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THE MECHANISM OF AFTER-DISCHARGES CAUSED BY VERATRINE IN FROG'S SKELETAL MUSCLES

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The veratrine alkaloids seem to affect a wide range of electrically excitable cells in the same way (Krayer and Acheson, 1946). A single stimulus, which in normal circumstances causes a single response, will in the presence of veratrine give rise to a prolonged after-discharge or series of repeated action potentials. The production of after-discharges by the veratrum alkaloids has been observed in peripheral nerve (Feng, 1941), in skeletal muscle (Kuffler, 1945) and in conducting tissue of mammalian ventricle (Goldenberg and Rothberger, 1936). To produce an after-discharge it is not necessary that the whole length of an electrically excitable cell should be bathed in veratrine solution; local application of the alkaloid followed by excitation of any portion of the cell's membrane is sufficient to produce a train of action potentials in response to a single stimulus (Wible, 1924). In skeletal nerve-muscle preparations, it appears that the regions most sensitive to the drug's action are the terminal branches of the motor nerve and the muscle fibres. The frog's sartorius nerve-muscle preparation will give repetitive responses to single motor nerve volleys in the presence of a veratrine concentration of approximately 10^{-7}. The frog's sciatic nerve trunk, however, requires soaking for one hour in a concentration of 10^{-5} veratrine before after-discharges can be produced reliably (Feng, 1941). The action of the drug at motor-nerve terminals does not seem to be due to interference with the normal mechanisms of humoral transmission. Moreover, the muscle fibre itself seems to be equally sensitive to the drug in all regions down its length (Kuffler, 1945). In skeletal muscle, as in peripheral nerve (Graham and Gasser, 1931), veratrine seems to cause a delay in repolarization following an action potential. It is during this process of delayed repolarization following the first driven action potential that the repetitive after-discharge occurs. Kuffler (1945) concluded that this maintained, partial depolarization of the muscle fibre was directly responsible for a state of membrane instability which could give rise to repeated action potentials. He says, "All the evidence available indicates that the effects of the negative after-potentials are analogous to those of cathodal currents applied artificially to nerve or muscle fibres."

Recent experiments with the cat's cerebral cortex (Burns, 1954, 1955) have shown that there is a system of cells in the cortical grey-matter which will normally give a prolonged series of after-discharges following a few driven responses. The experimental results lead to the conclusion that this cortical after-discharge occurred because the different parts of the cells involved did not repolarize at the same rate following their driven activity. It was postulated that after an action potential one end of the type-B neurones (Burns and Grafstein, 1952) repolarized some ten times more slowly than did the other. During this *differential repolarization*, current must flow from the more rapidly repolarizing end of the cells to the other end and this current may be sufficient to cause the development of an action potential. Following the first "spontaneous" or undriven action potential the recovery process is set back a step and the same cycle of events may be repeated, thus giving rise to a series of after-discharges. It was pointed out (Burns, 1955) that the mechanism of differential repolarization might play some part in causing the after-discharges produced by veratrine. This interpretation does not differ seriously from that proposed by Kuffler and quoted above, but would specify the analogy between the negative after-potentials due to veratrine and the effects of applied cathodal currents a little more precisely. Forced uniform depolarization of the cell's surface may lead to an instability of membrane potential and consequent development of a stationary "membrane action potential" (Hodgkin and Huxley, 1952). Alternatively, a local cathode may give rise to transmitted action potentials because it depolarizes

one section of cell membrane *relative* to neighbouring membrane beyond the reach of the polarizing current. In this case the final breakdown of membrane potential would be associated with current flow between normal and cathodally polarized membrane.

Both the mechanisms for after-discharge from veratrinized cells presuppose a persistent depolarization of the membrane, but one supposes that universal, partial depolarization is a sufficient cause for after-discharge, while the other postulates that there must be a depolarization gradient (or differential repolarization) down the length of the cell before repetitive action potentials can occur. We undertook the experiments described below in order to find the extent to which each of these two postulated mechanisms contributed to the formation of the after-discharge. It will be realized that if differential repolarization is the major cause of repetitive response, then the local application of veratrine should be far more effective in producing after-discharge than its universal application to the whole cell. Most of the techniques developed have therefore been concerned with making this comparison.

METHODS

The Preparation.—The sartorius muscle of the frog was used in these experiments. The muscle was first dissected free from the animal and then pinned out on a board. Small strips were cut from the muscle under a dissecting microscope so that the cuts ran parallel to the muscle fibres and from end to end. A strip usually contained some 50 muscle fibres and was about 1 mm. in cross-sectional diameter. Fine

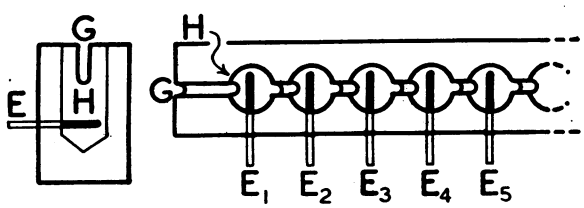

FIG. 1.—A diagram of the perspex bath used for mounting strips of the frog's sartorius muscle. For description see text.

silk thread was tied around each end of the strip of muscle and the preparation was lifted into the slot of the saline baths, with its distal end in bath 1 (Fig. 1). In order to insulate the baths from one another electrically, and to prevent interchange of fluid, vaseline was packed around the muscle in the groove joining the baths.

The Saline Bath.—The muscle bath was constructed from perspex (lucite) in such a way that we could either apply drugs to one section of the length of muscle fibres or apply them universally to the whole of the preparation (Fig. 1). Nine holes (H) of 6 mm. diameter were drilled in a perspex block to a depth of 13 mm. The centres of the holes lay in a straight line and were 6.5 mm. apart. The upper one-third of these holes communicated with one another through a milled groove (G) of width 1.5 mm. and depth 4 mm. which ran the whole length of the perspex block. Each hole contained at the lower end a chlorided silver electrode (E) which had been pressed into the perspex block so as to form a water-tight seal.

Solutions.—The composition of the Ringer solution used was: NaCl, 6.5 g., $NaHCO_3$, 0.2 g., KCl, 0.14 g., $NaH_2PO_4.H_2O$, 0.012 g., $CaCl_2$, 0.12 g., glucose, 2.0 g., dissolved in 1 litre of glass-distilled water. To the solution was added (+)-tubocurarine chloride obtained from Burroughs Wellcome and Co. Tests were made in the summer of the strength of tubocurarine solution necessary to produce complete neuromuscular block, and we found that a concentration of 10^{-5} was sufficient. Later in the year 10^{-4} was needed in some frogs. In order to demonstrate that the application of veratrine solutions to the whole length of muscle fibres does not produce after-discharges, it is necessary to have sufficient tubocurarine present to reduce the end-plate potentials to very small values. When the tubocurarine concentration is only just sufficient to prevent normal neuromuscular transmission in all fibres, large end-plate potentials may still occur. A stimulus which is intended for direct excitation of muscle fibres will still excite a few intramuscular nerve-twigs; the repetitive response of the latter in the presence of veratrine can give summation of end-plate potentials until a repeating neuromuscular transmission is established (*cf.* Kuffler, 1945). In the early experiments we used Ringer solution to which 10^{-4} (+)-tubocurarine HCl had been added, but for safety we would recommend the use of 10^{-3} tubocurarine, which was employed during the latter part of the work reported below.

Veratrine sulphate (obtained from Brickman & Co., Montreal) was made up as a 10^{-3} stock solution in tubocurarine-Ringer fluid. It will maintain its potency for about three weeks if stored in a refrigerator.

Recording System and Figures.—Most of the records made employed the silver-silver-chloride electrodes fixed in the base of the baths. Occasionally, however, we used a microelectrode filled with soft solder (Burns and Grafstein, 1952) for recording the repetitive activity from one or a few muscle fibres. Potentials from the electrodes were fed through cathode followers to two channels of amplification with coupling time-constants which could be varied from 1 msec. to infinity (=direct coupled). Records were made on film with a Cossor 1049 oscilloscope.

Voltage calibration for most of the records is not given since such calibrations had no particular physiological meaning. The magnitude of the potentials recorded was more dependent upon the perfection of

the vaseline seals than any other factors. A diagram of the recording arrangement is provided with each of the figures. Because in many of the experiments there was a great difference in the oscillograph spot velocities in the X and Y directions, many of the "spikes" have been touched up with white ink in order to make the figures clearer.

RESULTS

A Comparison Between Local and General Application of Veratrine

In most of our preparations a few muscle fibres began to give a repetitive response after local exposure to 10^{-7} veratrine for 5 min. The veratrine solution was usually applied in two adjacent baths (bath 1 and 2 of Fig. 1) of the seven baths through which the muscle passed; the threshold concentration for repetitive response to a single stimulus (given to the untreated portion of the muscle) varied from preparation to preparation but always lay between 0.5×10^{-7} and 0.5×10^{-6}. Concentrations of veratrine greater than 10^{-6} are liable to block transmission. Once the required threshold concentration for the local application of the drug had been found, a repetitive response to single stimuli could be maintained indefinitely. Test stimuli given once every half minute or minute would elicit the after-discharge in repeatable fashion for more than 5 hr. Occasionally, the repetitive response would disappear after about half an hour of testing, but we were always able to show that on these occasions the veratrine had leaked from the baths in which it was applied into a neighbouring bath. As a precaution against this failure to localize the action of the drug, we usually perfused the bath next to that which contained the veratrine solution (usually bath 3 of Fig. 1) with a slow flow of tubocurarine-Ringer solution.

When veratrine was supplied to baths 1 and 2 but the rest of the muscle was bathed with tubocurarine-Ringer solution, there was a great difference in the repolarization rates of the veratrinized and untreated lengths of muscle fibre, following a driven action potential. Records made from leads in baths 2 and 3 (with the amplifier direct coupled) showed a long-lasting relative negativity of bath 2, upon which the activity of repeating muscle fibres was superimposed (Fig. 5). Leakage of the veratrine from bath 2 into bath 3 caused an immediate decrease of this difference in repolarization rates, and usually produced a reduction in the after-discharge (see Fig. 2).

In contrast to the efficiency of the local application of veratrine in producing after-discharge, the

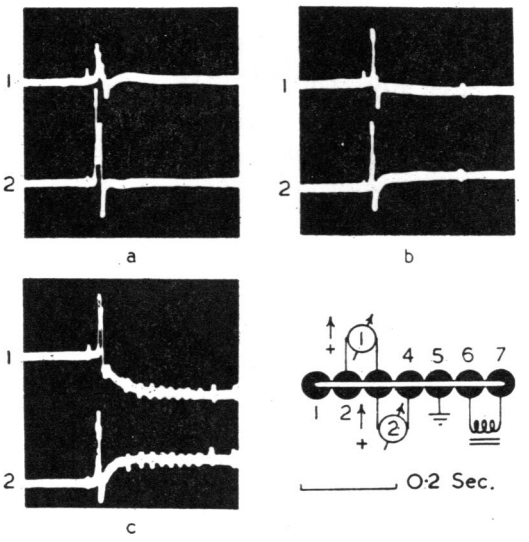

FIG. 2.—The responses to a single stimulus of the sartorius muscle to general and to restricted application of veratrine. (*a*) After immersion in 10^{-7} veratrine sulphate for 80 min. (*b*) 20 min. after beginning of continuous perfusion of bath 3 with tubocurarine-Ringer solution, in order to wash out the veratrine. (*c*) 130 min. later.

application of the veratrine in the same concentration to all the baths through which the muscle passed caused no repetition following a single stimulus. Soon after the application of veratrine to the whole length of the muscle fibre there was usually an increase in after-discharge, but within a few minutes the muscle fibres reverted to a state in which a single stimulus produced a single response, and stayed in this condition indefinitely. The results of Fig. 2, which are from a modification of this experiment, illustrate this point. Fig. 2a shows the response to stimulation of a strip of muscle which had been soaked in 10^{-7} veratrine solution for 80 min. before it was placed in the series of baths, all of which contained veratrine in the same concentration. The single stimulus produced no after-discharge. We then began washing out the veratrine in bath 3 by perfusion with tubocurarine-Ringer solution; Fig. 2b shows a single after-discharge occurring probably in one muscle fibre of the bundle, 20 min. after washing was begun. Fig. 2c shows the after-discharge recorded after bath 3 had been washed through for $2\frac{1}{2}$ hr. Fig. 2b and c also shows the development of a more rapid rate of repolarization for the muscle in bath 3 as the veratrine is washed out. For example, in Fig. 2 the driven action potential is passing from right to left along the muscle fibre, and channel I records its passage through bath 3 and 2 as an upward, followed by a downward,

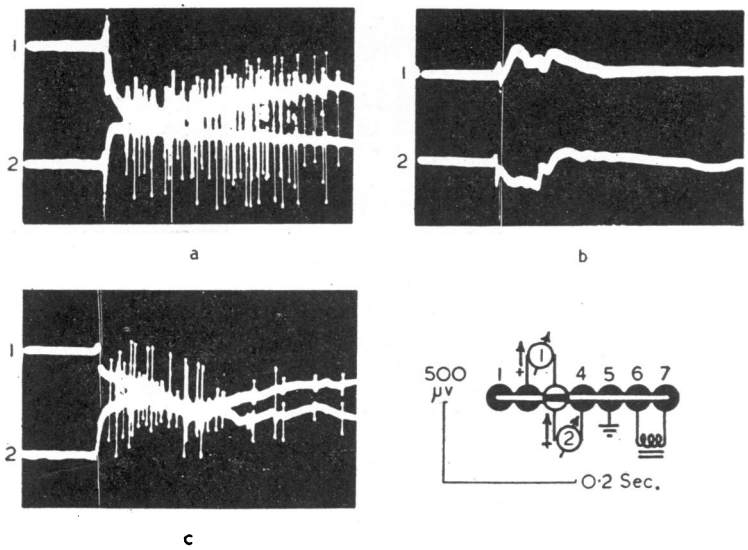

FIG. 3.—The responses to a single stimulus of the sartorius muscle to general and restricted application of veratrine. (*a*) 0.25×10^{-6} veratrine sulphate in all baths except 3. (*b*) 47 min. after 0.25×10^{-6} veratrine sulphate added to bath 3. (*c*) 70 min. after beginning continuous perfusion of bath 3 with tubocurarine-Ringer solution in order to wash out the veratrine.

deflection. The downward deflection implies that the muscle in bath 2 is depolarized at a time when that in bath 3 is recovered; any prolongation of this downward deflection (as in Fig. 2b or c, channel I) indicates that the muscle in bath 2 must be repolarizing much more slowly than that in bath 3.

Essentially the same result is obtained when the muscle is exposed to the same conditions in the reverse order. A reliable after-discharge in response to a single stimulus can be obtained when all of the baths, through which the sartorius strip passes, contain veratrine except one. The repetitive response shown in Fig. 3a was obtained as a repeatable phenomenon, when baths 1, 2, 4, 5, 6, and 7 contained 0.25×10^{-6} veratrine, although bath 3 contained tubocurarine-Ringer solution. The diphasic records make it clear that muscle fibres treated with this comparatively low concentration of veratrine can transmit a series of action potentials at high frequency. Soon after bath 3 had been filled with veratrine of concentration 0.25×10^{-6} so that the whole muscle was submerged in a uniform veratrine concentration, the after-discharge to the test stimulus disappeared (Fig. 3b). Finally, when the veratrine in bath 3 was washed out with Ringer solution, the after-discharge returned (Fig. 3c).

In the experiments described so far, the veratrine solution was either applied locally to one stretch of muscle fibre or was applied to the whole fibre. In other words, either there was a maintained concentration gradient of the drug down the length of the fibres or the concentration gradient was made zero. If the necessary condition for after-discharge is a sufficient concentration gradient, it should be possible to stop the after-discharges which occur when a threshold quantity of veratrine is in only one bath, by the addition of a *subthreshold quantity* of veratrine to the neighbouring baths. In the experiment whose results are given in Fig. 4, 0.5×10^{-7} veratrine was first added to baths 1 and 2. This concentration of veratrine was only just above the threshold for after-discharge of the fibres whose activity is shown in Fig. 4a and b. When 0.25×10^{-7} veratrine was added to bath 3 the repeated response to a single test stimulus soon disappeared (Fig. 4c and d), and only returned again when the veratrine in bath 3 was washed out (Fig. 4e and f). The experiment

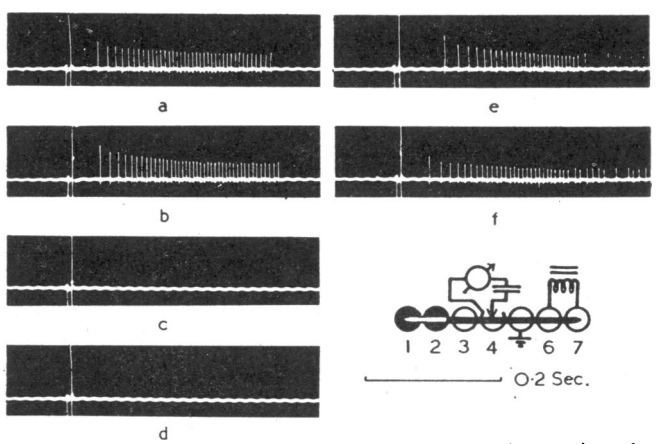

FIG. 4.—Responses to a single stimulus of a muscle fibre in the sartorius to local application of veratrine sulphate, recorded from a metal micro-electrode in bath 4. (*a*) 0.5×10^{-7} veratrine sulphate in baths 1 and 2 only (for 34 min.). (*b*) Same as (*a*) 4 min. later. (*c*) 2 min. after beginning of perfusion of bath 3 with 0.25×10^{-7} veratrine sulphate. (*d*) 3 min. later. (*e*) 6 min. after beginning perfusion of bath 3 with tubocurarine-Ringer solution in order to wash out veratrine. (*f*) 13 min. later.

of Fig. 4 required considerable patience during the initial administration of veratrine to baths 1 and 2 because each addition of the drug took some 15 min. to reach its full effect under our conditions of experiment. Moreover, it will be seen that the results of Fig. 4 can only be obtained if the concentration in these first two baths is only a little above threshold for the after-discharge. Only then can the subsequent concentration gradients between baths 2 and 3 and between baths 3 and 4 both be below threshold for after-discharge.

The Mechanism of Action of a Veratrine Concentration Gradient

The experiments described above make it clear that frog's skeletal muscle, physiologically isolated from its nerve supply, will only maintain a repetitive response to direct excitation if veratrine is locally applied, and not if it is uniformly applied over the length of the muscle fibres. It is presumably the well-known delayed repolarization of the veratrinized length of muscle which is responsible for this after-discharge. After the driven action potential has swept through the normal and veratrinized muscle, the latter repolarizes much more slowly than does the former (see Fig. 5b). The different rates of repolarization must cause current to flow in the extracellular spaces from normal to veratrine-treated muscle, and it is this current flow at the boundary of veratrinized and normal muscle which presumably causes the origin of an action potential. This concept implies that the threshold concentration of veratrine necessary to produce an after-discharge should be accompanied by a measurable threshold difference of membrane potential between normal and treated membrane. Fig. 5b, c, d, and e shows the development of such a potential difference as a concentration of 10^{-7} veratrine slowly attains its full effect. In Fig. 5b and c the potential difference during recovery from the driven action potential between veratrinized and normal muscle (baths 2 and 3) is not sufficient to trigger an after-discharge. Seven min. later this potential difference is adequate for after-discharge (Fig. 5d and e). The repetitive response recorded with a high velocity sweep from baths 2 and 3 showed that each action potential was diphasic with the first phase down. This indicated that the after-discharge originated in the neighbourhood of the treated stretch of muscle and its activity was therefore travelling in the opposite direction to that of the driven action potential. In Fig. 6 is shown the consequence of two driven action potentials on a similar preparation. The potential

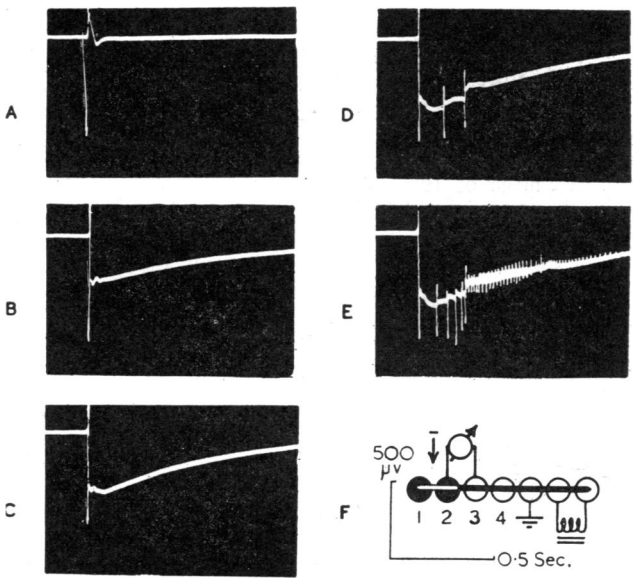

FIG. 5.—Responses to a single stimulus in a small bundle of fibres from the sartorius muscle during the progressive local application of veratrine sulphate. (*a*) Control. Whole of muscle in tubocurarine-Ringer solution. (*b*) 34 min after 0.75×10^{-7} veratrine sulphate in baths 1 and 2. (*c*) 12 min. later After a further 6 min. 10^{-7} veratrine sulphate put in baths 1 and 2. (*d*) 7 min later than previous record. (*e*) 1 min. later.

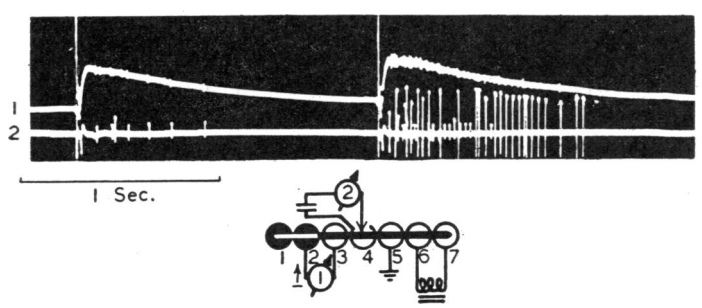

FIG. 6.—The responses of a small bundle of muscle fibres to two stimuli in the presence of local veratrine. Channel 1 shows a D.C. record from baths 2 and 3. Channel 2 records the activity of a few fibres with a metal microelectrode in bath 4. 10^{-7} veratrine sulphate in baths 1 and 2 only.

gradient between normal and veratrinized muscle is only sufficient to trigger a repetitive response from two fibres during recovery from the first volley, but the after-effects of the second volley add to those of the first so that the potential gradient is finally brought above threshold for after-discharge of four or five fibres.

Another way in which the dependence of after-discharge upon a suprathreshold potential gradient can be shown is by varying the frequency of the driving stimulus. A local concentration of veratrine which is sufficient to cause a repetitive response to stimuli given at one a minute may never cause after-discharge if the driving stimuli occur at two per minute. The magnitude of the after-potential (or delayed phase of repolarization) which follows a driven action potential increases measurably with increase of the rest allowed up to periods of about 2 min. As would be suspected, the probability of a stimulus causing an after-discharge is also increased by prolonging the rest which precedes the triggering stimulus.

Experiments with Polarizing Current

The experiments described so far make it clear that the veratrine after-discharges will only occur provided that during recovery from a driven action potential a sufficient gradient of membrane potential per unit length of fibre exists. For any one muscle fibre the value of this potential gradient will depend only upon the magnitude of the peak local concentration of veratrine, and upon the concentration gradient of veratrine down the length of the fibre.

This concept of the action of local veratrine application implies that one should be able to modify the veratrine after-discharge with polarizing currents applied to that part of the veratrinized muscle which lies close to the untreated length of the fibres. Polarization was effected by switching on a measured current, which entered one bath of the series in which the muscle lay, and left by another bath; the current entered and left these baths through non-polarizable electrodes separate from those used for recording purposes. When the preparation is treated with local veratrine, the addition of a cathode in the veratrinized bath (No. 2) will increase the veratrine after-discharge which follows a single stimulus given to the normal portion of the muscle. If the veratrine concentration just produced a repetitive response (as in Fig. 7a) then the cathodal polarization increased the after-discharge following a driven action potential (Fig. 7b). In the experiment of Fig. 7 this increase was slight. As would be suspected, anodal polarization of the veratrinized stretch of muscle fibre subtracts from the effect of veratrine so that the addition of the anode to bath 2 stopped the after-discharge (Fig. 7d). The reverse effects were seen when the polarization of the bath containing veratrine was switched off. Immediately after removal of the cathode the veratrine after-discharge was decreased (Fig. 7c); after removing an anode the discharge in response to the single test stimulus increased (Fig. 7e). It is known that the polarizations forced on the membrane by passing current through it for ½ min. persist for a minute or so afterwards (Burns and Paton, 1951). Presumably the inward current flow due to the relatively slow collapse of super-polarization after withdrawal of an anode acts like the applied cathode, and adds to the inward current produced by the relatively slow repolarization of veratrinized membrane; in this way the after-discharge is increased.

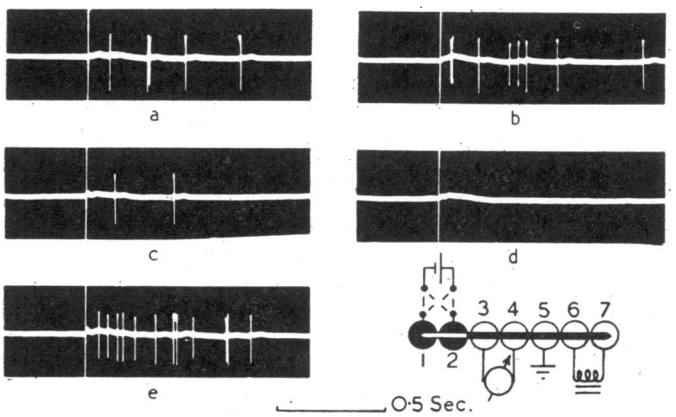

FIG. 7.—The responses to a single stimulus of (probably) one of a small bundle of muscle fibres during and after various polarizing currents in the presence of local veratrine. 2×10^{-7} veratrine sulphate in baths 1 and 2 only. Polarizing currents applied between baths 1 and 2. (*a*) Control. Polarizing current off. (*b*) 1 min. after switching on 7.5 μA current from bath 1 to bath 2. (Bath 1+). (*c*) 1 min. after switching current off. (*d*) 1 min. after switching on 7.5 μA current from bath 2 to bath 1. (Bath 2+). (*e*) 1 min. after switching off current.

DISCUSSION

In all of the electrically excitable tissues from which an after-discharge has been obtained veratrine appears to delay repolarization following an

action potential (Krayer and Acheson, 1946). It has been described by some as prolonging the negative after-potential; others have disliked this description on the grounds that an originally monophasic action potential can become one with two maxima of depolarization, repolarization thus occurring in two phases with a clear discontinuity between them. Whatever is the best way of describing events, repolarization is delayed by veratrine and in frog's muscle may occur with a time constant as great as 5 sec.

Although the mechanism by which veratrine delays repolarization is unknown, it is clear that the repetitive response to a single stimulus only occurs in the frog's sartorius muscle when the delay of repolarization is local. When veratrine is applied to the whole length of a muscle fibre, repolarization occurs equally slowly at all points along the fibre's length, and there is no cause for interstitial current flow. When the veratrine is applied locally, the veratrinized length of muscle fibre pursues the same slow time course of recovery as before, but current begins to flow in the surrounding fluid from the neighbouring and rapidly polarizing membrane toward the veratrinized region; it is this current flow which occurs during recovery from a driven action potential which may set up a "spontaneous action potential." The spread of this action potential, which is normally the first of a series, causes both veratrinized and normal membrane to depolarize a second time, following which the above cycle of events may be repeated, giving rise to the second action potential of the after-discharge. This is the characteristic mechanism of an after-discharge due to what has been termed differential repolarization (Burns, 1955).

Our results suggest that if the whole membrane of an electrically excitable cell is uniformly affected by veratrine, then no after-discharge can follow a driven action potential. On the other hand, after-discharges have been reported in nerve-muscle preparations submerged in veratrine solutions (Bacq and Brown, 1937) and can be obtained for many hours from nerve fibres which have been uniformly soaked in veratrine (Feng, 1941). It seems possible therefore that the mechanism of the drug's action is not the same for all susceptible electrically excitable cells. An alternative explanation of these findings may be that some excitable cells possess a membrane only certain parts of which are affected by the drug. Certainly some electrically excitable cells such as cardiac auricle do not show after-discharge in the presence of veratrine (Krayer and Acheson, 1946). In a nerve-muscle preparation, the region of the nerve terminals is known to be more sensitive than the proximal fibre (Dun and Feng, 1940). It was for this reason that we used tubocurarine-Ringer solution as a precaution against excitation through nerve twigs.

In our own experiments, the exposure of the whole length of the muscle fibres to veratrine solution lead to violent after-discharges following test stimuli, for the first 2 or 3 min. But these after-discharges always disappeared after a time interval consistent with the view that the concentration of the drug had finally become uniform at all points on the fibre's length. Consistent with this interpretation was the finding that a sudden decrease in veratrine concentration produced by the exchange of Ringer solution for veratrine solution would also cause after-discharges to test stimuli for a period of a few minutes.

The characteristics of an after-discharge due to differential repolarization have been described in connexion with the type-B cells of the cerebral cortex (Burns, 1954, 1955). Locally, veratrinized skeletal muscle seems to provide a simple biological model for the mechanism of this sort of after-discharge, and it could be said that the local application of veratrine converts the relatively phlegmatic muscle cell into a cell whose structure and function are as complex as those of some neurones found in the central nervous system. We have been able to demonstrate with veratrinized muscle many of the more important features of an after-discharge due to differential repolarization. Thus, when conditions are only just adequate for the production of repeated response to a single stimulus we found:

(*a*) An action potential starting from any point and travelling in any direction will trigger the after-discharge.

(*b*) The repetitive response tends to bear an all-or-nothing relationship to variation in the conditions required for its production. Because one "spontaneous" discharge resets the recovery cycle it produces the conditions necessary for a second "spontaneous" action potential; the second discharge produces a third, and so on. Thus the usual event is an after-discharge consisting of many action potentials and it is extremely hard to adjust conditions so that only one after-discharge occurs (Fig. 5).

(*c*) The latent period before the repetitive "spontaneous" firing breaks out is greater than the time interval between the first two action potentials of the after-discharge (Fig. 4).

(*d*) The maximum frequency of the repetitive response does not occur at the beginning of the after-discharge, but is usually reached after the first few action potentials (Fig. 4).

(*e*) When the after-discharge comes to an end its frequency does not decline asymptotically to zero, but after some reduction in the rate of discharge the series of action potentials stops suddenly (Fig. 4).

(*f*) The production of an after-discharge requires the presence of an adequate gradient of membrane potential along the length of the cell during recovery from driven activity (Figs. 2, 3, and 5).

These properties of after-discharge due to differential repolarization have been described at some length since they may ultimately serve to identify after-discharges of this type at other sites in the nervous system.

Locally veratrinized skeletal muscle offers a convenient preparation with which it should be possible to make a detailed analysis of the mechanism of this form of after-discharge. We have made no reference in this report to the precise site of origin of the after-discharge, other than to say that the origin is in the region of the border between veratrinized and normal muscle. The methods we have used in the experiments described are not adapted to precise localization, but the information available indicates that the after-discharge originates on the veratrine side of the vaseline junction separating veratrinized from normal Ringer solution. Because the vaseline junction is itself of the order of breadth of the muscle membrane's space constant, there must be gradients of both veratrine concentration and of recovery rate on either side of the vaseline barrier. This means that the point of slowest repolarization should be well inside the veratrinized bath. We are at present investigating this question in greater detail.

Summary

1. Experiments are described in which the effects of veratrine sulphate upon the fibres of the frog's sartorius muscle were tested.

2. Direct excitation of fully curarized muscle fibres gave rise to a repetitive after-discharge only when veratrine was not applied to the entire length of the fibres.

3. Veratrine delays repolarization of the muscle fibre's membrane after an action potential has passed through the veratrinized region.

4. The difference in repolarization rates of veratrinized and untreated lengths of muscle fibre is the immediate cause of the after-discharge.

5. The general properties of an after-discharge due to differential repolarization are discussed.

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