

## THE MODE OF ACTION OF ADRENALINE ON UNSTRIATED MUSCLE

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THE action of adrenaline on unstriated muscle is well known. Its action may be stimulatory or inhibitory. But it is not known why its action is twofold. In the present research an attempt has been made to elucidate these actions of adrenaline by studying their effect on the responses of frog's unstriated muscle to electrical stimulation. The action of adrenaline has been studied by recording its effect on tone or spontaneous contractions. There is very little on record to show its effect on the response of unstriated muscle to electrical stimulation. These responses of unstriated muscle correspond to the twitch contractions of striated muscle on which the effect of adrenaline is well known. Singh (1938 *a*, 1939, 1940) found that small concentrations of adrenaline increased the excitability of the muscle to electrical stimulation and decreased it in large concentrations.

Frog's stomach muscle was used. It was stimulated with alternating current, 12 volts, once a minute for 10 seconds. The inhibitory effect of adrenaline, 1 in 100,000 was tested.

### RESULTS

Adrenaline may either produce contraction or relaxation of unstriated muscle; it produces contraction or relaxation if the muscle is in lactic tone, and contraction or no effect if in alactic tone. Sometimes the inhibitory action of adrenaline is preceded by a contraction (Fig. 1). This figure gives a clue to the action of adrenaline, showing that adrenaline inhibition is really an adaptation (Singh, 1945). Substances which diminish adaptation, cause the production of this initial contraction, for example if the chloride of the saline is replaced with thiocyanate (Singh, 1938 *b*), or by reduction of temperature (Rao and Singh, 1940). It is, therefore, expected, that the inhibitory action of adrenaline will be converted into a stimulatory one by substances that diminish adaptation. This is well shown by the results described below.

*Effect of cyanide.*—Cyanide or asphyxia diminishes adaptation to electrical stimulation (Singh, 1938 *b*, 1949) and it converts the inhibitory

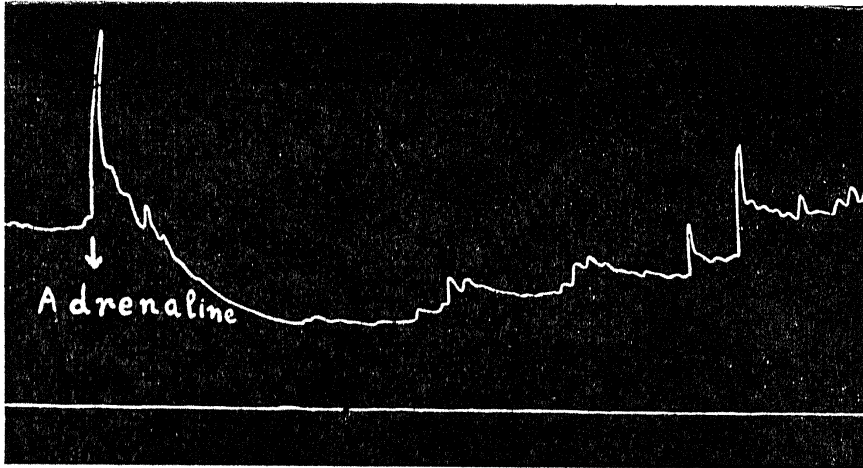


FIG. 1. Frog's stomach muscle. Contraction and inhibition produced by adrenaline (1 in 100,000).

effect of adrenaline into a stimulatory one (Fig. 2). Starkenstein (1941) found that asphyxia has similar action on excised intestines. This therefore

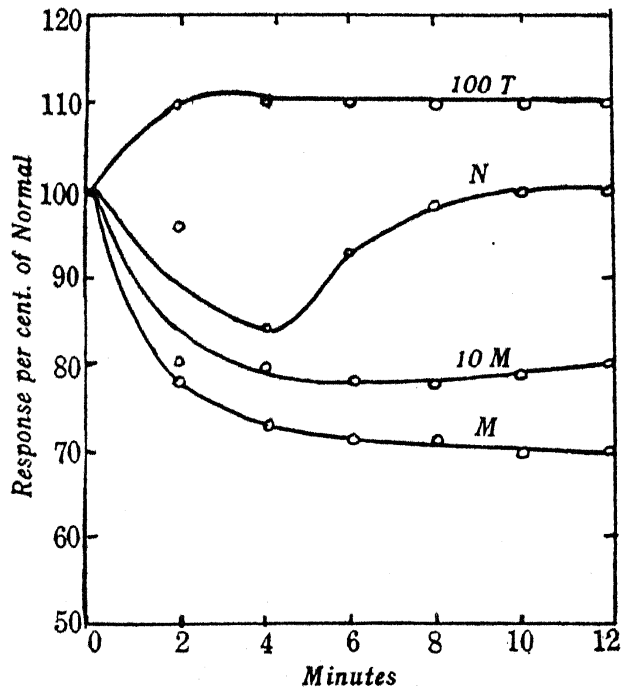


FIG. 2. Frog's stomach muscle. Effect of cyanide on the inhibitory action of adrenaline (1 in 100,000) on the responses of the muscle to alternating current. N, is normal curve. M, 1 in million sodium cyanide; 10 M, 1 in 10 million, 100 T, 1 in 100,000.

suggests that energy is required for the inhibitory action of adrenaline, just as it is required for the inhibitory effect of electrical current (Singh, 1949). Glucose, therefore, ought to increase the inhibitory action of adrenaline,

as it does with similar action of electrical current. In these experiments, however, glucose was found to have a stimulatory action or decreased the inhibitory action, the action of glucose being antagonised by iodoacetic acid (Fig. 3).

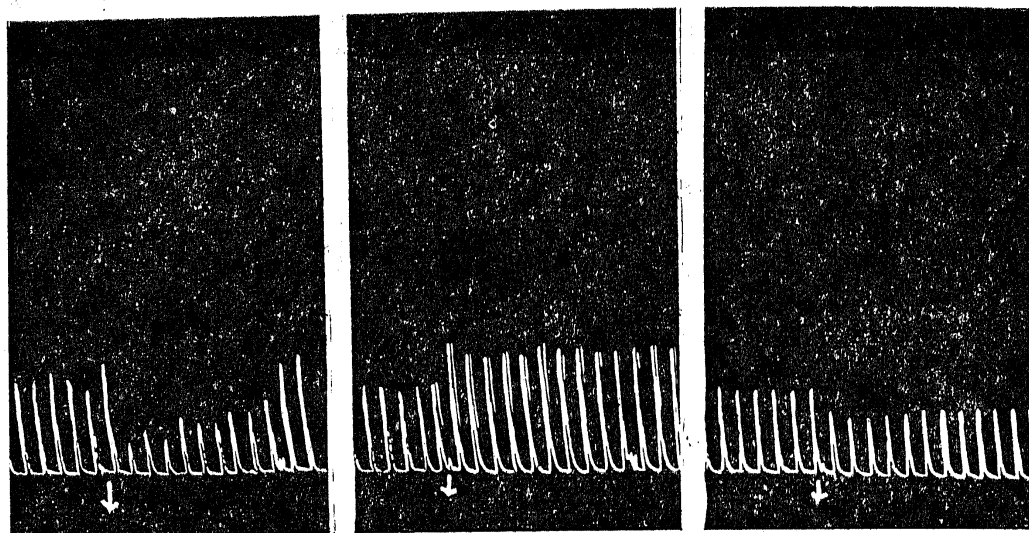


FIG. 3. Frog's stomach muscle. Effect of glucose and iodoacetic acid on the inhibitory action of adrenaline (1 in 100,000) on the responses of the muscle to alternating current. A, Normal response. B, Effect of 0.1 p.c. glucose. C, Effect of iodoacetic acid, 1 in 10,000. Adrenaline added at arrows.

Small concentrations of cyanide may increase the inhibitory action of adrenaline. This is akin to the asphyxial increase of excitability for contraction.

*Effect of ions.*—Ions have twofold action; stimulating ions decrease the inhibitory action of adrenaline, and eventually convert its inhibitory action into a stimulatory one. These ions also diminish adaptation. They are, bromide, nitrate, iodide, thiocyanate, barium, lithium, sodium and potassium (Fig. 4). The effect of sodium chloride was determined by partially replacing it in the saline by sucrose. The effect of ions increases in this order  $\text{Cl} > \text{Br} > \text{NO}_3 > \text{I} > \text{SCN} > \text{CN}$ . Ions which produce inhibition or increase adaptation, have the opposite action. These are hydrogen ions, ammonium, magnesium, calcium and strontium.

*Effect of drugs.*—Acetylcholine in small concentrations increases the inhibitory action of adrenaline (Fig. 5), but increases the rate of recovery. Eserine has a similar action (Fig. 6); by recovery the inhibitory effect may be converted into a stimulatory one. Pilocarpine has effects similar to those of acetylcholine. Ephedrine in small concentrations (1 in 10 million) potentiates and in larger concentrations (1 in 100,000) antagonises the effect

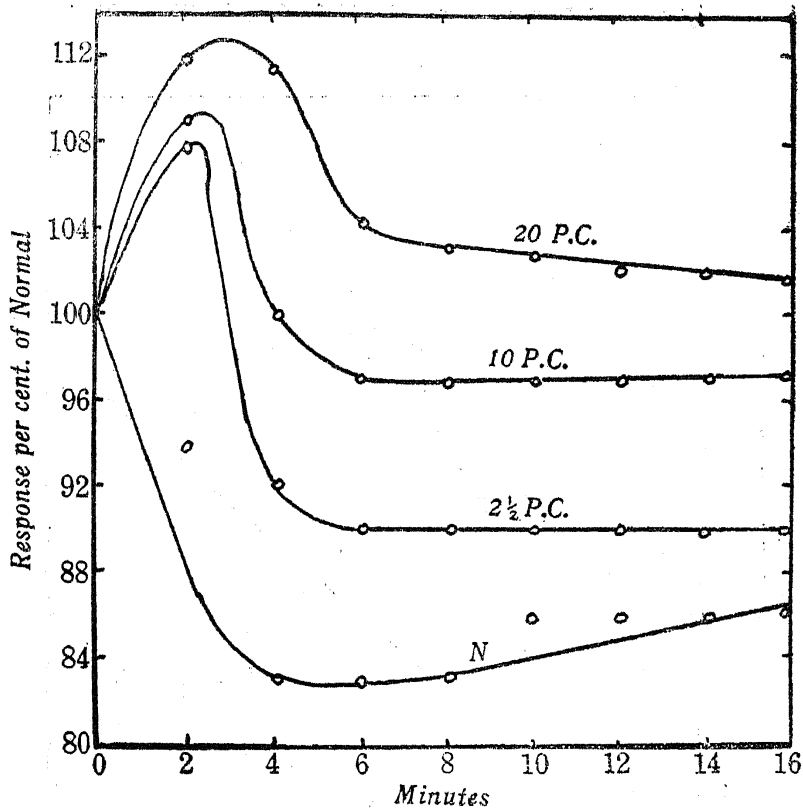


FIG. 4. Frog's stomach muscle. Effect of sodium thiocyanate on the inhibitory effect of adrenaline (1 in 100,000) on the responses of the muscle to alternating current. N, is normal curve. 2½, curve obtained in saline in which 2½ p.c. of the sodium chloride has been replaced with sodium thiocyanate, etc.

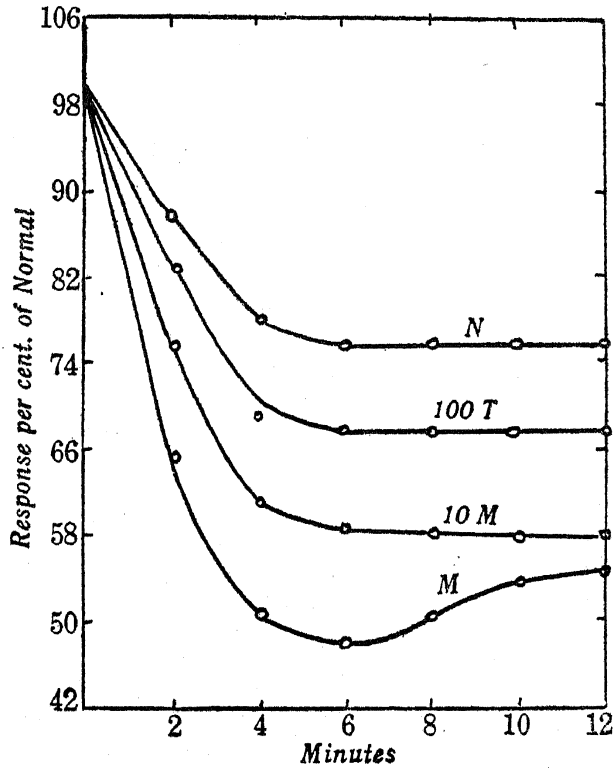


FIG. 5. Frog's stomach muscle. Effect of acetylcholine on the inhibitory action of adrenaline (1 in 100,000) on the responses of the muscle to alternating current. N, is normal curve; M, in acetylcholine, 1 in million; 10 M, 1 in 10 million; 100 T, 1 in 100,000.

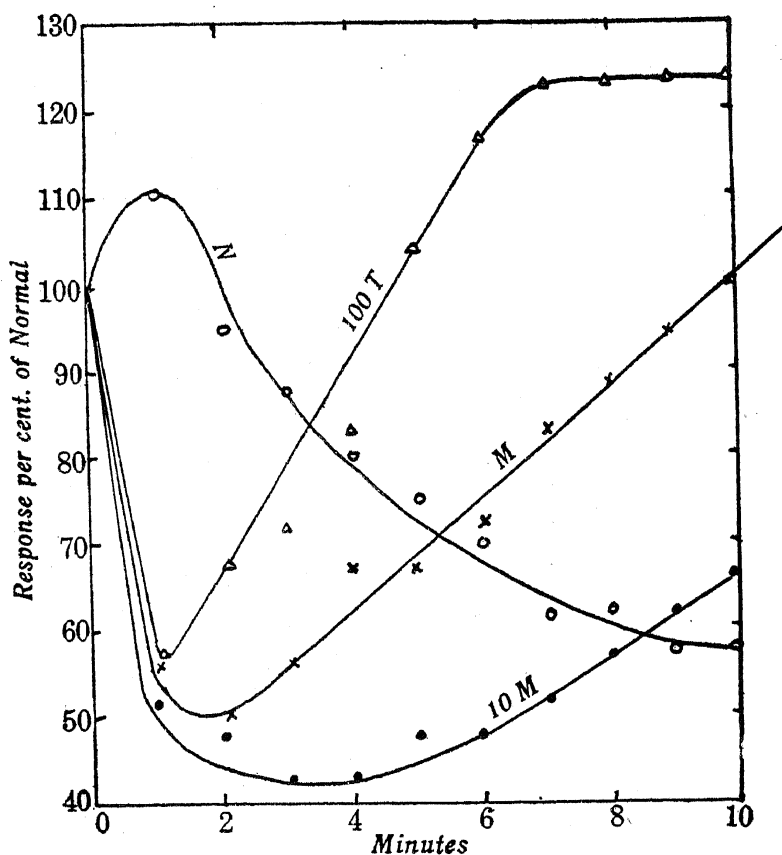


FIG. 6. Frog's stomach muscle. Effect of eserine on the inhibitory action of adrenaline (1 in 100,000) on the responses of the muscle to alternating current. N, normal curve. M, curve in eserine sulphate 1 in million, 10 M, 1 in 10 million; 100 T, 1 in 100,000.

of adrenaline. Nicotine has similar action as that produced by stimulating ions.

Increase in osmotic pressure of the saline up to twice normal increases the inhibitory effect of adrenaline. A slight decrease of osmotic pressure (20 p.c.) has the opposite action but a large decrease (40 p.c.) again increases the inhibitory effect of adrenaline.

*Action of fatigue.*—With repeated use, the inhibitory action of adrenaline may at first increase (beneficial effect of inhibition, Singh, 1942) and then decrease (fatigue), or the inhibitory action of adrenaline may decrease from the outset, the beneficial effect of inhibition being absent (Fig. 7).

*Action of tonus.*—As mentioned in previous papers there are two kinds of tonus in unstriated muscle (Singh, 1949; Singh and Singh, 1950). One is sensitive to adrenaline, and the other insensitive; the former is identical with lactic tone and the latter with alactic tone. When the muscle is in alactic tone, adrenaline may cause contraction. The amount of relaxation

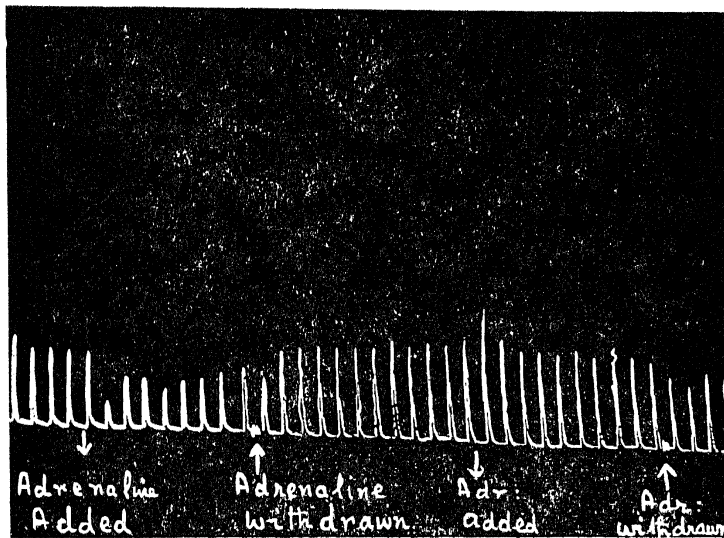


FIG. 7. Frog's stomach muscle. Inhibitory action of adrenaline (1 in 100,000) reversed on repetition.

by adrenaline (1 in  $10^7$ – $10^6$ ) may be used to judge the proportion of lactic and alactic tones in unstriated muscle, which is ordinarily inhibited by adrenaline. In dog's stomach muscle from the pyloric end, adrenaline may cause contraction, which does not subside on withdrawal of the drug such as is produced by acetylcholine (Singh and Singh, 1947). The pH of the saline should be 8, buffered with borate or bicarbonate and temperature about  $25^\circ\text{C}$ . to  $37^\circ\text{C}$ .

#### DISCUSSION

In considering the inhibitory and excitatory action of adrenaline it has to be taken into consideration that unstriated muscle is a complex structure and unless its variables are kept in mind, the results are likely to be conflicting. The two chief variables in unstriated muscle are (a) the excitatory mechanism, (b) the contractile mechanism. Not only the excitatory, but the contractile mechanism also is likely to vary in its properties, its most important variation being its inability to relax. Unstriated muscle shows two kinds of tone. One of these is susceptible to asphyxia (lactic tone) and the other resistant (alactic tone). If the muscle is in alactic tone, then the contractile mechanism is unable to relax, with the result that inhibitory substances such as adrenaline, would not be able to produce relaxation. The excitatory system appears to be normal as contraction by excitatory substances can be produced. Inhibition in unstriated muscle is also of two kinds, one active and the other passive. Active inhibition can be abolished by poisoning with cyanide, when only passive inhibition remains. There are other variables such as twitch and tone. Twitch is antagonistic to

lactic tone but not to alactic tone, so that twitch may be affected indirectly by changes in lactic tone.

The various factors which affect and cause reversal of the action of adrenaline may now be summarised. These have been previously mentioned in several papers (Singh, 1942, 1943, 1944 *a, b*, 1945, 1949; Singh and Singh, 1947, 1949 *a, b, c*; 1950).

The first of these factors is metabolism. Thus the inhibitory action of adrenaline may diminish in an asphyxiated or cyanide treated muscle. This shows that energy is required for inhibition and so glucose would increase the inhibition (Singh, 1949). Fatigue has been shown to cause reversal of the effect of adrenaline. Now, fatigue is of two kinds (Singh and Singh, 1949 *a*). One is due to exhaustion of energy stores and the other to the development of an opposite state or accommodation. In the case of inhibition, fatigue will be due to the development of an excitatory state, and as excitatory substances have been shown to convert the inhibitory action of adrenaline into an excitatory one, this explains the fatigue reversal. Glucose by increasing the excitatory state will also decrease or convert the inhibitory action of adrenaline into an excitatory one. This explains the dual action of glucose. Glucose increases inhibition if it aids the inhibitory ions; it decreases inhibition if it aids the excitatory ions. For the same reason glucose may increase or decrease accommodation to excitation. That metabolism plays a part is shown by the increase of inhibition with temperature, up to about 30° C. Thereafter the inhibition decreases owing to the increase in the action of excitatory ions causing tone. Excitation and inhibition, or contraction and relaxation are subserved by different metabolic mechanisms respectively, and the action of glucose and cyanide would depend upon the particular metabolic mechanism involved.

The second important factor affecting the action of adrenaline is sensitisation. The mechanism of this sensitisation appears to be the entrance of ions into the muscle fibres. The entrance of sodium into nerve during excitation is an established fact. Hodgkin and Katz (1949) have stressed its importance in the reversal of action potential. The entrance of ions from the saline into the muscle fibres appears to play a very important part in determining the type of response by unstriated muscle and drug reversals (Singh, 1944 *a*). The evidence for this is both chemical and physiological. Chemical analyses have shown that *Mytilus* unstriated muscle gains sodium during stimulation (Singh, 1939 *a*). The same muscle gains ions from the saline in the absence of calcium (Singh, 1938, 1939 *a*, 1944 *b*). The gain of ions by the muscle is increased by blotting.

Physiological evidence for the entrance of ions into the muscle fibres during stimulation is as follows. If *Mytilus* muscle is immersed in saline containing barium ions, it passes into a tonic contraction. At other times, especially in summer, the muscle may remain quiescent without any apparent effect. Barium ions are present in the saline in both instances, and yet the muscle remains quiescent in one instance and contracts in the other. The quiescent muscle can be made to produce a typical barium contraction by certain diverse procedures. These are (a) Passage of electric current for a short period which may be insufficient to cause a contraction or produce only a twitch, (b) Sudden heating or cooling, (c) Sudden stretch or release, (d) Stroking the surface with a hair brush or blotting paper, or vigorous aeration with air or an indifferent gas such as nitrogen, (e) Removal of calcium, (f) Addition of drugs such as adrenaline, acetylcholine, caffeine. These drugs, by themselves, may or may not produce a contraction.

The above factors appear to be unrelated, but there must be some common effect produced by them. The removal of calcium suggests increase in permeability and entrance of barium into the muscle. As mentioned above this is supported by chemical analyses. Blotting also, as shown by chemical analyses, increases the gain of ions by the muscle. Two conclusions follow from the above experiments (a) The common factor among the above agencies is that they produce excitation, and during such excitation, ions enter the muscle fibres from the saline. (b) To produce excitation ions must enter the muscle fibres. This latter conclusion is also supported by chemical and physiological experiments. The stimulating power of many ions is proportional to their ability to enter the muscle (Singh, 1938, 1939 a). The above argument regarding barium, applies to other ions in the saline, such as sodium and potassium. The cessation of excitation would be followed by extrusion of these ions by the muscle fibres.

It appears that not only excitatory, but inhibitory ions may also enter the muscle fibres and produce inhibition. Thus the action of sodium chloride in frog muscle is inhibitory, and removal of calcium produces relaxation instead of contraction. Similarly, adrenaline may produce excitation, if there are excess of excitatory ions in the saline, and inhibition if there are excess of inhibitory ions, such as hydrogen ions. This appears to be one cause of drug reversal.

The above sensitisation explains the action of many substances, such as calcium, hydrogen, sodium and other ions in causing reversal. During the action of adrenaline, the sensitisation will enable the excitatory ions in the saline to antagonise the inhibitory effect of a substance and increase the



excitatory effect; inhibitory ions will increase the inhibitory effect and decrease the excitatory effect. In the saline therefore there must be excitatory and inhibitory ions to account for the action of drugs (Singh, 1942). Sodium ions are presumably inhibitory and chloride ions excitatory. Adrenaline does not produce inhibition in the absence of sodium chloride; sodium ions are presumably responsible for inhibition and chloride ions, for excitation by adrenaline.

The third important factor in determining the action of adrenaline is excitability. Small concentrations of adrenaline which do not produce contraction, produce relaxation; this is found with all kinds of stimulation. Hence, as the excitability decreases, higher and higher concentration of the drug produces inhibition. This action of adrenaline is due to adaptation. Every excitatory stimulus is accompanied by an inhibitory state, so that if the excitatory state diminishes or is antagonised, the inhibitory state produces inhibition. Thus small concentrations will produce inhibition, and substances that decrease the excitability will also convert the excitatory into inhibitory action. In an inexcitable muscle, adrenaline would produce inhibition; it must be remembered that the muscle may be inexcitable to a particular substance and not to others. Substances that antagonise the inhibitory state and these are excitatory ions, would thus convert the inhibitory action of adrenaline into an excitatory one. Substances that increase accommodation to the inhibitory state would also cause reversal of the action of adrenaline (accommodation to accommodation or adaptation to adaptation, Singh, 1944 *a*).

It is known that small concentrations of adrenaline produce fall of blood pressure. After ergotoxine and dibenzamine, adrenaline produces fall of blood pressure. Weak stimulation of a mixed nerve causes vasodilatation, strong stimulus causing vasoconstriction.

It is interesting to note that adrenaline would cause stimulation by two methods; first, by direct stimulatory action, and secondly, indirectly by accommodation to accommodation or inhibition. Thus eserine which increases accommodation into the inhibitory action of adrenaline, converts its inhibitory action into an excitatory one. As inhibition is due to accommodation to excitation, all responses begin as excitation. If this excitation is masked by accommodation, then inhibition only would be produced and if this accommodation is further masked by subsequent accommodation, then only contraction would occur. The process of accommodation to excitation, and then subsequent accommodation to accommodation if repeated, will produce rhythmic stimulation.

Increase in initial length of the muscle would change the inhibitory action of adrenaline into an excitatory one as it increases the action of excitatory ions (Singh and Singh, 1950).

The loss of the inhibitory action of adrenaline during alactic tone is due to interference with active relaxation. Low temperatures also produce such an effect. The effect of lactic tone is to convert the excitatory action of a substance into an inhibitory one causing contraction of a relaxed muscle and relaxation of a contracted one. This acts by two methods: first, increase of tone decreases excitability; secondly, it is accompanied by increase in the latent-inhibitory state due to accommodation insufficient to cause relaxation. This latent inhibitory state sums up with the inhibitory action of the substance and thus causes relaxation. That increase in tonus increases the latent inhibitory state is shown by the fact that moderate increase in tonus increases inhibition; greater increase in tonus decreases inhibition owing to its direct antagonistic action (Singh, 1942).

Lastly, the action of ions inside the muscle fibres is antagonistic to that outside, hence increase in osmotic pressure of the saline converts the inhibitory action of adrenaline into an excitatory one (Singh, 1945), but if it antagonises the excitatory ions outside, the inhibitory action of adrenaline would increase; this explains the opposite effects of change in osmotic pressure of the saline.

#### SUMMARY

1. The inhibition produced by adrenaline in unstriated muscle may be preceded by a contraction. This shows that inhibition by adrenaline is due to adaptation to excitation.

2. Substances that decrease adaptation to excitation, convert the inhibitory effect of adrenaline into a stimulatory one; these are cyanide, thiocyanate, bromide, nitrate, iodide, barium, lithium, sodium and potassium.

3. Substances that increase adaptation to inhibition, convert its inhibitory effect into a stimulatory one; this is produced by eserine.

4. Substances that cause stimulation, convert the inhibitory effect of adrenaline into a stimulatory one.

5. Substances that cause inhibition, or increase adaptation to excitation, increase the inhibitory of adrenaline. These are hydrogen ions, ammonium, magnesium, calcium and strontium.

6. Glucose decreases the inhibitory effect of adrenaline, and may convert its inhibitory effect into a stimulatory one. Iodoacetic acid has the opposite action.

7. Acetylcholine, eserine, pilocarpine in small concentrations increase the inhibitory action of adrenaline.
8. Ephedrine in small concentrations, potentiates the inhibitory effect of adrenaline. In large concentrations it may antagonise it.
9. Nicotine acts like other stimulatory substances.
10. Increase in osmotic pressure of the saline to twice normal increases the inhibitory effect of adrenaline. Decrease of osmotic pressure by about 20 p.c. has opposite action, but further decrease, increases the inhibitory effect of adrenaline.
11. Fatigue may convert the inhibitory action of adrenaline into excitatory one.
12. Alactic tone abolishes the inhibitory action of adrenaline; lactic tone converts the excitatory action into inhibitory one.

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