very important showing that excitation releases the adaptation factor and inhibition suppresses it.

In the guinea pig uterus, eserine aids the suppression of the adaptation factor, as with adrenaline the preliminary inhibition is followed by a contraction and on withdrawal another contraction occurs (Agar, 1940) with the exception of the withdrawal contraction, a similar action is produced by deficiency of sodium chloride in the case of potassium (Singh, 1942*b*).

Contraction produced on withdrawal of potassium.— There are two kinds of contractions, one produced by withdrawal of small concentrations in *Mytilus* muscle, and the other by large concentrations in the guinea pig uterus. The former contraction is akin to the calcium withdrawal contraction, potassium having a calcium-like action. The latter contractions are of two kinds: (1) like those produced by withdrawal of ammonium; (2) like

those as shown in Fig. 4 of Singh (1943*g*). There is no preliminary inhibition, but contraction on addition as well as withdrawal of potassium, like the D.C. contraction in *Mytilus* muscle. For this contraction, no explanation is satisfactory, except that the preliminary inhibitory phase may be regarded as transient or latent.

The contraction described above on withdrawal of potassium is a tonic contraction as shown by the following considerations. On frequent stimulation the initial contraction is replaced by inhibition, and on withdrawal of potassium, the usual re-development of tone occurs; the original contraction has disappeared, but the withdrawal contraction remains in the above form.

That potassium should cause inhibition or contraction in the same muscle, shows that the factor determining inhibition or contraction resides in the cell. It is possible to antagonise one of these, and so produce the other result.

Contraction produced on withdrawal of anions.—The preliminary inhibition by thiocyanate is well marked (Fig. 13). In *Mytilus* muscle, with sulphate there is no inhibition, this is probably due to changes in the calcium concentration or the adaptation factor.

Contraction on withdrawal of drugs.—The preliminary inhibition is well emphasised; adrenaline and acetylcholine produce a withdrawal contraction in *Mytilus* muscle only if the muscle is insensitive to these drugs. In dog stomach, and the guinea pig uterus, the preliminary inhibition is proded by adrenaline. In *Mytilus* muscle, veratrine produces withdrawal contraction only if the preliminary contraction is little marked (at pH 6·5) or is abolished by A.C.

In guinea pig uterus inhibitory substances produce a withdrawal contraction, and excitatory ions like chloride, bromide, acetylcholine produce a withdrawal inhibition. The former is probably due to inhibition and the latter to liberation of the adaptation factor; the latter is identical with the inhibition of tone found just after stimulation. This inhibition of tone really results during stimulation, and outlasts the latter.

A withdrawal stimulus may not actually produce a contraction, but may retard the relaxation of the contraction like veratrine in skeletal muscle. This is shown by Fig. 13. Bromide produces no withdrawal contraction, and the relaxation of the bromide contraction is rapid. Nitrate, thiocyanate and ammonium produce withdrawal contractions, the potency being in the order, $NO_3 < SCN < NH_4$; relaxation is also retarded in the same order.

Slow relaxation after chemical stimulation is also due to passive changes probably increase in viscosity. This is suggested by the fact, that the contraction persists though the stimulating substance is withdrawn, the muscle relaxing slowly, alike in the presence or absence of the stimulus.

Thus after chemical stimulation, as after electrical stimulation, the slow relaxation may be due to an active contraction, or may be a passive phenomenon, due to structural changes in the muscle.

Fatigue.—Fatigue with intermittent stimulation and adaptation with continuous stimulation are identical; the former is due to the persistence of the adaptation factor. Hence, as there are two kinds of adaptation, there will be two kinds of fatigue. In one kind of fatigue, the excitability to potassium will decrease, and in the other kind, it will increase.

The two kinds of fatigue to A.C. are beautifully shown in Figs. 1 and 2. In the former the A.C. off contracture, hence the sensitivity to ions without increases, and in the latter, it decreases. The former occurs in potassium sensitive muscles, and the latter in comparatively insensitive muscles. The former fatigue is due to increased sensitivity to ions outside so that contracture producing substances, such as adrenaline (1 in 10^5), convert the first kind of fatigue into the second kind, by antagonising the factor of adaptation, and increases the second kind of fatigue (see Fig. 5, Singh, 1939*b*).

As mentioned above, the neutralisation of adaptation is probably due to entrance of ions from the solution into the fibres. The entrance of ions would account for the different effects produced by change of the direction of direct current on fatigue (Heilbrunn, 1937, for references). As anions are antagonistic to A.C. the accumulation of cations at one end of the muscle fibres will be antagonised by the anions; at the other end the fibre will be poorer in anions, and when the current is reversed, the excitability will be greater.

The above experiments clearly show that fatigue could not be due to consumption of substances responsible for contraction, but it can be due to accumulation of waste products if the latter increase the permeability and thus make the muscle more sensitive to action of ions without.

The increase of fatigue at high temperatures are also due to the same two factors, *viz.*, increase in adaptation. The second factor also comes into play, as the excitability to ions also increases. As these two factors are antagonistic, the result will depend on their interaction. Variable results of temperature have been obtained by Bernstein (1908) and others.

It is evident that calcium will affect the two kinds of fatigue oppositely.

Beneficial effect of contraction.—This is produced by the liberation of the adaptation factor in potassium sensitive *Mytilus* muscle, as this is antagonistic to the action of ions outside; in potassium insensitive muscles, the same factor will produce fatigue. The above conclusion is supported by the fact that in potassium insensitive muscles the beneficial effect of contraction is marked if the concentration of calcium is decreased (Fig. 4), thereby increasing the action of ions outside. It is also supported by the fact that the rate of relaxation increases so that bigger the contraction, the more quickly it relaxes, the slow relaxation being due to the ions outside (Singh, 1938*e*). This is the reason why the contractions cannot be superposed on each other (Bozler, 1930; Ritchie, 1931), and neutralisation of the tonic contraction in freshly dissected muscle by stimulation (Bayliss, Boyland and Ritchie, 1930). As with fatigue, the two excitabilities may be similarly or oppositely affected.

In frog muscle, the beneficial effect of contraction is due to antagonising the inhibitory action of ions outside, instead of excitatory, as in *Mytilus* muscle.

Recovery of excitability.—The liberation of the adaptation factor would result in a stage of hyper-excitability during recovery; this conclusion is supported by the fact that in potassium insensitive muscles, the stage of hyper-excitability is absent. As the ions outside have not much effect, on recombination of the adaptation factor, there will be no depression of excitability. The beneficial effect of contraction and the supernormal phase during recovery are thus due to similar causes, and are so affected identically (Adrian, 1921); for the same reason they are most marked in the same muscle.

Relaxation.—The muscle adapts at different times to slow relaxation and to the primary A.C. tension, so that these are produced by two distinct agencies relaxation is slow if the current is continued for 3–4 seconds, and becomes rapid thereafter, but the primary tension continues to increase. The primary A.C. tension is produced by ions within and the slow relaxation by ions without the fibres.

The slow relaxation with stimuli of short duration would thus be due to lack of adaptation as " V " rises more rapidly than " U ". As the action of ions outside would increase with increasing voltage, the slow relaxation at higher voltage would be due to neutralisation of adaptation or " adaptation to adaptation ".

The secondary A.C. contracture.—The liberation of the adaptation factor during stimulation with A.C. would diminish tone; when the adaptation

factor recombines, the ions outside would exert their action, producing a tonic contraction. The muscle would, however, readapt, thus producing the secondary A.C. contracture.

Magnesium shortens the latent period of the secondary A.C. contracture (Singh, 1938*b*), so that, like other inhibitory ions, it suppresses adaptation, or makes the fall of " U " quicker.

Inhibition.—There are two kinds of inhibition (1) external inhibition or by ions without the fibres; (2) internal inhibition or that produced by electric current and increase of osmotic pressure, that is, ions within the fibres. There are several kinds of external inhibition (*a*) tone diminishes, the excitability to A.C. as well as potassium is increased; this is produced by eserine, 1 in 200,000 in *Mytilus* muscle; (*b*) tone diminishes, the excitability to A.C. is increased and that to potassium decreased; this is produced by calcium in *Mytilus* muscle; (*c*) tone diminishes, the excitability to A.C. is decreased and that to potassium increased this is produced by veratrine in *Mytilus* muscle in acid solutions; (*d*) tone diminishes, the excitability to A.C. as well as potassium is decreased. This is produced by adrenaline in frog stomach.

The above shows the complicated nature of inhibition. When tone is diminished, and the excitability to A.C. as well as potassium is increased, it may be inferred that the substance favours action of cations, and suppresses that of anions, tone presumably in the case being due to chloride. When tone is diminished, the excitability to A.C. is decreased and that to potassium increased, it may be inferred that the substance favours the action of cations outside and suppresses that of anions, tone again presumably due to chloride. In case (*d*) above, both anions as well as cations are suppressed. In case (*b*) the action of ions outside is suppressed.

In frog stomach inhibition affects both the actions of A.C. as well as potassium identically, but in *Mytilus* muscle the action of A.C. is enhanced. This latter result is probably an indirect effect, produced by antagonising the action of ions outside. In frog stomach this does not occur, as the sodium chloride content of the saline is low, but can be made to occur by enhancing the stimulating power of the ions of the frog saline replacing part of the sodium with potassium. Inhibition, therefore, like adaptation, affects the two excitabilities identically.

When the various actions described above are analysed, tone appears to be caused by chloride.

Antagonism between sodium and chloride.—When unstriated muscle is immersed in isotonic solution of sucrose of glucose, a contracture develops.

In frog stomach, guinea pig uterus and *Mytilus* muscle, introduction of sodium chloride causes inhibition ; this shows that sodium chloride contains an inhibitory factor. In frog stomach contraction may ensue showing that it also contains an excitatory factor. In guinea pig uterus, after adaptation sodium chloride causes contraction, the inhibitory factor being antagonised by adaptation; thus, here too sodium chloride contains an excitatory factor.

In *Mytilus* muscle sodium chloride deficiency in the saline may either increase or decrease the excitability to A.C. inhibitory cations in the *Mytilus* saline, such as calcium and potassium increase the excitability to A.C. If the chloride of the saline is replaced with excitatory ions such as bromide, nitrate, iodide, then their partial removal always increases the excitability to A.C. This shows that in *Mytilus* muscle increase in excitability in sodium chloride deficient solutions, must be due to removal of the chloride, and decrease to removal of the sodium. Thus the actions of sodium and chloride are antagonistic.

In frog stomach small concentrations of anions increase the excitability by antagonising the inhibitory action of cations, larger concentrations decrease the excitability as in *Mytilus* muscle owing to their excitatory action.

Plain muscle exhibits two kinds of tone: (1) by ions within the fibres, (2) by ions outside the fibres (Singh, 1939*b*). Tone produced by ions without, again, is of two kinds; (1) with high viscosity, (2) with low viscosity (Singh, 1943*a*). In guinea pig uterus cations in small concentrations produce the tonus with low viscosity. It therefore appears that tonus with high viscosity is due to anions, and that with low viscosity to cations; chloride and sodium probably produce these two kinds of tone respectively.

Discussion

There are two kinds of adaptation to excitation; one is slow and the other quick. The quick adaptation is probably brought about by liberation of calcium. If the length of the muscle is decreased, then the quick adaptation does not seem to occur, and the adaptation that occurs after prolonged soaking appears to be due to diffusion of ions into the interior of the muscle fibres.

The equilibrium between " U " and " V " suggests liberation of calcium by ions.

That practically all known types of stimulating agents can cause release of calcium from protoplasm is shown by the work of Mazia and Clark

(1936). They were able to show that ultraviolet radiation, electric shock, mechanical impact, heat hypertonic solutions all caused a release of calcium from the protoplasm.

The liberation of calcium assumed to take place during A.C. stimulation (Singh, 1938*b*, 1939*b*, 1940) is probably also be due to the same factor, that is increase ionic concentration; this is the reason that " U " rises as well as declines more slowly than " V ".

If adaptation is brought about by the action of ions outside, then the excitability of the muscle to potassium should increase during the passage of the current as the ions outside increase the excitability to potassium (Singh, 1938*b*). If adaptation is brought about by the second factor, that is increase in the calcium ion concentration, then the excitability to potassium should decrease.

There is a relation between the permeability of the muscle and excitability. If adaptation is brought about by ions outside, then the permeability should increase; if by calcium, it should decrease. Decrease in membrane resistance with excitation indicating increased permeability, has been found by Duboisson (1934), Ebbeck (1932), McClendon (1912, 1927), Ray and Rapport (1927), Buchthal (1934), Hogben and Gordon (1930), Achelis (1932). Bozzler, Bozzler and Cole (1935) found an increase in the resistance or impedance of the muscle, Hartree (1935) found no change. Variable results are to be expected.

Noonan, Fenn and Haege (1941) found that in frog skeletal muscle, there was no increase in permeability to radio-active potassium during electrical stimulation of frog skeletal muscle. This is in agreement with the views mentioned previously, that electrical stimulation is due to ions within the fibres; no increase in permeability would be necessary. As mentioned above, a decrease of permeability should be expected.

In plain muscle, too, there should be a decrease in permeability, though this should be preceded by a transient increase, according to the sensitivity to ions without (Singh, 1938*e*). In *Mytilus* muscle however, the decrease of permeability should be followed by an increase if it shows a tonic contraction on electrical stimulation. Similarly with chemical stimulation, a decrease of permeability should occur if adaptation occurs; otherwise an increase should occur. In diffusion experiments these opposing tendencies might neutralise each other.

With nervous stimulation, these changes are to be expected, if the mechanism of stimulation is the same.

A decrease in the concentration of potassium within the fibres, should produce tonic results, such as slowing of the rate of relaxation or increase in viscosity, with frequent stimulation. Both these changes are known to occur.

Summary and Conclusions

(1) There are two kinds of adaptation to alternating current. During one, the sensitivity to potassium decreases, and during the other, it increases. One is probably produced by calcium, and the other by ions outside.

(2) There are two kinds of adaptation to chemical stimulation; one is similar to the first kind of adaptation to A.C. and the second one is probably due to diffusion of ions into the muscle fibres. This kind of adaptation also occurs to A.C.

(3) Adaptation may be diminished by (*a*) leakage of the adaptation factor, (*b*) neutralisation of the same or (*c*) or inhibition of adaptation. This last appears to be responsible for tonic withdrawal contractions.

(4) Adaptation increases with length.

(5) Adaptation and fatigue are identical.

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