STIMULATION OF UNSTRIATED MUSCLE BY CHANGE OF TEMPERATURE AND OTHER EFFECTS OF TEMPERATURE

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THE reports on the effect of change of temperature on unstriated muscle are conflicting. Cooling may cause unstriated muscle to contract (Botazzi and Grunbaum, 1899; De Zilva, 1901; Starling, 1912; Roskam, 1920 *a*; Verbitzky, 1922; Evans, 1923; Botazzi, 1925; Phillips and Mckenzie, 1934; Hertzman and Roth, 1942; Singh and Singh, 1944 *b*; Perkins, *et al.*, 1950). Cooling may cause relaxation of unstriated muscle (Samkowy, 1874; Roskam, 1920 *b*, *c*; Verbitzky, 1922; Rao and Singh, 1940; Aschoff, 1943). Starling (1912) and Evans (1926) emphasize the fact that rate of cooling is a more effective stimulus than gradual cooling, though the latter also may be effective. The muscle may or may not accommodate to the change of temperature.

These conflicting results are due to the fact that unstriated muscle contains several variables, and the description of the effect of temperature is usually confined to one of these variables. The two main variables are contraction and inhibition. Inhibition is of two kinds; in one the relaxation is active and in the other it is passive. Contractions are of two kinds, tonic and phasic. Tonic contractions are of two kinds; one is susceptible to asphyxia and the other is resistant. The latter tonus is of two kinds; one is susceptible to inhibition and the other is resistant. The last is the basic tone found in all muscles (Singh and Singh, 1949 *a*, *b*; 1951). The twitch contractions are of four kinds produced respectively by alternating current, nervous stimulation, ions and drugs. Inhibition again is of two kinds, tonic and phasic (Singh, 1942). The twitch inhibition is also of four kinds produced respectively by electric current, nervous stimulation, ions and drugs. Singh and Singh (1949 *b*) have shown that agencies which depress excitability, cause contraction of unstriated muscle; cooling belongs to this category of stimulants.

In the present experiments, transverse pieces from frog's stomach muscle (Singh, 1939), from dog's stomach muscle (Singh, 1940) and fowl's gut (Singh, Singh and Muthana, 1947) were used. The muscles were stimulated with alternating current, 10 volts for 10 seconds, potassium or acetylcholine.

RESULTS

Change of temperature produces two kinds of responses. One group of them are functions of a particular temperature; the other group are functions of the rate of changes of temperature. Change of temperature may produce contraction or relaxation which may be temporary or permanent. The latter effects are due to change in the tonus of the muscle and the former effects may be tonic or phasic. To understand these tonic and phasic effects, the permanent effects of temperature or tonus have to be first considered.

Effect on tonus.—The permanent effects are variations in tonus and many of them have been described previously. They are a function of the temperature, and not of the rate of change of temperature.

If frog's stomach muscle is cooled to about 2° C., it relaxes, but dog's stomach muscle at first relaxes and then contracts; for these experiments an isotonic lever should be used. Frog's muscle mainly shows lactic tone, and dog's stomach muscle, alactic tone. Therefore the contractile system which requires energy from chemical stores, relaxes, and the other contractile system, which requires energy for relaxation, contracts due to the action of cold (Singh and Singh, 1949 *b*).

In dog's stomach muscle, the agencies that diminish metabolism, such as asphyxia, cyanide, iodoacetic acid and extreme cold, first cause the muscle to relax and then cause contraction; the relaxation is due to the action on the first contractile mechanism, and contraction due to action on the second contractile mechanism. In frog's stomach muscle the action is predominantly that of relaxation, as the second contractile system is feeble, though not entirely absent, as the above agencies may produce a feeble contraction (Singh and Singh, 1948). In dog's stomach muscle, the preliminary relaxation is sometimes absent, and relaxation on warming is active (Singh and Singh, 1949 *b*). When dog's stomach muscle is put under tension at low temperatures, on warming up to 25° C., it relaxes. On recooling its contraction is very feeble. An unloaded dog's stomach contracts when cooled (Singh and Singh, 1949 *b*).

In the frog's stomach muscle, the tonus curve shows a maximum at 20° and 45° C., the tone beginning to increase after 35° C. (Rao and Singh, 1940). Therefore if a muscle is cooled below 20° C. or warmed above 35° C. either gradually or suddenly it may show a tonic contraction. The tonus at 20° C. is susceptible to asphyxia; therefore, it is lactic tone. This is supported by oxygen consumption experiments (Rao and Singh, 1940). The contraction above 35° C. is also lactic tone, as it is susceptible to asphyxia and is diminished by iodoacetic acid. If some of the sodium of the saline is replaced with potassium or the chloride with other stimulating anions, such as bromide, nitrate, iodide, thiocyanate, then the tone increases continuously with temperature, as these substances antagonise adaptation and the decline of tone after 20° C. being due to adaptation (Rao and Singh, 1940). The tonus produced by these anions is lactic tone, as it is diminished by asphyxia. Thus frog's muscle predominantly shows lactic tone.

Mytilus muscle also shows maximum at about 15 to 20 and 45° C. Dog's stomach muscle behaves differently. If it is put under stretch, so that it exerts tension at about 10° C., and is then gradually warmed, it relaxes upto 25–30° C.; thereafter it begins to contract. The tone at high temperatures is not susceptible to asphyxia, and may pass into that stage when it is not susceptible to inhibition. So these are the reactions of alactic tone. Lactic tone can be induced in dog's stomach muscle by replacing some of the sodium of the saline with potassium or barium, or by replacing the chloride with other anions such as iodide, thiocyanate. Tonus then shows a maximum at about 30° C.; it may continue to increase up to 37–40° C., when it passes into the alactic stage (Fig. 1). In the fowl's gut and human appendix, the tone continues to increase with temperature up to 37–40° C., and is susceptible to asphyxia (Singh, Singh and Muthana, 1947; Khan and Singh, 1947).

Some of the above effects on tonus of unstriated muscle have also been observed on isolated proteins. Varga (1946) has observed that actomyosin threads shorten maximally at 16–18° C. Other similarities between isolated proteins and muscle have been described previously (Singh, 1943; Singh and Singh, 1951). Astbury and Dickinson (1940) have shown that isolated strips of myosin from unstriated muscle show thermal contraction below 40° C.

Optimum temperature for twitch responses.—This has been described by Winton (1926) and others (Singh, 1940; 1942, 1944 *a*; Rao and Singh, 1940; Narayana and Singh, 1944, Singh, Singh and Muthana, 1947; Khan and Singh, 1947). In frog's muscle it has been found, that the optimum temperature is a function of the surrounding temperature, that is, the tissue exhibits adaptation or acclimatisation; this is found in mammalian and avian muscle also to some extent, as shown in the above papers.

Frog's stomach muscle was stimulated by alternating current (8 volts for 10 seconds every 10–15 minutes), potassium (20 p.c. of the sodium of

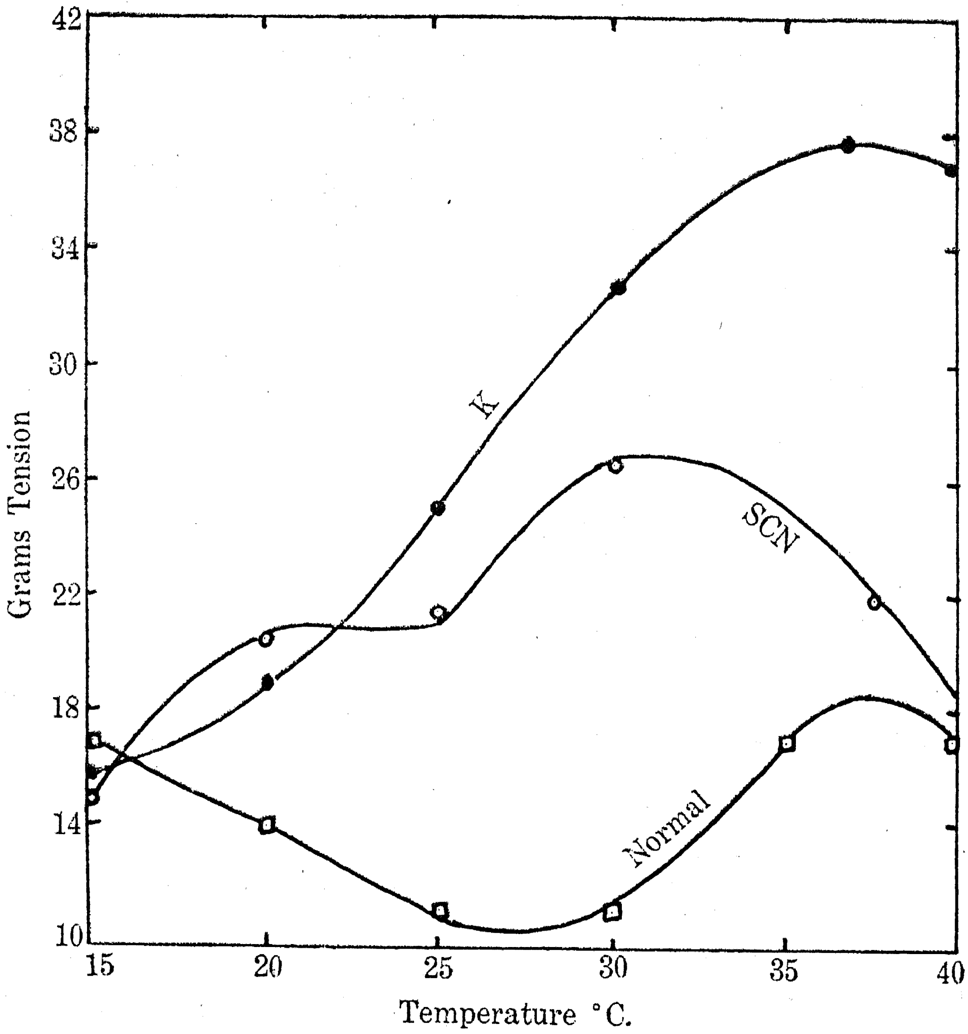

FIG. 1. Dog's stomach muscle. Effect of temperature on normal tone and tone produced by potassium (40 p.c. of the sodium of the saline replaced with potassium) and thiocyanate (chloride of the saline replaced with thiocyanate).

the saline being replaced with potassium) and acetylcholine (1 in 5,000). Two methods were employed; in the first method, the temperature was gradually raised to 30–35° C. and then gradually lowered back agian. In the second method, the temperature was raised gradually and then suddenly lowered to the original value, and after an hour or so the observations were repeated.

When the temperature is increased, the response to alternating current at first increases and then declines (12 experiments); when the temperature is lowered, the opposite happens (Fig. 2). This shows that the muscle tries to counteract the effect of temperature. Increase of temperature would hasten the chemical processes, and so increase the response, but an inhi-

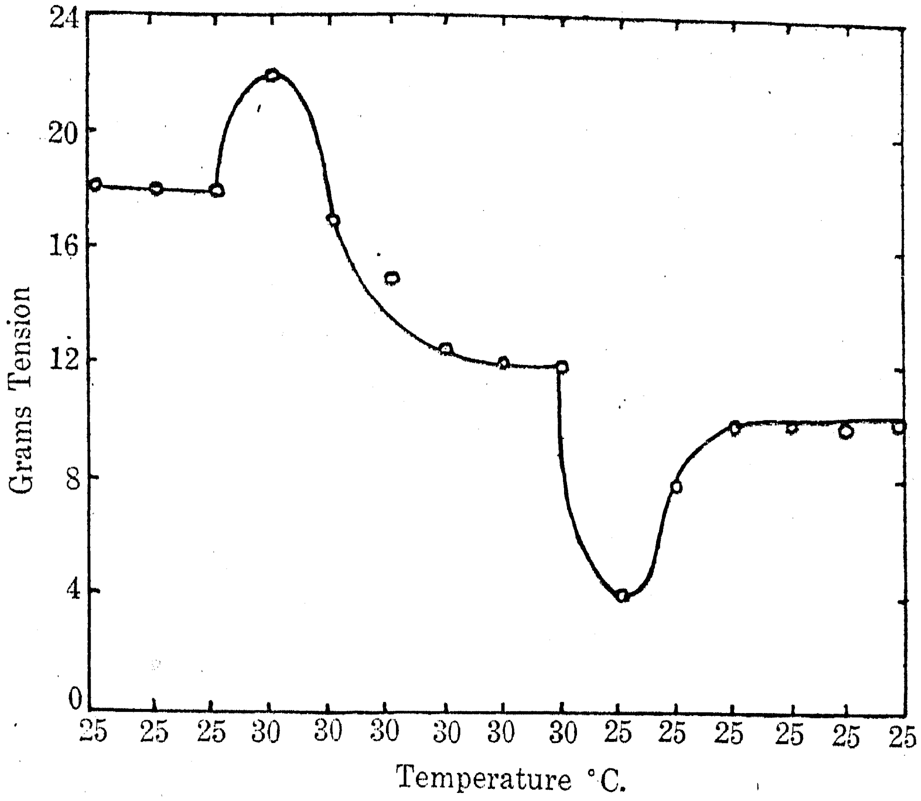

FIG. 2. Frog's stomach muscle. Effect of temperature on the response to alternating current, 6 volts in 10 seconds every 15 minutes.

bitory influence sets in to oppose this action. With decrease of temperature the chemical processes would diminish, but some accelerating influence sets in so that the muscle recovers.

The above temporary increase of excitability on increase of temperature can be produced by increase in the concentration of calcium in the saline by about 3 to 6-fold, and the opposite effects can be produced by decrease in the concentration of calcium. Increase of temperature appears to liberate calcium (Singh, 1944 *a*; Rao and Singh, 1940). It is probable that in protoplasm, the dissociation of calcium is a function of temperature. The above increase in excitability can be produced to a less extent by increase of potassium and sodium ions in the saline, potassium about two-fold and sodium by about 40 p.c. by addition of sodium chloride to the saline, but the opposite effect has not been observed with these ions, so that the change in excitability due to change of temperature are probably a calcium effect.

If the temperature of frog's stomach muscle is gradually increased, then again gradually lowered, the optimum temperature may increase (Fig. 2). This especially happens if the tmeperature is raised high, such as 35° C. The cause of this shift appears to be adaptation, as shown by the following experiment. The temperature is at first gradually increased as before, but instead of decreasing the temperature back again to its original value gradually, it is done suddenly and the muscle is allowed to remain at this temperature for about an hour or so. It is then found that the optimum temperature retains its original value. Six experiments were performed with acetylcholine and four with potassium; the alterations in excitability were noted as with alternating current.

In dog's stomach muscle, the optimum temperature for twitches produced by alternating current, potassium, acetylcholine, nervous stimulation and lactic tone, is about 25–30° C.; for alactic tone it is about 37–40° C.

Optimum temperature for asphyxial hyperexcitability.—The optimum temperature for the asphyxial hyperexcitability to alternating current in frog's stomach muscle is 30° C. (Singh and Singh, 1948). In dog's stomach muscle, the optimum temperature for asphyxial hyperexcitability to alternating current is 20–30° C.; that for potassium and tone it is 20° C. (Singh and Singh, 1949 *a*). The metabolic mechanisms for the responses to these two stimulants during asphyxia are therefore different. The fact that the optimum temperature for asphyxial hyperexcitability to alternating current can also be 20° C., that is the same as that for potassium, indicates that a common metabolic mechanism may subserve the response to both potassium and alternating current during asphyxia. The optimum temperature for asphyxial hyperexcitability should be kept in mind, as it may not be obtained at 37° C., the temperature at which mammalian muscles are usually studied.

Rhythmic stimulation by increase of temperature.—Increase of temperature produces a very interesting effect. If the temperature of the saline is gradually raised at the rate of $\frac{1}{2}$ to 1° C. per minute from 15 to 20° C., or from 20 to 30° C., or from 30 to 37° C., dog's stomach muscle executes powerful rhythmic contractions, about one per minute, and lasting for about 10 minutes, before it settles down to a steady state during which the rhythmic contractions are only about 5 to 10 p.c. of the previous ones (Fig. 3). Same phenomenon is produced by alternating current with about 10 volts per second every minute. The above contractions are produced by change of temperature as shown by the fact that they are absent if the temperature is raised very gradually. about 1° C. in about 10 minutes, the minimum rate at which the temperature produces the rhythmic state being about 2° C. rise in 10 minutes (6 experiments). This is also shown by the fact, that if the rhythmic contractions are not produced by rise of temperature at the rate of 1° C. per minute, they may be produced if the rate of rise of temperature is

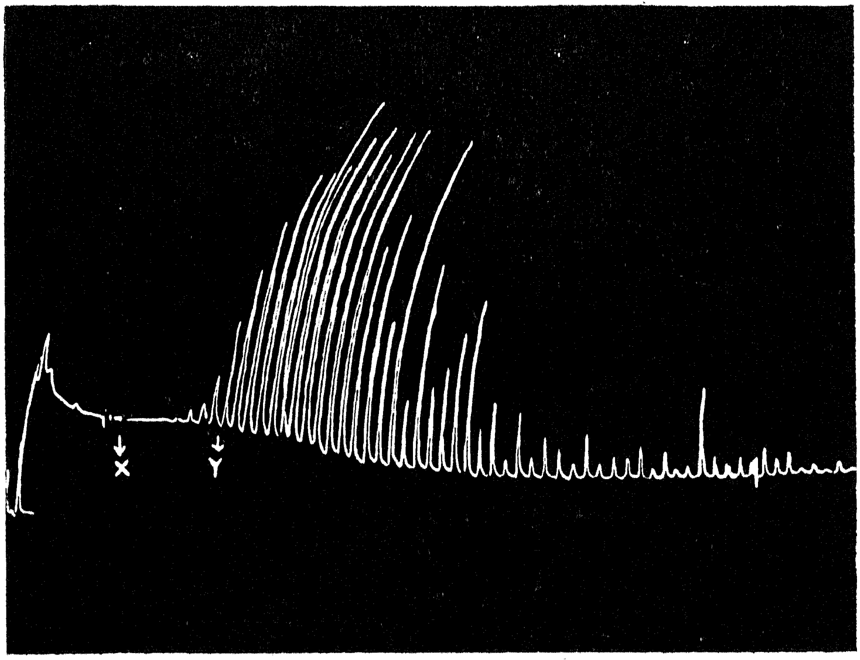

FIG. 3. Dog's stomach muscle. Rhythmic stimulation of dog's stomach muscle by increase of temperature from 15° C. to 25° C. at the rate of 1 °C. rise per minute. Warming begins at X and finishes at Y.

more rapid, about 2° C. per minute. These contractions are increased by 0·1 p.c. glucose. These rhythmic contractions are not the same as those produced in frog's muscle, as no corresponding decrease in rhythmic contractions have been noticed in dog's stomach muscle on decrease of temperature.

The above rhythmic contractions resemble those produced during asphyxial hyperexcitability, or when glucose is added to a muscle which has been asphyxiated or treated with cyanide. Glucose produces a temporary increase in excitability (Singh and Singh, 1949 *c*, 1949 *d*). These experiments suggest that during asphyxia, or increase of temperature a new metabolic mechanism is brought into action.

Temporary contractions.—Besides the contractions which may result by change of temperature described above, other tonic and twitch contractions are also produced.

Change of temperature produces a twitch contraction in dog's and frog's stomach muscle (Fig. 4). Both sudden heating and cooling produces the contraction. The muscles show wide variations in excitability to change of temperature. Some muscles do not show any response, whilst others are quite sensitive. With dog's stomach muscle, the

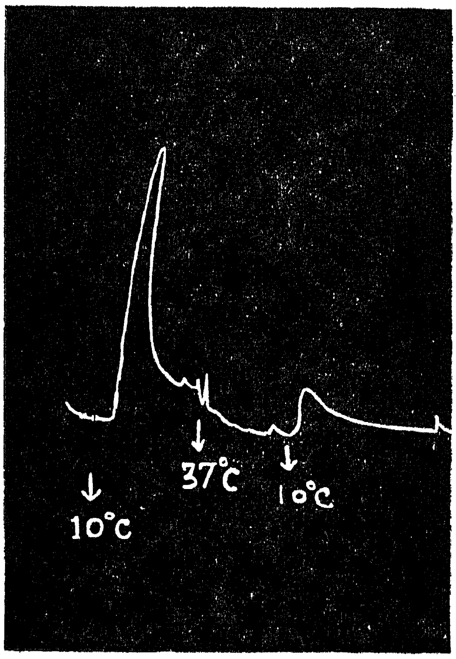

Fig. 4. Dog's stomach muscle. Twitch contraction produced by sudden cooling. Initial temperature 25° C.

sensitivity is greatest in freshly dissected muscles, as happens with excitability to other forms of stimulation. This is probably due to the fact, that with keeping glycogen breaks down into lactic acid and thus the energy reserves are diminished (Bharadwaj and Singh, 1951).

The muscle may be stimulated by different variations of temperature. Thus it may be cooled from 20 to 5° C., or 35 to 20° C. and also warmed similarly. Generally the muscles are more sensitive to cooling, though at other times they are more sensitive to warming. The sensitivity to cooling and warming may vary in opposite directions. If a certain change of temperature does not cause contraction, a greater change may be effective. Relaxation of contraction produced by warming is much rapid than that produced by cooling. If the muscle is stimulated by warming, it usually gives the maximum response if the temperature is raised from 15° C. (6 experiments). If the muscle is cooled below 15° C., then the response declines (Fig. 5). This is due to the fact, that excessive cooling lowers the excitability of the muscle, so that though change in temperature causes stimulation, to do so, the muscle must be excitable. The muscle may adapt to low temperature so that with frequent cooling it may become more excitable; then the muscle may be cooled below 15° C. (experimental range, 5° C.), and then warming causes a maximum response. Similarly if stimulated by cooling, the response may decline if the muscle is cooled below a

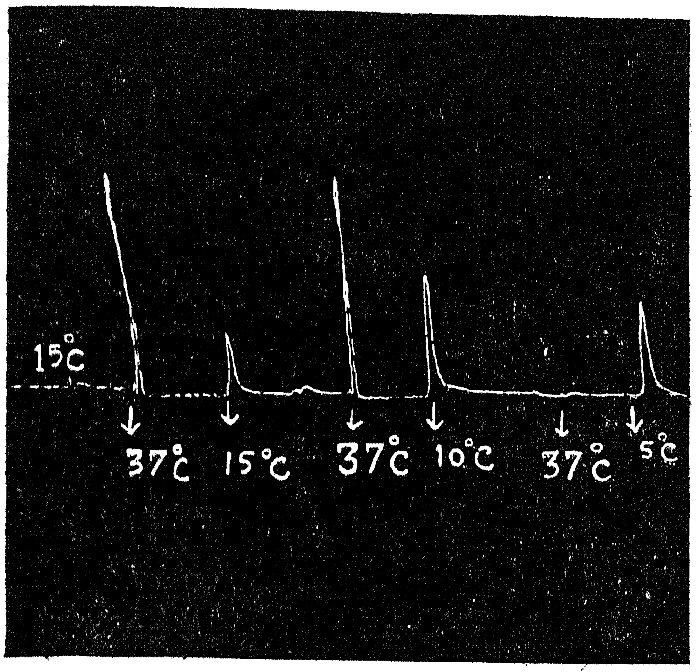

FIG. 5. Dog's stomach muscle. Contractions produced by sudden warming and cooling. Initial temperature 15° C.

certain temperature (in dog's muscle, 10° C.) as found by Perkins, *et al.* (1951). This then will be due to depression of excitability by excessive cooling. In hyperexcitable muscles, the muscle may be cooled below 10° C., without decline of the response.

Though a tonic contraction may not be produced on cooling, after a twitch due to cooling, there may be left residual tone on subsidence of the twitch contraction.

Tonic contraction is also produced on change of temperature in those muscles which show lactic tone, such as frog's stomach muscle (Fig. 6). In dog's stomach muscle which shows alactic tone, lactic tone can be induced by potassium, or anions such as iodide, thiocyanate, and the muscle then shows a tonic contraction on change of temperature.

Cause of thermal contraction.—One way in which change of temperature acts is to lower the threshold of excitability to ions outside the muscle fibres; the change of temperature sensitises the muscle to ions, as is done by stretching, drugs, touching and electrical stimulation (Singh, 1938 *a*, *b*, 1944 *b*, 1945; Singh and Singh, 1947). This is shown by the following experiments: (1) Simultaneous excitation of dog's or frog's muscle by heating or cooling and potassium potentiates the response to the latter (Fig. 7). (2) If the muscle is inexcitable to potassium, then sudden heating

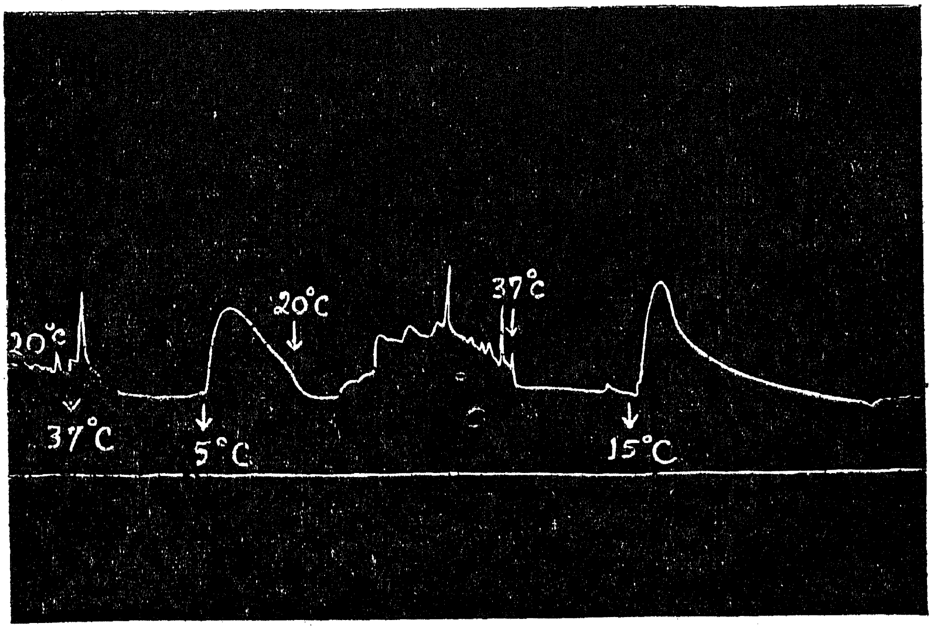

FIG. 6. Frog's stomach muscle. Tonic contraction produced by cooling.

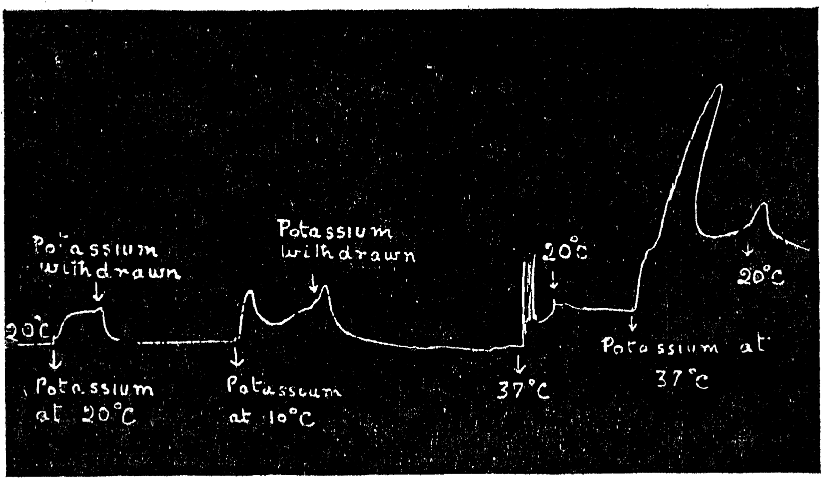

FIG. 7. Dog's stomach muscle. Normal response to potassium at 20° C. (20 p,c. of the sodium of the saline replaced with patassium). Addition of potassium saline the temperature of which was 10° C. Initial temperature 20° C. Potassium removed. Temperature of saline increased to 37° C. Saline added at 20° C. Potassium saline at 37° C added. Note that both cooling and warming potentiate the response to potassium.

or cooling enables potassium to act, and produces a tonic contraction in dog's muscle (Fig. 8). (3) The response to change of temperature in dog's stomach muscle is potentiated by excess of calcium, as is the response to potassium (Singh, 1940).

For the production of thermal contractions, the excitability of the muscle must be normal. Thus excess of potassium in frog's muscle

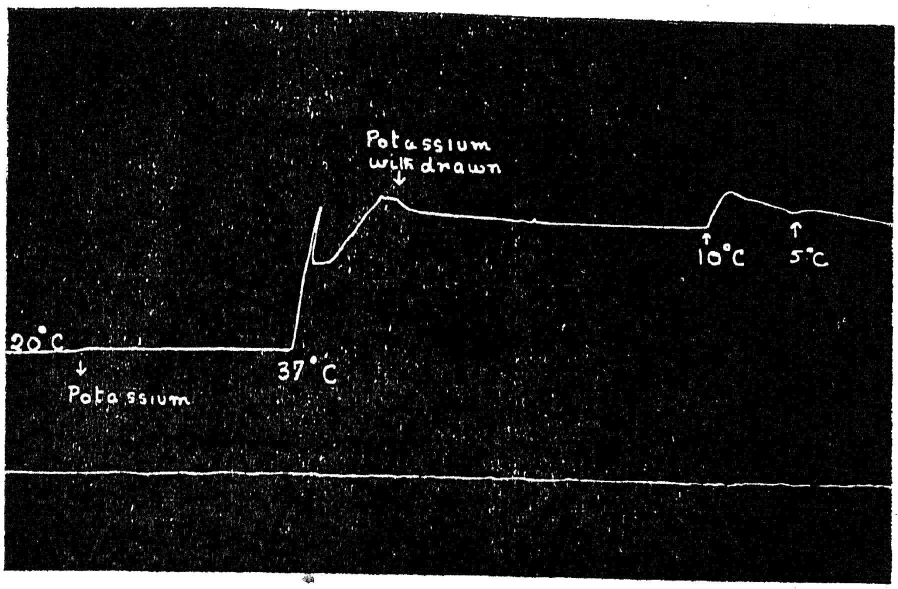

FIG. 8. Dog's stomach muscle. Potassium added at initial temperature of 20° C Temperature then suddenly increased to 37° C. Potassium withdrawn and temperature decreased to 10 and 5° C. (20 p.c. of the sodium of the saline replaced with potassium to cause stimulation).

depresses excitability and also the thermal response; in dog's muscle, excess of hydrogen ions beyond pH 6·5 depress the response. The twitch contractions are susceptible to asphyxia, and the tonic contractions in excess of potassium described above may or may not be susceptible, as happens with the potassium contraction.

In the guinea pig's uterus, most agencies that cause excitation in other muscles cause inhibition (Singh, 1942). It is interesting to note, therefore, that in this muscle change of temperature may produce an inhibitory twitch. The pH of the saline was 8 (borate buffer) and temperature was increased from 30° C. to 38° C. suddenly or lowered from 38° C. to 25° C. As the tone declines with decrease of temperature, the inhibitory twitch on sudden cooling is followed by slow decline of tone, so that the sequence of events on sudden cooling are as follows: first, there is relaxation and then contraction which together constitute the inhibitoty twitch; this is followed by slow decline of tone. Greater cooling tends to cause contraction, though in some muscles an inhibitory twitch is produced, even if the temperature is suddenly lowered from 38° C. to 5° C.

DISCUSSION

Change of temperature probably affects the muscle membrane, making it more permeable or affects its calcium concentration. The increased sensitivity to ions induced by change of temperature, is similar to that induced by change in the calcium concentration of saline, by change of tension or by drugs. It is known that mere presence of ions outside the muscle membrane is not sufficient to cause excitation. Thus *Mytilus* muscle may be immersed in a barium-rich saline, without any contraction resulting. Barium will produce its typical response if the muscle is suddenly stretched or released, or if calcium is excluded from the saline, or if electric current is passed for a second, or if drugs such as adrenaline or acetylcholine are added. Similarly, potassium can be present in the saline without stimulating dog's muscle, but stimulation occurs on sudden change of temperature, or by the above procedures.

The ions normally present in the saline in predominant numbers are the sodium or chloride ions, and these are likely to cause excitation. Both inside and outside the muscle there are inhibitory or excitatory ions. Excitation can be caused if the action of inhibitory ions is suppressed or if the action of excitatory ions is increased. Cooling, therefore, may suppress the action of inhibitory ions, thus causing excitation (Singh and Singh, 1949 *b*), and heating may increase the action of excitatory ions; the latter is actually shown to occur in Fig. 8.

During change of temperature, ions from the saline probably move into an outer zone to cause excitation (Singh, 1944 *b*).

SUMMARY

1. Temperature produces two kinds of effect on unstriated muscle. One of these is a function of a particular temperature and the other is a function of the rate of change of temperature. The former produces changes in tonus, and the latter produces both tonic and phasic effects.

2. Excessive cooling increases tonus of muscles showing alactic tone, and causes relaxation of muscles showing lactic tone.

3. Temperature affects lactic and alactic tones differently. Lactic tone increases with temperature up to 30–37° C. Alactic tone at first decreases with temperature up to 25–30° C. and then increases.

4. The optimum temperature for tonus and twitch responses is different.

5. Frog's stomach muscle shows adaptation to temperature.

6. In dog's stomach muscle, the optimum temperature for asphyxial hyperexictability is 20° C. for potassium, and 20 to 30° C. for alternating current.

7. Increase of temperature at the rate of 2 to 10° C. per minute causes a temporary rhythmic stimulation of dog's stomach muscle. This rhythmic

stimulation resembles similar stimulation by asphyxia or by glucose in an asphyxiated muscle.

8. Change of temperature at certain rate produces both a tonic and phasic contraction of unstriated muscle.

9. Change of temperature sensitises the muscle to ions outside the muscle fibres.

10. Cooling and warming may cause contraction by direct action on the contractile mechanism.

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