THE HEAT-PRODUCTION AND THE MECHANISM OF THE VERATRINE CONTRACTION. By W. HARTREE[1] AND A. V. HILL.

(*From the Physiological Laboratories of Cambridge and Manchester.*)

THE remarkable action of veratrine on skeletal muscle has been the subject of many investigations, but its mechanism remains obscure. The following pages show that, whatever that mechanism, the liberation of energy in the veratrine contraction bears, qualitatively and quantitatively, the same relation to the mechanical response as it does in an ordinary prolonged contraction produced by a succession of shocks.

It was shown by Fick and Böhm (1) in 1872 that the veratrine contraction, in response to a single shock, causes a production of far more heat than the normal twitch. They concluded that the prolonged veratrine contraction is due to a greater intensity of the "chemical processes," and not simply to a slowing of the "restitution processes." They showed also that a short tetanus leads to a greater production of heat than a single shock, as in ordinary muscle. They approached the question of whether the prolonged veratrine contraction is of a tetanic nature, by laying the nerve of a second muscle-nerve preparation upon an excited veratrinised muscle; the absence of any response in the former led them to conclude that the veratrine contraction is not of an oscillatory character. This question has been the subject of many later investigations. Hoffmann (2) found that muscles subjected to weak doses of veratrine show an electric response of a pronounced oscillatory character, as does a tetanised, or a voluntarily innervated, muscle: while muscles treated with stronger doses show a smooth, non-oscillatory electric response, as well as a continuous mechanical one. In our investigation we have employed only solutions which, in Hoffmann's nomenclature, would be "strong."

In an ordinary prolonged isometric contraction, induced by a rapid succession of shocks, we have shown (3, p. 144) that, after a very short interval during the development of the contraction, the *rate* of heat-production (called here for simplicity the "heat-rate") is proportional

[1] Working for the Medical Research Council.

to the force maintained. Expressing the heat-production in work units (grm. cm.), in order to make it directly comparable with the product Tl, T being in grms. wt. the force maintained, and l in cm. the resting length of the muscle, we showed (3, p. 147) that, at 15° C., in the sartorius muscle of the frog (*Rana temp.*), the ratio (heat-rate)/Tl, rapidly attains the constant value 0·61. We argued that a prolonged stimulus causes a steady production of lactic acid, which is "removed" at a rate depending upon its concentration, the mechanical response being due to the momentary presence of the free acid in the neighbourhood of certain sensitive structures in the muscle: in this way the force maintained, once a steady state is reached, will be proportional to the *rate* at which the lactic acid is being formed and removed, *i.e.* to the heat-rate.

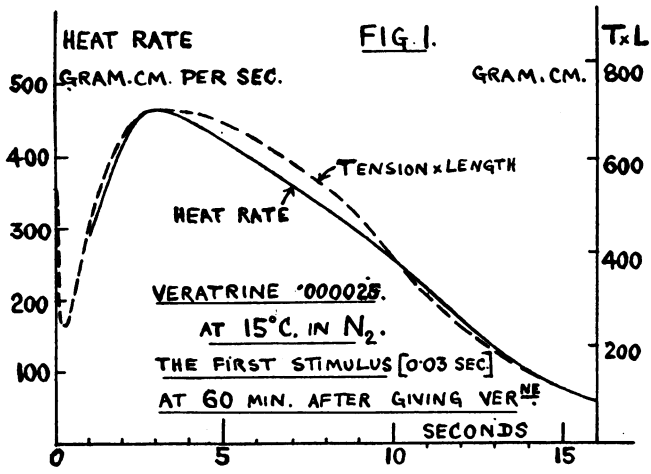
It was possible that veratrine might act at one or other of several different points in the mechanism: it might for example delay the "removal" of the acid either (*a*) by slowing the normal chemical processes of relaxation, or (*b*) by rendering the sensitive structures of the muscle abnormally receptive to lactic acid, and so much slower in parting with it: or, on the other hand, it might cause the liberation of acid, once started by a shock, to proceed unchecked by the normal "escapement" which limits the liberation of acid and energy in a twitch. Now anything which acts upon the rate of "removal" of the acid must affect the ratio (heat-rate)/Tl: the "efficiency" with which a prolonged contraction is maintained, which is the inverse of this ratio, is increased by anything which slows relaxation, *e.g.* by a fall of temperature or by fatigue (3, p. 148). Hence, if by any means veratrine were to slow the normal processes of relaxation, the ratio (heat-rate)/Tl should be far smaller than normally: the maintenance of the contraction should be far more efficient. If, on the other hand, the action of veratrine be, either to produce a tetanus or to abolish, in whole or in part, the normal check to an unlimited and continual breakdown in response to a shock, leaving the physico-chemical nature of the mechanical response to that breakdown, and of relaxation, quite unaltered, then we should expect to find, during the prolonged isometric contraction of a veratrinised muscle, (*a*) a prolonged production of heat as Fick and Böhm found, (*b*) that the curves of heat-rate and of tension should run parallel, and (*c*) that the ratio, (heat-rate)/Tl, should have the same absolute value as in a prolonged contraction produced at the same temperature by a succession of shocks. All three of these expectations have been verified.

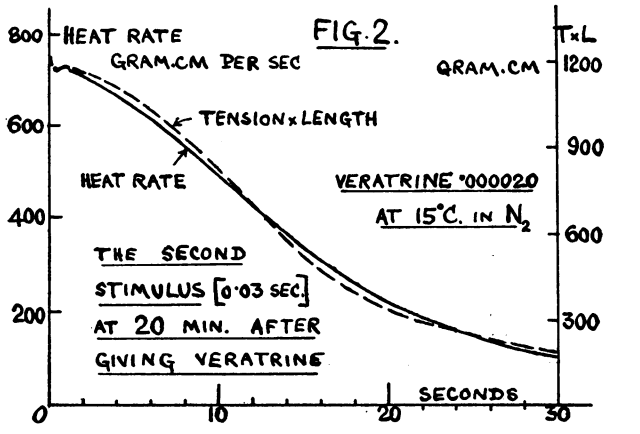
Method. A pair of sartorius muscles of *Rana temp.* was mounted on a thermopile, and subjected to a small initial tension, and the course

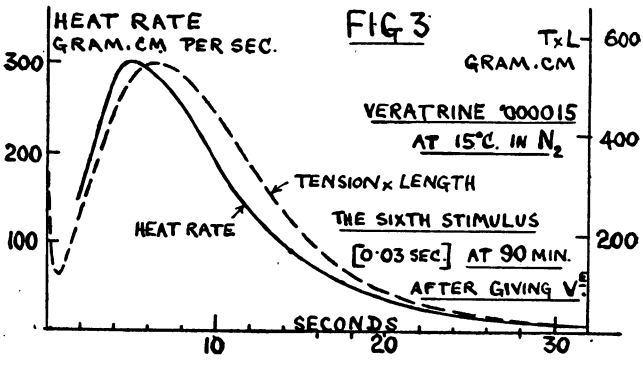
of the heat-production after a stimulus found by analysing the photographic curves of galvanometer deflection, as described previously (4, p. 102). Most of the experiments were made at 15° C., the muscle being in nitrogen which had been freed from oxygen by passing it through two bottles of alkaline pyrogallol: in this way, heat-production due to oxidative recovery is entirely eliminated. A suitable interval for the analysis was found to be 1 sec. during the first 20 secs. of the contraction. After that a 5 sec. interval could be used without loss of accuracy. The tension was recorded on a smoked drum by a tension lever, the contractions being practically isometric.

After a few preliminary stimuli, to determine, in the normal muscle, (*a*) the strength of the maximal stimulus (always a short tetanus of 0·03 sec.), and (*b*) the corresponding heat-rate and tension curves, the veratrine solution was introduced into the chamber, and left in for four or five minutes, after which it was blown out by nitrogen. The strength of the veratrine, made up in Ringer's solution, was usually ·002 p.c. or less: with stronger solutions the galvanometer zero was very unsteady for an hour or more, and in this case, as is well known, see (5), the first tension curves made show a sharp fall immediately after the initial quick response followed by a subsequent rise, little, if any, greater than that due to a very short tetanus. With weaker solutions the galvanometer zero was steady and records could be taken about a quarter of an hour after the veratrine solution had been removed. In order to record the relatively enormous heat-production of the veratrine contraction, the deflection was reduced by the introduction of a resistance about 15 times that of the thermopile and galvanometer together, the sensitivity of the galvanometer itself being left unchanged. Control curves were made on the dead muscle and the analysis conducted as usual.

Results. It is known that, with relatively weak solutions of veratrine, the contraction is very similar, in height and form, to a tetanus obtained by discontinuous stimulation (Fig. 2): whereas, with stronger solutions, a sharp twitch is found preceding the prolonged contraction (Fig. 1). With weaker solutions, the first one or two contractions are of the simple prolonged form; in later contractions, however, the initial twitch, followed by the later prolonged rise, is pronounced (see Fig. 3, which shows a sixth contraction). The unsteadiness of the galvanometer, together with the spontaneous fibrillation which Prof. Langley informs us is often visible, in a muscle treated with a strong solution of veratrine, may be regarded as an exaggeration of the weakening of normal control of breakdown caused by weaker solutions. Such spontaneous breakdown


HEAT RATE
GRAM.CM. PER SEC.
FIG. 1.
T×L
GRAM.CM.
500
400
300
200
100
800
600
400
200
TENSION × LENGTH
HEAT RATE
VERATRINE ·000025.
AT 15°C. IN N_2.
THE FIRST STIMULUS [0·03 SEC.]
AT 60 MIN. AFTER GIVING VERINE
SECONDS
0
5
10
15


HEAT RATE
GRAM.CM PER SEC
FIG. 2.
T×L
GRAM.CM
800
600
400
200
1200
900
600
300
TENSION × LENGTH
HEAT RATE
VERATRINE ·000020
AT 15°C. IN N_2
THE SECOND
STIMULUS [0·03 SEC.]
AT 20 MIN. AFTER
GIVING VERATRINE
SECONDS
0
10
20
30


HEAT RATE
GRAM.CM PER SEC.
FIG 3
T×L
GRAM.CM
300
200
100
600
400
200
VERATRINE ·000015
AT 15°C. IN N_2
TENSION × LENGTH
HEAT RATE
THE SIXTH STIMULUS
[0·03 SEC.] AT 90 MIN.
AFTER GIVING V.E.
SECONDS
10
20
30

would have the same effect as artificially induced activity, in changing the character of the response: consequently the first contraction of a muscle subjected to a strong solution is like the later one of a muscle treated with a weaker.

On comparing the heat-rate with the tension at any moment, it is found that, throughout the contraction, and certainly as a first approximation, the two are definitely proportional. See Figs. 1 to 3, in which the heat-rate and the product Tl are plotted throughout in the same units of energy (grm. cms.), to such scales that their maxima coincide: in this way the close agreement of the two curves is convincing evidence that, in a veratrinised muscle, the prolonged contraction is accompanied by a proportional prolonged heat-production.

When, as in Fig. 3, the agreement between the curves of heat-rate and of Tl is not quite exact, it is found that the fall of the latter curve tends to lag behind that of the former: this divergence is more pronounced when the muscle has been fatigued by earlier contractions. We have shown elsewhere (3, p. 148), that any factor, such as fatigue, which tends to slow the processes of relaxation, results in a diminution of the ratio, (heat-rate)/Tl, and if the slowing effect of fatigue on the "removal" processes be considered to occur during the single veratrine contraction, it is natural that the tension curve should fall less rapidly than that of heat-rate. Any want of absolute agreement between the curves of heat-rate and of tension, is probably due to this effect of oncoming fatigue.

In the figures, the quantities plotted are expressed per grm. of muscle.

As regards the absolute value of the ratio, (heat-rate)/Tl, its average during relaxation in the *third* contraction in five different experiments at 15° C., was as follows:

0·60, 0·53, 0·54, 0·61, 0·60; Mean, 0·58;

the unit being sec^{-1}.

For the first stimulus (which was not always well observed, owing to the unknown magnitude of the expected galvanometer deflection) the corresponding number was about 0·63, whereas for the fifth stimulus (in which the tension was sometimes too small to be well observed) it was about 0·53. Here again the effect of fatigue is to diminish the ratio. The higher values compare very exactly with those found previously by ourselves at 15° C. (3, p. 147) for the case of a prolonged tetanic contraction, the mean deduced from a number of observations made by another method being about 0·61. There can be no doubt therefore that the efficiency with which the prolonged contraction is maintained is almost exactly the same in the veratrine contraction as in the normal tetanus.

As a verification of the method one set of experiments was carried out in oxygen at 15° C., giving a long tetanus either of diminishing or of

constant strength, to a muscle untreated with veratrine, and completing the analysis as in the veratrine experiments. In this case also it appeared that the heat-rate throughout is very nearly proportional to Tl, and that about the same absolute value of the ratio is obtained.

We see therefore that the action of veratrine is limited to its effect upon the extent and duration of the liberation of energy, and presumably therefore of lactic acid, following a stimulus. In the normal twitch at 15° C. the liberation of energy is exceedingly rapid: even at 10° C., as we have shown (4, p. 112), it appears to be complete within 0·2 sec., although in a twitch some of the energy is stored in the potential mechanical form, and reappears after a short interval during relaxation. There is clearly some passage of escape, for the energy and lactic acid, which is opened by a shock and which, in normal muscle, rapidly closes again. This "passage" may not be of a mechanical or a structural nature, though we have supposed (3, p. 140), for the sake of definiteness, that it is opened and closed by a momentary change in the permeability of some membrane within the fibre. The "passage" can remain open, in a normal muscle, only in consequence of a rapid succession of shocks. In the muscle subjected to a large dose of veratrine the instability of the galvanometer zero shows that the "passage" is so nearly open that small spontaneous outbursts of energy may occur. In the muscle subjected to a weaker dose, the "passage," once opened by a shock, appears to remain open for some time, allowing considerable quantities of lactic acid to pour out, with a prolonged development of tension and heat. In very weak doses the oscillatory nature of the electric response (Hoffmann) suggests that the passage is opened discontinuously by something of the nature of a tetanus. According to Lamm (6), the action of veratrine resides in a change which it produces in the permeability of some membrane, since the effect of veratrine may be largely antagonised by that of calcium salts. Such evidence of course is only circumstantial, and the description of the regulating mechanism itself as a membrane is hypothetical, though possibly useful as giving definition to the undoubted fact that some regulating mechanism certainly exists, cutting short and controlling the liberation of energy following a single shock. Our experiments show that the effect of veratrine is to be ascribed to its action on this regulating mechanism, causing the liberation of energy, for some reason or another, to be much more extensive and prolonged.

SUMMARY.

In the prolonged isometric contraction of a veratrinised muscle there is a prolonged evolution of heat, the rate of heat-production being proportional throughout to the force maintained. The absolute value of the ratio, (heat-rate)/(force) × (length), is the same as that found in the ordinary prolonged contraction set up by a tetanic stimulus. These facts show that the effect of veratrine cannot be ascribed to a slowing of relaxation, or of the chemical processes by which the acid which excites the mechanical response is removed from the site of its activity; it can only be supposed that it puts out of action, more or less completely, the regulating mechanism by which the duration and extent of the liberation of energy (and lactic acid) following a single shock, are limited and controlled.

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