THE MODE OF ACTION OF VERATRINE ON SKELETAL MUSCLE

by

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I. HISTORICAL AND GENERAL INTRODUCTION[x]

This work is concerned with a study of the action of veratrine sulphate on the electrical responses of skeletal muscle. All the experiments performed in this study involve the use of frog's skeletal muscle. However, because of the similarity of the actions of this alkaloid on nerve and muscle, much of the discussion and many of the references given will concern studies performed on nerve fiber preparations. About 10 years ago, an excellent review on the pharmacology of the veratrum alkaloids appeared (Krayer and Acheson, 1946). Since the history of this subject has been exhaustively covered in this review, I feel that it is both unnecessary and wasteful to cover the same field again. Therefore, in the discussions, I will concentrate on recent work and on the more important of the earlier studies.

A. A brief description of the veratrum alkaloids and their effects on biological materials

According to Krayer and Acheson (1946), "The veratrum alkaloids are obtained from liliaceous plants belonging to the sub-order Melanthaceae. The species most throughly investigated are Veratrum album, Linn., native to Europe; Veratrum viride, Aiton, native to the United States and Canada; and Schoenocaulon officinale, Gray, also called Asagroea officinalis, Lindley, or Veratrum Sabadilla, Retz., the Mexican or West Indian Sabadilla."

The name "veratrine" usually refers to the total alkaloids obtained from the Sabadilla seeds, and "veratrum" refers to an unknown num-

[x] Some of the experimental results included in this thesis have already been published (Burns, Frank and Salmoiraghi, 1955).

ber of the total alkaloids from either of the other two species. Veratrine contains primarily the two ester alkaloids, veratridine and cevadine, and a small amount of the alkamine cevine. Upon hydrolysis, both veratridine and cevadine yield cevine plus various organic acids. Shanes (1952a) has studied the ultraviolet spectra of veratridine, cevadine and veratrine and found that in the ultraviolet spectra of veratrine could be distinguished the spectra of the other two. He also found that these two alkaloids had slightly different effects on crab nerves. These differences were of a quantitative nature. (See Krayer and Acheson, 1946., for a more detailed description of the chemistry of the veratrum alkaloids.)

At present, interest in this group of alkaloids is based on their use in the treatment of hypertension and eclampsia. Krayer and Acheson (1946), give a detailed description of their toxicities and their actions on circulation and respiration. These alkaloids (particularly veratrine) are also frequently used as pharmacological tools in physiological studies. Recently, Aviado and Schmidt, (1955), have reviewed the literature on the study of the stretch receptors of the thoracic viscera. These studies provide a beautiful example of the use of a pharmacological agent in the study of a physiological problem. In much the same manner, in the past century, the veratrum alkaloids have been used in the study of nerve and muscle. Unfortunately, the rewards have been scant. The results obtained have more often served to confuse issues than to elucidate basic physiological phenomena.

According to Krayer and Acheson (1946), the effect of veratrine on the skeletal neuromuscular system was first clearly described

and analyzed by Prévost in 1866 and von Bezold and Hirt in 1867. At first, it was felt that veratrine acted only on muscle and had little or no effect on nerve. Only later was it shown that, except for the mechanical response, the action of veratrine on nerve was identical to its effect on muscle.

I would like to quote the description given by Krayer and Acheson (1946) of the classical mechanical response of frog skeletal muscle to veratrine treatment. "The resting muscle, treated with an appropriate dose of the drug, shows no obvious abnormality. If, however, a brief stimulus be delivered to the nerve or muscle, the usual quick contraction of the muscle is followed by a phase of slow relaxation which may last for more than twenty seconds. The myograms resulting from this type of response have varied shapes. Weak veratrinization leads to a twitch-like initial contraction and partial relaxation, followed by a slow secondary rise and fall. With stronger poisoning, the dip in the myogram diminishes, and the height of the secondary rise grows. With still stronger doses, the curve presents a smooth rise to a maximum several times the normal twitch height, followed by a long, slow fall to the base line." Such records can be found in almost any paper dealing with the mechanical responses of veratrine treated skeletal muscle.

B. Contracture vs. contraction

A contracture might be defined as an increase of tension or a shortening of a muscle without the presence of propagated electrical responses (Gasser, 1930). Most of the earlier workers felt that the response of a veratrine-treated muscle was a true contracture following a muscle twitch (Krayer and Acheson, 1946; Gasser, 1930).

There are two main procedures by which this question can be settled. One would be to show the presence of propagated electrical responses during the veratrine muscle response; the double myograph technique is the other. In the double myograph technique, a long thin muscle, such as the frog sartorius, is gently clamped in its center, both ends being used for myograph recording. Veratrine is then applied to one half of the muscle with care being taken to assure that there is no spread of the veratrine to the other half. Then either end of the muscle is stimulated and recordings are made to see if the response is limited to the poisoned half (Feng, 1936, 1938).

For some reason, the earlier workers who attempted this experiment came to the conclusion that the veratrine response was not transmitted from the treated half to the other. Later workers, however, even more surely reached the opposite conclusion (Feng, 1936b, see Krayer and Acheson for a more detailed list of the other workers who used this technique).

Although most of the earlier workers felt that the veratrine muscle response was a contracture, it would be unfair to imply that this was a universally held view. Buchanan (1899), for example, attempted

to show that the mechanical response of a veratrine treated muscle was the result of a tetanic electrical response of the muscle by showing that the mechanical response of the veratrine treated muscle could be duplicated by the tetanic stimulation of an untreated muscle. She goes on to say, "Our own subsequent experiments with regard to the electrical response of veratrinised muscle showed that this also has the character of that of tetanus produced by rapidly repeated stimulation, not that of a twitch". The actual records, however, (Burdon-Sanderson, 1899) made by means of the mercury capillary-electrometer leave much to be desired. These papers are important in that they show that the question of contracture versus contraction could not be definitely settled until better methods of electrical recording were developed.

According to Krayer and Acheson (1946), it was not until 1912 when Hoffmann was able to demonstrate oscillations following a single stimulus on the electromyogram recorded by using the string galvonometer, that the tetanic nature of the response of veratrine-treated muscle was shown. With stronger veratrine concentrations, the duration of the oscillations decreased although the mechanical response persisted. This work has been confirmed by many others, particularly with the aid of the oscilloscope for recording the electrical responses of the muscle (see Krayer and Acheson, 1946).

Krayer and Acheson (1946) felt that this question was entirely settled. The response of veratrine-treated muscle is not a contracture and only severe veratrine treatment of amphibian muscle produces contracture. There are two observations in the literature concerning this point that I would like to discuss.

One is by Feng and Li (1941) who believed that the mechanical response of amphibian muscle far outlasted the propagated electrical responses and, therefore, at least the latter part of the mechanical response was a true contracture. On the other hand, they found that when they studied the response of veratrine-treated mammalian muscle (cat's soleus muscle) the mechanical record could be entirely accounted for on the basis of a tetanic response of the nerve-muscle preparation. This might explain some of the different results obtained by authors who worked with different preparations.

The other observation is one made by Kuffler (1945, and 1946) while working with a single muscle-fiber preparation. In order to obtain a repetitive response, he placed a drop of veratrine solution on the fiber at the point of stimulation. As he moved his recording electrodes away from the point of stimulation, he found that some impulses originating at the stimulated point died out while being conducted along the fiber. This observation might explain some of the earlier results showing that the veratrine response was not completely conducted from the site of origin.

Although the mechanical response of veratrinized frog muscle is always reported to last several seconds, I have rarely observed tetanic responses lasting more than about a second. The usual duration of the repetitive response was 0.5 sec. or less. On the other hand, the veratrine negative after-potential generally lasted several sec. (Fig. 18). This result is in accord with the belief of Feng (1938) and Kuffler (1946) that the latter part of the mechanical response of veratrinized frog's skeletal muscle must be considered a contracture. It is also possible that the proponged negative after-potential is involved in

the production of this contracture (Kuffler, 1945, and 1946).

Since this thesis is primarily concerned with the electrical phenomena associated with veratrine treatment of frog skeletal muscle, I have only discussed this question because of its importance in the history of this subject. Most of the initial records of the electrical changes produced by veratrine treatment were made during investigations of this problem.

C. Changes in the electrical properties of veratrine-treated nerve and muscle

Thus far, the mechanical properties of veratrinized muscle have been described. The electrical properties have been mentioned only insofar as they have helped in the interpretation of the altered mechanical response. I would now like to mention several of the changes in electrical properties of nerves and muscles that occur following veratrine treatment. Since the effects are similar in nerve and muscle, they will rarely be set apart in the following discussion.

It is common knowledge that nerve and muscle fibers are polarized structures. The outsides of these fibers have a uniform positive charge relative to their insides. The voltage of this steady charge is referred to as the resting potential. Until fairly recently, the only way in which this potential, or changes in this potential, could be estimated, was to damage the surface of the fibers at a single point or to place a drop of concentrated KCl on a point along the fibers, and to record the potential difference between this point and the undisturbed surface of the fibers. The potential recorded in this manner is called the demarkation potential.

When the fibers are stimulated, a wave of negativity, or action

potential is conducted away from the point of stimulation at a relatively constant velocity (the conduction velocity). Originally, it was felt that the action potential represented a brief reduction of the resting potential to zero. When the action potentials of squid giant axons were measured with inside-the-cell electrodes (Hodgkin and Huxley, 1939, 1945; Curtis and Cole, 1940, 1942), it was found that during the action potential there was actually a reversal membrane of potential. This advance was followed by the development of inside-the-cell microelectrodes (Graham and Gerard, 1946; Ling and Gerard, 1949; Nastuk and Hodgkin, 1950) for recording the resting and action potentials of smaller cells. In the past 10 years, by the use of these and other techniques, it has been found that the reversal of membrane potential during an action potential is a property of most excitable tissues (Eccles, 1953).

Following an action potential several events occur before the membrane potential resumes its resting level. If, following an action potential, the potential across the cell membrane is not at resting level but for a time is slightly less, then this event is called a negative afterpotential. In a like manner, if the potential is greater than normal, it is called a positive afterpotential. In some preparations, notably the squid giant axon, there is frequently an oscillation of the potential about the resting level following an action potential (Hodgkin and Huxley, 1952d; Shanes, 1949a). This effect has also been reported to occur following a subthreshold stimulation of this preparation (Shanes, 1949a). Under certain circumstances, a single driven action potential may be followed by a whole series of action potentials without the stimulus being maintained. This is often referred to as an

after-discharge. When action potentials occur without a stimulus being applied to the tissue, this activity is referred to as spontaneous activity.

As mentioned above, an action potential normally arises only when the fibers are stimulated. When a stimulus is of just sufficient strength and duration to cause the production of an action potential, it is called a threshold stimulus. Any process which decreases the threshold is called facilitation, and any process which raises the threshold is called inhibition. If, while a stimulus is maintained, the threshold of the fiber rises due to some mechanism inherent to the fiber, accommodation is said to have occurred.

Although rather long, the above description of membrane electrical (or better, membrane) phenomena has been given for the sake of brevity. Every property or process mentioned is modified by veratrine treatment. Even more surprising is that, depending on the experimental conditions, almost all of these properties and processes can be either increased or decreased. I do not intend to go into a detailed description of the literature concerning these changes at this time, for most of them will be discussed in the appropriate sections below. (See Krayer and Acheson, 1946, for a detailed description of these changes). Rather, I would like to describe some of the outstanding changes that I have observed during my studies with veratrine.

When a frog's muscle is treated with a very weak concentration of veratrine, no change is observed until the muscle is stimulated. It is then found that the negative afterpotential is considerable prolonged. With stronger veratrine concentrations, not only is it prolonged, but it is also increased in size and, under certain conditions, it may be ac-

companied by a series of action potentials (or after-discharge). With still stronger concentrations of veratrine the resting potential will decrease and the conduction of action potentials along the fibers may be blocked.

These three changes, the prolonged and increased negative after-potential, the repetitive after-discharge, and the block of conduction with strong concentrations, are the ones most consistently reported.

D. Theories of the mechanism of after-discharge production by veratrine treatment of nerve and muscle

Since the beginning of this century, attention has been shifting away from simply describing the action of veratrine on nerve and muscle to trying to explain the changes that have been found. The effect which has held the greatest interest is the after-discharge, which was called the veratrine contracture before adequate electrical records were made. Several mechanisms have been proposed to explain this effect. These mechanisms might roughly be divided into two groups, the electrical mechanisms and the chemical mechanisms. As will become obvious later on in this introduction, the mechanism proposed in this thesis must be classed as an electrical mechanism.

The chemical theories propose that in the presence of veratrine the initial response produces changes in the environment of the fibers that lead to the veratrine response. One of the first of these theories, proposed by Lamm in 1911 and von Frey in 1912, assumed that the veratrine itself was changed by the initial response into a substance which caused further contraction of the muscle (Krayer and Acheson, 1946). This theory seems to be based on the assumption that

the veratrine response was a contracture and little attention was given to the possibility of changes in the electrical response.

A slightly more acceptable theory was proposed in 1914 by Venzar and Felter (Krayer and Acheson, 1946). In this theory, it was assumed that some substance is released during the initial response and that the veratrine makes the muscle more permeable to this substance.

The modern counterpart of this theory is the humoral transmission theory for the veratrine contraction, first proposed by Szent-Gyorgyi, Bacq, and Goffart in 1939. Using the cross-perfusion technique, they found that when the veratrine solution coming from a frog that was strongly stimulated for several minutes was perfused through a second frog, the muscles of the second frog contracted (Bacq and Goffart, 1939). They also found that the presence of veratrine in a solution perfusing a frog increased the concentration of K^+ in the solution leaving the animal. Bacq (1939a) next studied the effect of veratrine on the contraction of the rectus abdominis muscle of the frog produced by the addition of KCl to the bathing solution and he concluded that veratrine sensitized the muscle to the effect of potassium. He next studied the effects of changing other ion concentrations on the veratrine response and he concluded that the veratrine response was not dependent simply upon the external K^+ concentration but upon the following relation: $K + Na / Ca + Mg$ (Bacq, 1939b). Probably the main hypothesis of this theory is that the reason the veratrine contraction is only evident following an initial contraction, is because the initial contraction liberates active K^+ ions to which the muscle fibers are hyperexcitable due to the action of veratrine (Bacq, 1939b).

There are several difficulties with this theory and recently

another chemical theory has been proposed based upon studies of electrical phenomena (Gordon and Welsh, 1948; Shanes, 1951, 1952a). In this theory, it is proposed that Ca^{++} is released from the cell membrane during the passage of an impulse and that the veratrine in some manner binds this calcium or blocks recalcification of the membrane.

Just as there have been several chemical theories put forward to explain the veratrine after-discharge, several electrical mechanisms have also been proposed. One of the first of these was proposed in 1928 by Kodera and Brucke. (Krayer and Acheson, 1946). They felt that the veratrine after-discharge was similar to the "closing tetanus" seen in many normal nerves and muscles. They, therefore, proposed that veratrine acted on the same mechanism responsible for the "closing tetanus". Veratrine might therefore be considered to interfere with normal accommodation. This theory was put forth in a more generalized form by Schriever and Cebulla (1938). They proposed that the excitability characteristics of rhythmically and non-rhythmically responding nerves were quite different. In particular, they felt that the "Eischleichzeit" of rhythmically responding nerve was always much greater. Feng (1941) tested this theory on veratrine-treated frog nerve and found that it did not apply to the rhythmic response of this preparation. Another difficulty with this explanation is that one of the most striking facts about the veratrine after-discharge is that it occurs long after the external stimulus has ended.

Most of the other electrical theories have sought to link the after-discharge with the increased and prolonged negative after-potential. Some authors have felt that the negative after-potential is associated with a phase of supernormal excitability, while others have felt that the

prolonged and increased negative after-potential was analogous to an externally applied cathode (Krayer and Acheson, 1946). Since the theory proposed in this thesis is also dependent upon the altered negative after-potential, these theories and their difficulties will be discussed in greater detail below.

E. Our reasons for investigating this problem

In some recent experiments with the cat's cerebral cortex, Burns (1954, 1955) has 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 neurons (Burns and Grafstein, 1952) repolarized some ten times slower than 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 suggested by Burns (1955) that the mechanism of differential repolarization might play some part in causing the after-discharges produced by veratrine.

It is obvious that this theory should be classed as one of the electrical theories as defined in the last section. It is also clear that this is one of the theories in which the prolonged negative after-potential

is given a prominent role in the production of after-discharges. This theory is similar to the one proposed by Kuffler, (1945). He concluded that the maintained, partial depolarization of the muscle fiber following an action potential 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 fibers."

The differential repolarization mechanism differs from the one proposed by Kuffler (1945) only in that it would specify the analogy between the negative after-potential 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 single stationary "membrane action potential" (Hodgkin et al., 1952). Alternatively, a local cathode may give rise to transmitted action potentials because it depolarizes one section of the cell membrane relative to neighboring 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. It must be remembered that while the differential rate of repolarization may initiate a second impulse, it will only initiate a series of impulses if each of the "spontaneous" impulses set back the recovery process, so that the negative after-potential does not act as a maintained local cathode which would permit the cells to accommodate.

F. Approach to the problem

The differential repolarization mechanism, like most other electrical mechanisms proposed to explain the veratrine after-discharge, depends on the persistent depolarization of the membrane which follows the initial response, but whereas the other mechanisms suppose that a universal, partial depolarization is sufficient cause for the after-discharge, the differential repolarization mechanism postulates that there must be a depolarization gradient down the length of the fiber before repetitive activity can occur. It will be realized that if differential repolarization is the major cause of the after-discharge, then local veratrine application should be far more effective in producing after-discharge than its universal application to the whole fiber. Therefore, the initial approach to the problem was a comparison of local and universal veratrine application. In line with this study, it was of interest and importance to study the modification of the veratrine after-discharge by other factors and to see how these changes agreed with the hypothesis.

When this study was started, it was felt that if the veratrine after-discharge was caused by the proposed mechanism, the muscle fiber treated locally with veratrine might well serve as a model for the type-B neurone in the cat's cerebral cortex. The next step, therefore, was to study the repetitive response in greater detail. In order to do this, it was felt that it was necessary to see in what way treatment with veratrine modified the properties of the muscle membrane.

I also became interested in the problem of just how veratrine prolonged the negative after-potential. The best approach to this problem was to study the membrane properties by means of inside-the-

cell electrodes. Such studies were performed on resting and active fibers. Many of the studies were carried out using universal veratrine application to avoid the after-discharge. This was followed by a study of the potential changes along the fiber's surface during a veratrine after-discharge. The studies with inside-the-cell electrodes were not only useful in the study of the prolonged negative after-potential, but also yielded useful information concerning several of the other mechanisms proposed to explain the veratrine after-discharge.

II. GENERAL MATERIALS AND METHODS

A. The preparations

The sartorius muscle of the frog, rana pipiens, was used in most of the experiments. In a few experiments the extensor digitis longis IV was used. The exact methods of preparation and of mounting the muscles will be described below (pp26-28). All the experiments were performed on isolated muscles. Many of the experiments were performed in the winter, about an equal number were performed in the spring and autumn, and very few were performed in the summer. In general, seasonal changes did not seem to affect the results of these experiments, although quantitative comparisons were not attempted. During the course of this work parasites were found in the muscles of the frogs. At first, only a few cases were noted and when this occurred the muscles were discarded. However, during the second half of the experiments the incidence of infection became so bad that no frogs could be found that did not have the parasites, and most of the experiments with inside the cell electrodes had to be performed using these frogs.

Since so many of the experiments were performed using these infected frogs I felt that it was important to find out exactly how the parasites acted on the frogs and what changes, if any, were produced in the muscles isolated from such frogs. I therefore spent some time investigating this question (Frank, 1956). The main conclusions reached in this investigation were that the parasites had in some manner upset the water balance mechanisms of the frogs and that the parasites had no direct effect on the muscles isolated from

infected frogs. The result was that the tissue fluids of infected frogs, both extracellular and intracellular, were hypertonic. This had the effect of reducing both the resting potentials and the active membrane potentials which were recorded soon after the muscles were isolated. However, when these muscles remained in Ringer's solution, there was a gradual improvement (increase) of the potentials recorded, and after a few hours they reached normal values. On the other hand, this condition had its advantages. In those experiments in which it was necessary to compare some other value with the resting potential, fibers were available having a wide range of resting potentials without the addition of another experimental variable.

In some cases, experiments were checked by leaving the muscles isolated from infected frogs overnight at 6° C. in a large volume of Ringer's solution. Any parasites that remained in the muscles were dead the following morning and the potentials recorded were considerably higher than those recorded from freshly isolated muscles from infected frogs. Qualitatively, the results of such comparisons were always the same and any quantitative differences could be entirely accounted for on the basis of the altered ionic environment. Further reference will be made to this question in the discussions below, and a more detailed discussion can be found in the paper mentioned above (Frank, 1956).

B. Solutions

The compositions of the various solutions used are shown in Table 1. The reduced Na^+ solutions were made by mixing appropriate amounts of Choline Ringer's with Normal Ringer's solution. Thus a 30% Na^+ solution was made by mixing 70% Choline Ringer's with 30% Normal Ringer's solution. The resulting solution had a Na^+ concentration which was slightly more than 30% of normal but the difference was slight.

In most of the experiments d-tubocurarine chloride (Burroughs Wellcome & Co.) was added to the solutions to make a final concentration of 10^{-4}. In some experiments, nerve-muscle preparations were used, and in other experiments altered solutions were applied universally to the whole muscle. In most of these cases the d-tubocurarine was not added to the solutions.

TABLE 1

Composition of the various solutions used (in millimoles per liter).

Type	NaCl	KCl	$CaCl_2$	$NaHCO_3$	NaH_2PO_4	Choline chloride	Glucose
Normal Ringer	111.3	2.47	1.08	2.38	0.087	------	11.1
Low K^+	111.3	----	1.08	2.38	0.087	------	16.0
1.5 K^+	111.3	3.76	1.08	2.38	0.087	------	11.1
2.0 K^+	111.3	4.94	1.08	2.38	0.087	------	11.1
1.5 Na^+	161.3	2.47	1.08	2.38	0.087	------	11.1
Choline Ringer	-----	2.47	1.08	2.38	0.087	111.8	11.1

C. Baths, recording systems and figures

Because of the wide variety of tests performed, several different procedures were used. Some of the arrangements, such as a moisture chamber or the suspension of the muscle in mineral oil were quite standard. Others involved the use of baths specially designed for this work. In order to avoid confusion, the various arrangements used will be described in detail in the appropriate sections.

The same might be said of the recording arrangements. However, the basic design of the recording system was the same in all experiments. 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). Most of the records were made on film with a Cossor 1049 oscilloscope. As will be seen below most of the recording was direct coupled.

Voltage calibration for most of the records obtained with external electrodes is not given since such calibration had no particular physiological meaning. A diagram of the recording arrangement is provided with most 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.

III. GENERAL PROPERTIES OF THE REPETITIVE RESPONSE OF VERATRINE-TREATED MUSCLE FOLLOWING A SINGLE STIMULUS

A. Introduction

Ever since veratrine treatment of nerve was clearly shown to produce a prolonged and increased negative after-potential (Graham and Gasser, 1931) it has been assumed that the repetitive response was in some manner responsible for the veratrine after-discharge. It has been shown by many workers that the prolonged negative after-potential in both nerve and muscle is associated with a phase of supernormal excitability (Graham and Gasser, 1931; Graham, 1934; Gasser and Grundfest, 1936; Bremer, 1955) and it was felt by some workers that the repetitive response was caused by the supernormality of which the negative after-potential was a sign (Krayer and Acheson, 1946). However, it has also been found that a prolonged and increased negative after-potential may occur without the presence of a phase of supernormality (Graham and Gasser, 1931; Acheson and Rosenblueth, 1941) and the relation between the veratrine after-discharge and supernormality is of a somewhat doubtful nature.

Of course, the development of a phase of supernormality need not be the only way in which the altered negative after-potential might produce the repetitive response. Kuffler (1945), for example, felt that the negative after-potential was analogous to an externally applied cathode. He also found that small doses of veratrine which failed to produce an after-discharge in single muscle fibers did however increase the negative after-potential, and larger doses of

veratrine which caused the appearance of after-discharge increased the negative after-potential even more.

There are other difficulties in accepting a cause and effect relation between the negative after-potential and the repetitive response. Thus, although Acheson and Rosenblueth (1941), in their study of the effects of veratrine on circulated mammalian nerves, found a general correlation between the amplitude of the negative after-potential and the rate of the repetitive response, Dun and Feng (1940a), studying the retrograde discharges from motor nerve endings in veratrinized muscle found quite another relation. They found that the frequency of the after-discharge was quite independent of the veratrine concentration, that the smallest concentration that led to after-discharge produced the longest after-discharge, and that the duration of the after-discharge decreased with increasing veratrine concentration.

Acheson and Rosenblueth (1941), also reported that with increasingly severe veratrinization the negative after-potential increases while the repetitive response may decline. When frog nerves are painted with veratrine solutions the after-discharge appears for a short time before the negative after-potential is very large, but it disappears while the negative after-potential is increasing (Feng, 1941). Feng (1941) also found that upon aeration following anoxia, the negative after-potential increased to a value greater than before anoxia while the repetitive response remained depressed.

In their extensive survey of the literature, Krayer and Acheson (1946), have found that while an increased negative after-potential with veratrine treatment has been reported to occur without

the after-discharge, after-discharge has never been reported without the increased negative after-potential. They conclude: "A general correlation certainly exists among these three aspects of the veratrine response, namely, negative after-potential, supernormality, and repetitive response. The discrepancies noted above, however, indicate that other factors not now understood play a significant role."

I would suggest that the conditions required for an after-discharge as prescribed by the differential repolarization mechanism might well serve to resolve many of the "discrepancies". Wible (1924 a, and b)showed that a local veratrine treatment of a nerve was sufficient for the production of a repetitive response following s stimulus applied to the nerve and many workers have since taken to applying veratrine locally. However, if the veratrine is free to diffuse along the surface of the fibers, the effect of the local application in the production of the after-discharge might well be annulled, while the amplitude of the negative after-potential at the point of application is increasing.

Another way in which it is possible to obtain after-discharge is by the uniform treatment of fibers which are not uniformly sensitive to veratrine along their surfaces. When Feng (1941), applied veratrine uniformly to frog sciatic nerve he found that the finer end of the nerve was more sensitive to veratrine (as indicated by the size of the negative after-potential), and that the repetitive action potentials originated in this region of greater sensitivity no matter which portion of the nerve received the initial stimulus. Dun and Feng (1941a), found it easy to obtain after-discharge from motor

nerve endings in the muscle which were also considerably more sensitive to veratrine than the attached nerve trunk.

In this respect, the nerve-muscle preparation, which is frequently used in veratrine experiments, is unusually complex. Dun and Feng (1941a), have reported that the order of veratrine sensitivity in this preparation is: muscle > motor nerve endings > nerve trunk. Since the motor nerve endings can after-discharge so readily, they have to be considered even after the motor nerve has been cut. The relation between the end plate potential set up by the repeating nerve endings and the effect of curare treatment is rather complex and it will be discussed later after some experiments concerning this phenomenon have been presented.

A general outline of the experiments to be presented in this section has been given above (pg 15). A major concern of these experiments was a comparison of local and universal veratrine treatment. The experiments show just how the increased and prolonged negative after-potential causes a repetitive discharge. In so doing they will help to explain many of the discrepancies mentioned above. They also show that the veratrine after-discharge is primarily due to a differential repolarization mechanism.

B. Materials and methods

1) Preparations. - In a few of the experiments described in this section the Extensor digitis longis IV was used. It was used only in the perfusion experiment that will be described below and when this procedure was abandoned the muscle was no longer used because it was too short to be used in most of the other recording arrangements.

In all the other experiments, the sartorius muscle of the frog was used. In some experiments nerve-muscle preparations were obtained and used. The usual procedure, however, was to disect the muscle free from the animal and then pin it out on a board. Small strips were cut from the muscle under a disecting microscope so that the cuts ran parallel to the muscle fibers and from end to end. A strip usually contained some 50 muscle fibers and was about 1 mm. in cross-sectional diameter. Fine silk thread was tied around each end of the muscle strip and the preparation was placed in the bath arrangement to be used.

2) Bath arrangements. - The saline bath used in most of the experiments described in this section is shown in Fig. 1. The bath was constructed from perspex (lucite) in such a way that we could either apply drugs to one section of the length of muscle fibers or apply them universally to the whole of the preparation. Nine holes (H) of 6 mm. diameter were drilled in a perspex block to a depth of 13 mm. The centers 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.,

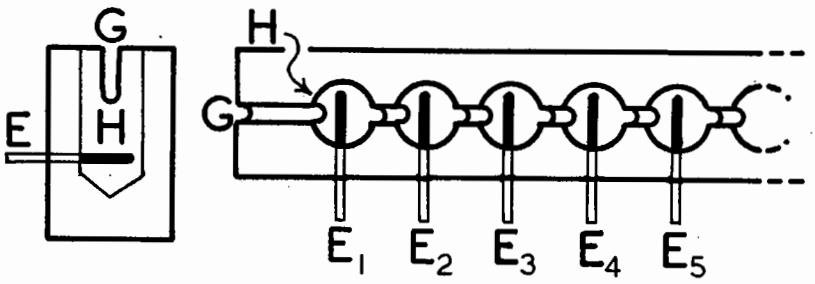

Figure 1. - A diagram of the perspex bath used for mounting strips of the frog's sartorius muscle. For description see text.

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.

The muscle strips which were prepared as described above, were lifted into the slot of the saline baths, with the distal end in bath 1. In order to insulate the baths from one another electrically, and to prevent interchange of fluid, petroleum jelly was packed around the muscle in the groove joining the baths.

In some experiments, muscle strips prepared as described above were mounted in a moisture chamber or in mineral oil. Neither method proved very successful. The muscle strips and even whole muscles seemed particularly sensitive to mounting in mineral oil.

Another method of mounting the muscle strips was to suspend the muscle in a vertical position. The threads attached to the ends of the muscle strip were fastened to opposite sides of a perspex frame which looked very much like a picture frame. The frame was held in an upright position and solutions were dripped onto the top of the muscle strip and onto some other point along the strip (Fig. 9). A thin strip of filter paper was placed onto the muscle strip in order to assure a smooth flow of fluid down the strip. Although this method proved very successful in allowing me to obtain repetitive responses with veratrine treatment, in other respects it proved very difficult to control and so was not frequently used.

3) Solutions . - In all the experiements to be described in this section, Normal Ringer's solution (Table 1), containing d-tubocurarine chloride sufficient to make a concentration

of 10^{-4} was used. In some of the earlier experiments, 10^{-5} d-tubocurarine chloride was used, but this was found to be insufficient to properly block neuromuscular transmission. (For a further discussion of this point see pp 30-32).

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

4) Recording system. - The recording system was essentially the same as described above (pg 21). Most of the records were made using the silver-silver chloride electrodes fixed in the base of the baths. Occasionally, however, a microelectrode filled with soft solder, (Burns and Grafstein, 1952), was used for recording the repetitive activity from one or a few muscle fibers.

In the experiments in which the saline bath was not used, silver-silver chloride electrodes were placed directly on the muscle strips. These electrodes were used for recording, grounding and stimulating the muscle. Occasionally, however, platinum electrodes were used for stimulating.

C. Experiments and Results

1) A comparison of local and general veratrine application. - From the above discussion, it will be realized that if differential repolarization is the major cause of the repetitive response, local veratrine application should be far more effective in producing after-discharge than its application to the whole fiber. For this reason, the initial experiments were designed to make this comparison. The sartorius muscle was chosen for this experiment both because it contained long parallel fibers that extended from one end of the fiber to the other and because its fibers were uniformly sensitive to veratrine (Kuffler, 1945). However, since the motor nerve endings are likely to produce a repetitive response if the intrinsic nerve supply is stimulated along with the muscle when the preparation is treated with veratrine, it was necessary to block neuromuscular transmission.

Accordingly, experiments were performed to determine the concentration of d-tubocurarine necessary to completely block neuromuscular transmission and it was found that a concentration of 10^{-5} was sufficient. It was soon found, however, that with this concentration of d-tubocurarine, muscle strips uniformly treated with veratrine usually produced after-discharges and that a region roughly corresponding to an end-plate region seemed more sensitive to veratrine as determined by the size of the negative after-potential. It was, therefore, necessary to determine the effect of various concentrations of d-tubocurarine on the response of the sartorius muscle uniformly treated with veratrine. Fig. 2 shows the result of such an

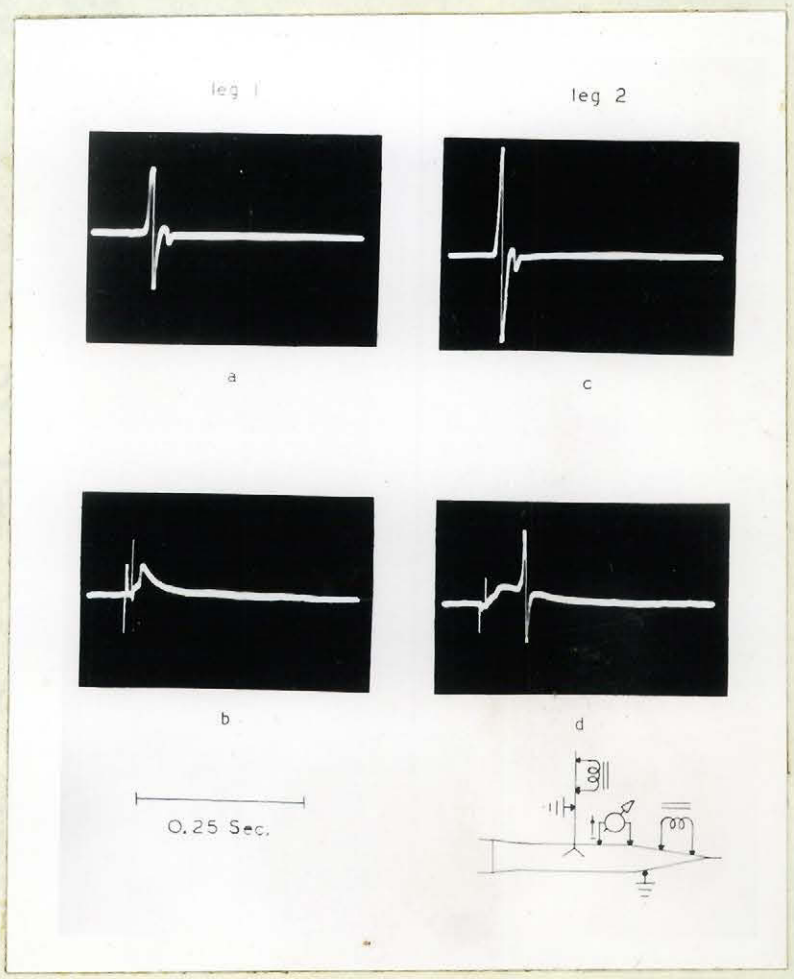

Figure 2. - Effect of veratrine treatment on the end-plate potential of the sartorius muscle. (a) and (c) control responses of the two muscles from a single frog. (b) response of leg 1 after immersion in 10^{-7} veratrine sulphate plus 10^{-5} d-tubocurarine for 15 min. followed by 5 min. in mineral oil. (d) response of leg 2 after immersion in 2.5×10^{-7} veratrine sulphate plus 10^{-5} d-tubocurarine for 15 min. followed by 5 min. in mineral oil.

experiment.

Fig. 2a and c show the response of the two sartorii from a single frog to indirect stimulation. The sartorius, from leg 1, was next placed in Ringer's solution containing 10^{-7} veratrine sulphate and 10^{-5} d-tubocurarine and the sartorius from leg 2 was placed in Ringer's solution containing 2.5×10^{-7} veratrine sulphate and 10^{-5} d-tubocurarine. After 15 minutes, these solutions were replaced by mineral oil. Fig. 2b and d show the response of the muscles to indirect stimulation five minutes after being placed in the mineral oil. The end-plate potentials of both muscles show a staircase like rising phase which is probably caused by the repetitive response of the motor nerve endings. The repetitive response in the preparation treated with the stronger veratrine lasts longer and eventually leads to a response of the muscle which involves a large portion of the muscle fibers. The muscles were then stimulated directly. After-discharge was seen in the response of the muscle from leg 2.

Similar experiments were performed using paired muscles but only direct stimulation was used. The muscle pairs were soaked in 10^{-7} veratrine sulphate solutions for periods of 30 to 45 minutes and one of each pair was simultaneously treated with d-tubocurarine. A repetitive response was always found when no d-tubocurarine was applied. It was also found that while 10^{-5} d-tubocurarine reduced the repetitive response, 10^{-4} was needed to completely eliminate it. Therefore, 10^{-4} tubocurarine was used in most of the experiments reported below. However, for reasons which will be given below (pg 50), it might have been better to use 10^{-3} d-tubocurarine.

With muscle strips mounted in the saline bath, a few fibers began to give a repetitive response after local exposure to 10^{-7} for 5 min. The veratrine solution was usually applied in two adjacent baths (baths 1 and 2 of Fig. 1) of the seven baths through which the muscle passed; the threshold concentration for the repetitive response to a single stimulus (usually 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 10^{-6} or greater usually blocked transmission. Once the required concentration for a threshold response had been found, the repetitive response could be maintained indefinitely. Test stimuli given once every half minute or minute would elicit the after-discharge in repeatable fashion for more than five hours. Occasionally, the repetitive response would disappear after about half an hour of testing, but it was always possible to show that on these occasions the veratrine had leaked from the baths to which it was applied into a neighboring bath. As a precaution against this failure to localize the action of the drug, the bath next to that which contained the veratrine solution (usually bath 3 of Fig. 1) was usually perfused with a slow flow of tubocurarine-Ringer's solution.

When veratrine was supplied to baths 1 and 2 but the rest of the muscle was bathed with tubocurarine-Ringer's solution, there was a great difference in the repolarization rates of the veratrinized and untreated lengths of muscle fiber 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 fibers were 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.

In contrast to the efficiency of the local application of veratrine in producing after-discharge, the application of the veratrine in the same concentration to all the baths through which the muscle passed caused no repetition following a single stimulus. This same effect was noted in the previous experiments when the whole sartorius muscle was soaked in solutions containing the same concentration of veratrine (10^{-7}) and sufficient d-tubocurarine.

Soon after the application of veratrine to the whole length of the muscle strip there was usually after-discharge, but within a few minutes the muscle fibers reverted to a state in which a single stimulus produced a single response, and stayed in this condition indefinitely. The results of Fig. 3, which are from a modification of this experiment, illustrate this point. Fig. 3a shows the response to stimulation of a muscle strip which had been soaked in 10^{-7} veratrine solution of 80 minutes 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. Removal of the veratrine from bath 3 was then begun by perfusion with tubocurarine-Ringer's solution; Fig. 3b shows a single after-discharge occurring probably in one muscle fiber of the bundle, 20 min. after

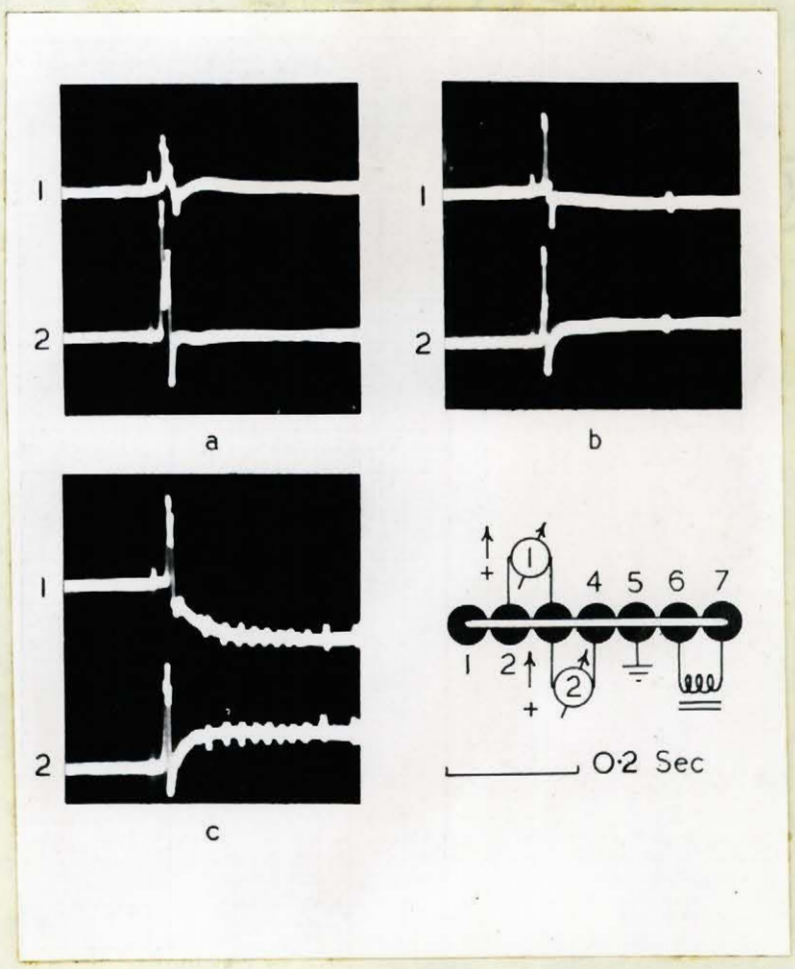

Figure 3. - The responses to a single stimulus of the sartorius muscle to general and to restricted application of veratrine. (a) After immersion in 10^{-7} veratrine sulphate for 80 min. (b) 20 min. after beginning of continuous perfusion of bath 3 with tubocurarine-Ringer solution, in order to wash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

washing was begun. Fig. 3c shows the after-discharge recorded after bath 3 had been washed through for 2 1/2 hr. Fig. 3b and c also show 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. 3 the driven action potential is passing from right to left along the muscle strip, and channel 1 records its passage through baths 3 and 2 as an upward, followed by a downward, 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. 3b and c, channel 1) 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 muscle strip passes, contain veratrine except one. The repetitive response shown in Fig. 4a was obtained as repeatable phenomenon, when baths 1,2,4,5,6 and 7 contained 0.25×10^{-6} veratrine solution, and bath 3 contained only tubocurarine-Ringer's solution. The diphasic records make it clear that muscle fibers treated with this comparatively low concentration of veratrine can transmit a series of action potentials at a high frequency. Soon after bath 3 had been filled with the same concentration of veratrine so that the whole muscle was submerged in a uniform veratrine concentration, the after-discharge to the test stimulus disappeared (Fig. 4b). Finally, when the veratrine in

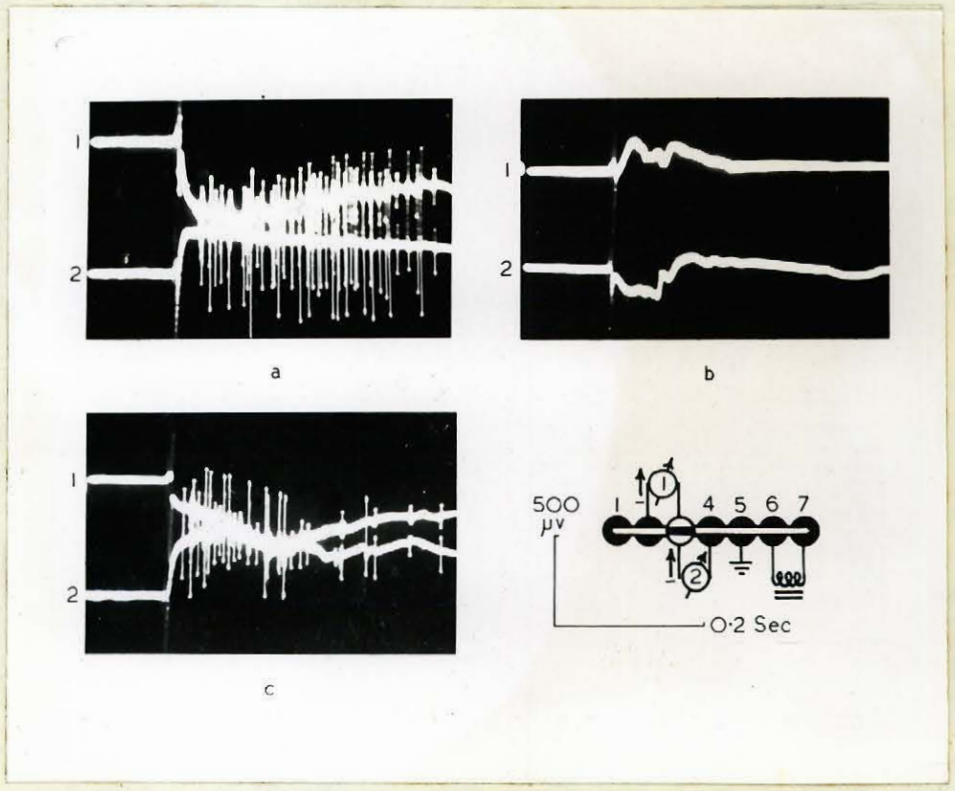

Figure 4. - The responses to a single stimulus of the sartorius muscle to general and restricted application of veratrine. (a) 0.25×10^{-6} veratrine sulphate in all baths except 3. (b) 47 min. after 0.25×10^{-6} veratrine sulphate added to bath 3. (c) 70 min. after beginning continuous perfusion of bath 3 with tubocurarine-Ringer solution in order to wash out the veratrine.

bath 3 was washed out with Ringer's solution, the after-discharge returned (Fig. 4c). Note that the difference in repolarization rates also returned.

2) 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 a single stimulus if the veratrine is locally applied, and not if it is uniformly applied over the length of the muscle fibers. 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 veratrinized muscle, and it is this current flow at the boundary of veratrinized and normal muscle which presumably causes the origin of an action potential. (But see section V below.) 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 between veratrinized and normal muscle during recovery from the driven action potential is not sufficient to trigger an after-discharge. Seven min. later the potential difference is adequate for after-discharge (Fig. 5d and e).

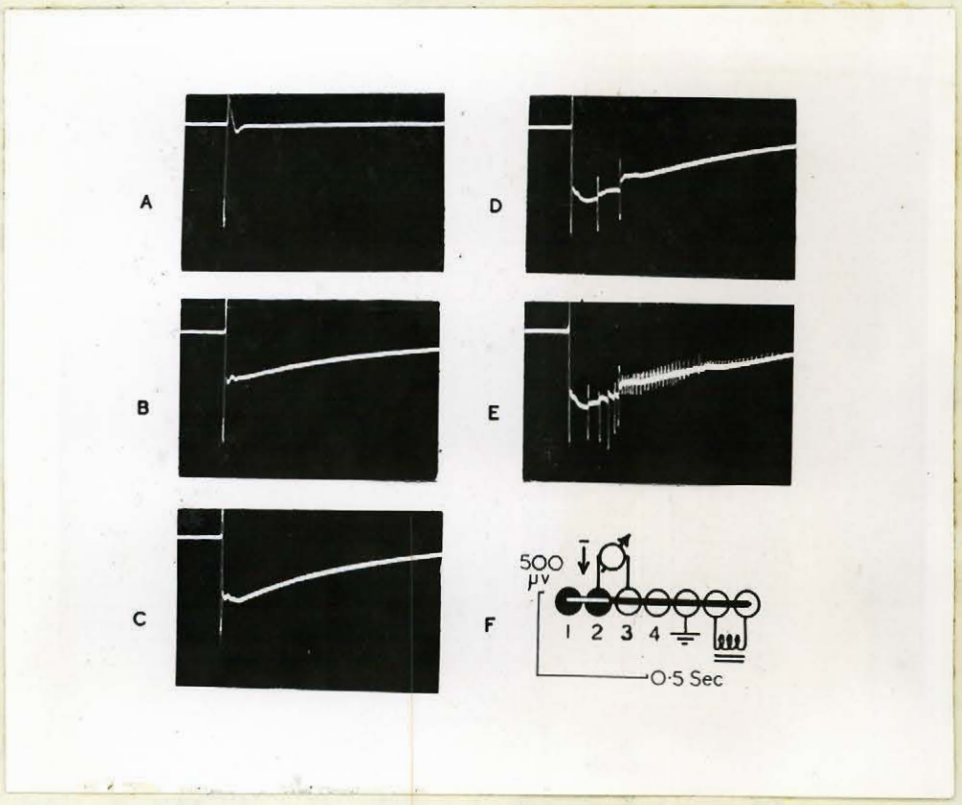

Figure 5. - Responses to a single stimulus in a small bundle of fibers from the sartorius muscle during the progressive application of veratrine sulphate. (a) Control. Whole of muscle in tubocurarine-Ringer solution. (b) 34 min. after 0.75×10^{-7} veratrine sulphate in baths 1 and 2. (c) 12 min. later.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

That the threshold shown in Fig. 5 is not simply a difference in repolarization between any two points on the surface of the fibers, but a sufficient gradient of membrane potential between two adjacent points, can be shown by an experiment such as the following: In the experiments described so far, the veratrine solution was either applied locally to one stretch of muscle fiber or was applied to the whole fiber. In other words, either there was a maintained concentration gradient of the drug down the length of the fibers or the concentration gradient was made zero. If the necessary condition for after-discharge is a sufficient concentration or potential 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 neighboring baths. In the experiment whose results are shown in Fig. 6, 0.5×10^{-7} veratrine was first added to baths 1 and 2. This concentration of veratrine was only just above threshold for after-discharge of the fibers whose activity is shown in Fig. 6a and b. When 0.25×10^{-7} veratrine was added to bath 3 the repeated response to a single stimulus soon disappeared, (Fig. 6c and d), and only returned again when the veratrine in bath 3 was washed out, (Fig. 6e and f). The experiment of Fig. 6 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 the experimental conditions. Moreover, it is easy to see that the results of Fig. 6 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 po-

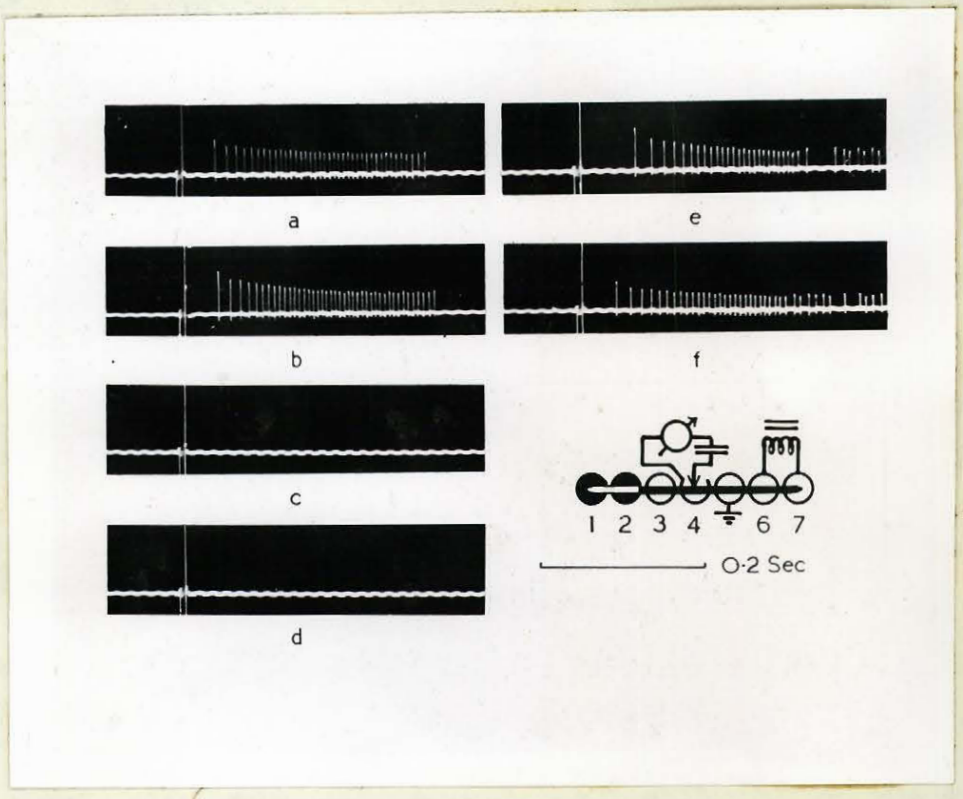

Figure 6. - Responses to a single stimulus of a muscle fiber in the sartorius to local application of veratrine sulphate, recorded from a metal micro-electrode in bath 4. (a) 0.5×10^{-7} veratrine sulphate in baths 1 and 2 only (for 34 min.). (b) Same as (a) 4 min. later. (c) 2 min. after beginning of 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.

tential gradients between baths 2 and 3 and between baths 3 and 4 both be below the threshold for after-discharge.

A similar effect was noted when we attempted to obtain after-discharges using muscle strips mounted horizontally in a moisture chamber. The muscle was kept in tubocurarine-Ringer's solution for at least an hour before mounting. A drop of veratrine solution was placed on one end of the muscle, usually by applying a small piece of filter paper which was moistened with the solution. After a few minutes after-discharge followed each stimulus (given every 1/2 to 1 min.), but this condition lasted only 5 to 10 min., after which there was a single response to each stimulus. In this condition there was a large difference in repolarization rates between the two ends of the muscle, but there was only a gradual potential gradient in between. Once a muscle strip was in this condition, adding more veratrine to the end of the muscle or adding it in a stronger concentration usually did not bring back the after-discharge.

On the other hand, the potential gradient could be increased in the following manner. Fig. 7 shows the consequence of two driven action potentials on a preparation mounted in the saline bath. The potential gradient between normal and veratrinized muscle is only sufficient to trigger a repetitive response from two fibers 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 fibers.

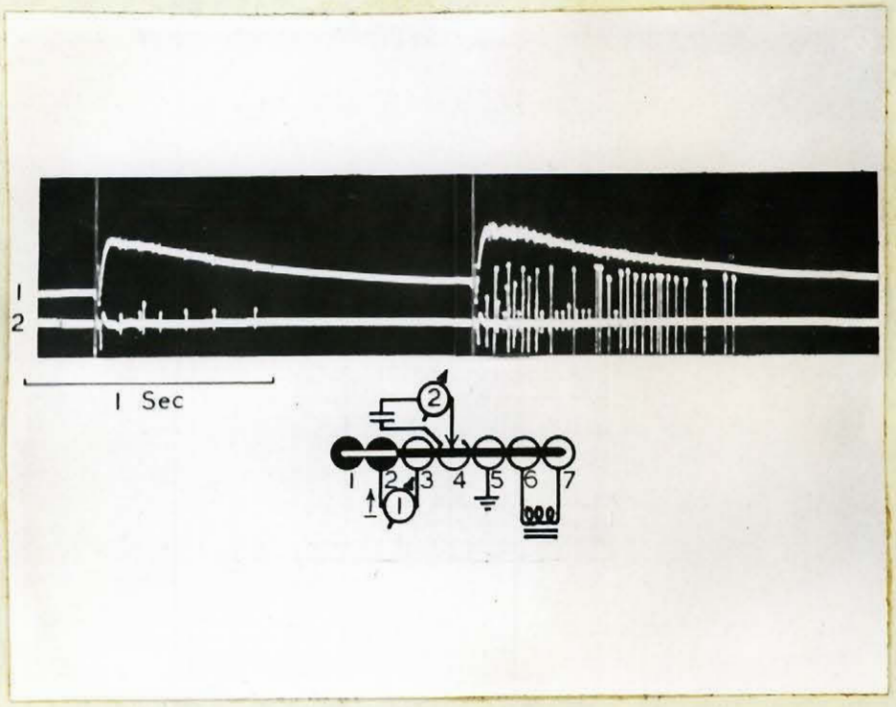

Figure 7. - The responses of a small bundle of muscle fibers to two stimuli in the presence of local veratrine. Channel 1 shows a D.C. record from baths 2 and 3. Channel 2 records the activity of a few fibers with a metal microelectrode in bath 4. 10^{-7} veratrine sulphate in baths 1 and 2.

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 expected, the probability of a stimulus causing an after-discharge is also increased by prolonging the rest that precedes the triggering stimulus.

3) Modification of the after-discharge 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 of fiber exists. For any one muscle fiber the value of this potential gradient will depend upon the magnitude of the peak local concentration of veratrine, the effect of this concentration on the negative after-potential, and upon the concentration gradient down the length of the fiber.

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 fibers. 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. 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 an after-discharge (as in Fig. 8a) then the cathodal polarization increased the repetitive response following a driven action potential (Fig. 8b). In the experiment of Fig. 8, this increase was slight. As would be expected, anodal polarization of the veratrinized stretch of muscle fiber subtracts from the effect of veratrine so that the addition of the anode to bath 2 stopped the after-discharge (Fig. 8d). The reverse effects were seen when the polarizing currents were switched off. Immediately after removal of the cathode the veratrine after-discharge was decreased (Fig. 8c); after removing an anode the discharge in response to the single test stimulus increased (Fig. 8e). It is known that the polarizations forced on the membrane by passing current through it for 1/2 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 causing the relatively slow repolarization of veratrinized membrane; in this way the after-discharge is increased. (See Section IV below).

4) Another method of local veratrine application. - Aside from the technique used in most of the experiments described above, two other methods of veratrine application that lead to the production of after-discharge, have been described. One is to

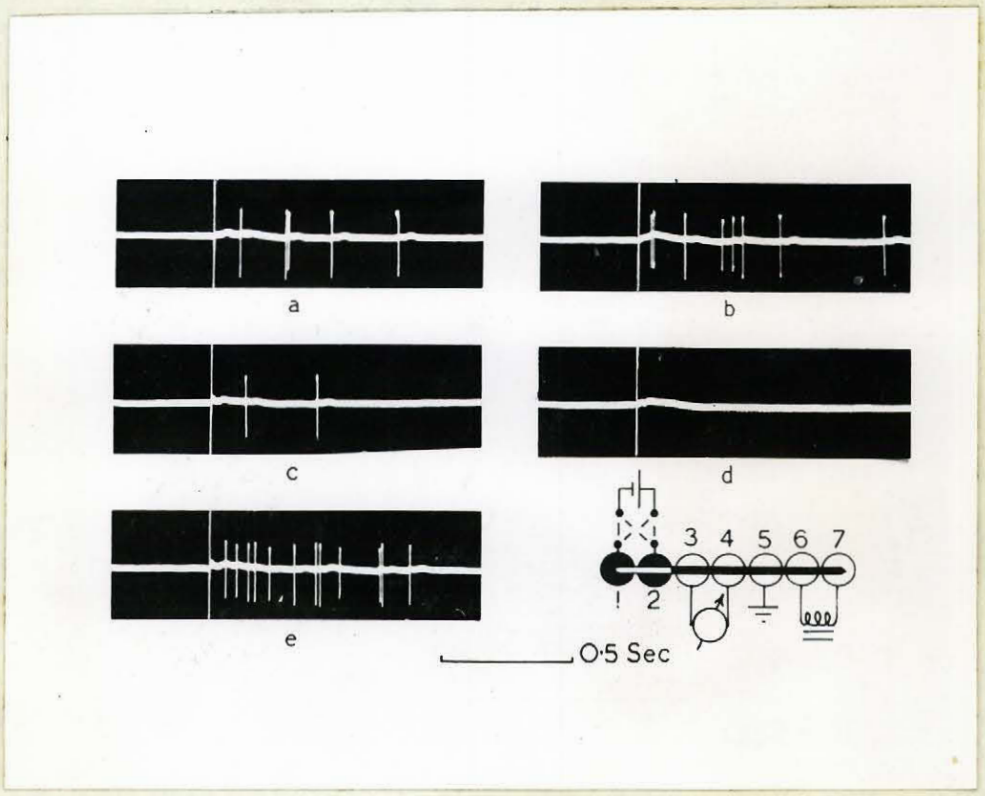

Figure 8. - The responses to a single stimulus of (probably) one of a small bundle of muscle fibers during and after various polarizing currents in the presence of local veratrine. 2 X 10^{-7} veratrine sulphate in baths 1 and 2 only. Polarizing currents applied between baths 1 and 2. (a) control. Polarizing 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.

place a drop of veratrine solution onto a muscle strip, horizontally suspended in mineral oil or in a moisture chamber, and the other is the universal application of a veratrine solution to a preparation which is not uniformly sensitive to veratrine. Fig. 9 presents another method for the local application of veratrine solutions. Although with this method of local veratrine application, repeatable and reproducible after-discharges could be obtained, the technique proved to be as unsatisfactory as the other two methods, for most of the experiments I wished to perform. However, some interesting observations were made while this method was being tested for its usefulness.

Tubocurarine-Ringer's solution, from the appropriate reservoir, was run onto the top of the strip of filter paper attached to the muscle at the rate of a few cc. per min. The veratrine solution was applied in much the same manner to a point further down the muscle. The constant flow of fluid from above prevented any significant spread of the veratrine above its point of entry along the muscle fibers. This point was checked both by the stability of the after-discharge phenomenon, and by the lack of any delay in repolarization above the point of veratrine application. (A delay in repolarization only occurs in portions of the membrane which are directly exposed to veratrine.).

As was mentioned above, in the experiments using the saline bath, when the conditions were such that a single stimulus applied once every minute always produced an after-discharge, stimuli given at the rate of two per min. would never produce an after-

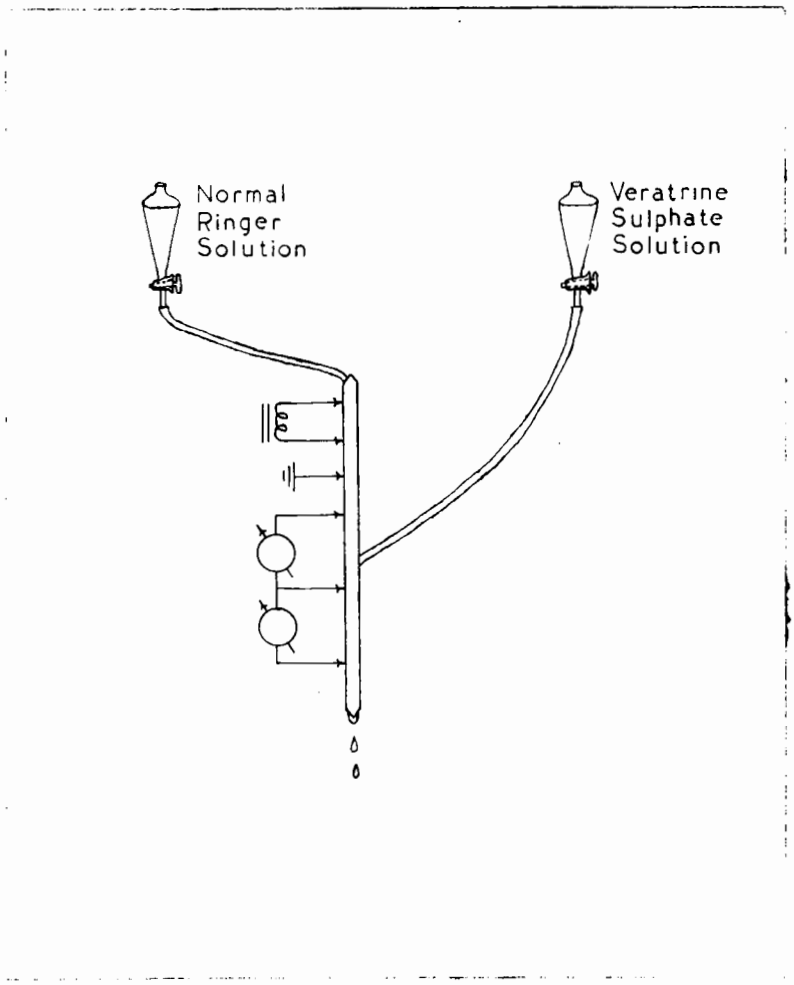

Figure 9. - Perfusion arrangement for obtaining local veratrine application. The lower two recording electrodes were moveable to locate the site of origin of repetitive responses and the potential gradient along the muscle.

discharge. The shortest time interval that I ever found to produce more than one after-discharge in any experiment using the saline bath was 15 sec. But at this rate of stimulation the after-discharges soon failed to appear, and it was more common to obtain preparations which needed a two minute interval between stimuli. This finding is so common that the necessity of a fairly large rest period between stimuli, has been considered to be a basic property of the veratrine repetitive response (Krayer and Acheson, 1946). However, using the method illustrated in Fig. 9, it was possible to obtain an after-discharge following each stimulus when the muscle was stimulated once every 3 sec. This was never seen to fail, however the longest test that was timed lasted 3 min. Thus some 60 after-discharges were obtained in a period of 3 min. without any sign that they were going to stop. Longer periods were tried but unfortunately not timed since the experiments being performed with this technique were of an exploratory nature.

D. Discussion of Results

In this paper concerning "The mechanism of after-bursts in cerebral cortex", Burns (1955) suggested that the repetitive response of veratrinized muscle following a single stimulus, might be brought about by the mechanism which he called "differential repolarization". The experiments described in this section support this hypothesis. The results of these experiments, moreover, have yielded some additional information concerning the details of this mechanism. Since the following sections also contain experimental results which have a bearing on this question, discussion will be deferred until the end of the thesis when I will be able to use all the available pertinent information.

However, there are some points that are best dealt with here. It has been suggested that veratrine has a "decurarization" effect on neuromuscular preparations (Copee, 1943; Goutier, 1950). These authors found that when the frog sartorius nerve-muscle preparation was treated with sufficient curare to just block neuromuscular transmission, subsequent treatment with veratrine would cause the muscle to contract following indirect stimulation. They also observed the "staircase like" end-plate potential (see Fig. 2) that has been observed by others (Dun and Feng, 1940a; Kuffler, 1945). Since Eichler (1938) had reported that there were no retrograde discharges from motor nerve endings, it was felt that the veratrine acted directly on the end-plate to cause a rhythmic response following indirect stimulation. That a repetitive response of motor nerves

treated with veratrine can occur following a single stimulus was clearly shown by Wible (1924a and b), and that retrograde discharges from motor nerve endings in veratrinized muscle do occur was clearly shown by Dun and Feng (1940a). Certainly, the assumption that the end-plate response under these conditions is independent of nervous activity, can no longer be held without additional proof. Moreover, just the opposite condition seem to be the case.

The term "decurarization" implies, as the authors who use this term probably mean it to imply, that the action of curare is inhibited. Since curare specifically inhibits the action of acetylcholine and when given in reasonable doses has no other action on the neuromuscular junction (Eccles, 1953), one would expect that if some substance inhibits the action of curare its effect would be to permit the normal action of the acetylcholine which is released following stimulation of the nerve. Thus if the action of curare was completely inhibited a normal action potential would occur following a single stimulation of the nerve, if the action of curare was only partially blocked then an action potential smaller than normal, due to transmission across only some of the junctions, would be observed, and if the curare action was only slightly inhibited then all that would be expected would be an augmented end-plate potential. But under the conditions that we have been discussing this is not observed. The end-plate potential is not initially augmented but it is prolonged (Dun and Feng, 1940a; Coppee, 1943; Kuffler, 1945; this thesis, Fig. 2). Then it must be assumed that rather than decreasing the effect of curare, the veratrine must in some way prolong the

action of the acetylcholine that is released. Eserine is known to prolong the action of acetylcholene and when a curarized nerve-muscle preparation is treated with eserine the end-plate potential is increased and prolonged (Feng, 1940; Eccles, Katz and Kuffler, 1942). But in this case the end-plate potential is quite smooth in appearance with a gradually rising phase. In fact, the end-plate potential of the curarized and veratrinized nerve-muscle preparation looks far more like the end-plate potential produced in curarized muscle by tetanic stimulation of the nerve (Feng, 1940) than produced by eserine treatment (which does not produce retrograde discharges in the motor nerve - Dun and Feng, 1940b).

This point has been dwelt upon in length not because of its importance to the over-all problem of this thesis, but because it is a typical example of the faulty reasoning and bad terminology that has consistently hampered the study of the effects pf veratrine on nerve and muscle. Even if we were to exclude the possibility of a repetitive nerve response, nowhere was it shown that veratrine had specifically inhibited the action of curare. When it is shown that two substances have opposite effects on a preparation, it does not necessarily mean that one is inhibiting the effect of the other. In fact, when both substances have effects when they are given separately and these effects are opposite in their nature, it is generally assumed that they are antagonists rather than inhibitors. In much the same way when two substances have similar effects when given separately, it is not reasonable to assume without further proof that when they are given together one simply potentiates the action of the other or simply sensitizes the preparation to the action of the other.

A typical case in point is the well known theory (Bacq, 1939a and b; Bacq and Goffart, 1939) that the sole action of veratrine is to sensitize the muscle to the action of external K^+ ions. Bacq (1939b) further states that the reason why the veratrine contracture is only evident following a contraction, is because the initial contraction liberates active K^+ ions, ions to which the muscle fibers are sensitized by the action of veratrine. The sensitization was shown in a very simple manner as follows: a dose of KCl is found, which, when added to the solution bathing an isolated frog rectus muscle, will just cause the muscle to contract slightly. The muscle is next bathed in a solution containing veratrine. The muscle does not contract during this time, but when the same concentration of KCl that previously caused a small response is now added to the bath the strength of the muscle contraction is several times greater than before.

But this result is just as easy to explain if we completely reject the hypothesis that veratrine sensitizes the muscle to the action of K^+ ions. It is well known that the tetanic veratrine response or the veratrine "contracture" does not occur unless there is a previous response of the muscle (Bacq, 1939b, Krayer and Acheson, 1946) and it is further known that this initial response releases K^+ ions (Bacq, 1939b) and presumably so do the subsequent impulses of the after-discharge (Hodgkin and Huxley, 1947). Now, if we further assume that the veratrine does <u>not</u> sensitize the muscle to the external K^+ ions, what is the sequence of events during this experiment? When the small dose of KCl is administered it caused the response of a few of the fibers. This response causes both an

increase in external K^+ concentration and the tetanic veratrine response. The tetanic veratrine response causes a larger contraction of the few muscle fibers involved and also a greater release of K^+ ions. The increased external K^+ concentration would now excite those fibers that just failed to be stimulated at the initial K^+ concentration and more fibers would become involved in the veratrine response. Thus the response would grow to several times the size obtained without the presence of the veratrine.

The fibers involved in the repetitive response may be either the intrinsic nerve fibers (Brown and MacIntosh, 1939), the muscle fibers, or both. Actually, the spread of excitation in the series of events that I have described is not a necessity in the explanation of the results of the experiment, since a tetanic response in the same number of fibers would also lead to a several fold increase in the strength of the muscle contraction. In this respect, it is interesting that the perfusion experiment (Fig. 9), in which the conditions were most unfavourable for any increase in external K^+ concentration, presented the most favourable conditions for the production of after-discharge.

It is interesting to speculate on just why this experiment (Fig. 9) presented such favourable conditions for the production of after-discharges. It may be that the K^+ ions that leak out of the fibers during the passage of an impulse actually hinder the production of after-discharges and that the constant perfusion removes the excess K^+ ions. It has been reported that excess K^+ ions hinder the repetitive response in veratrinized nerve fibers (Dun and Feng,

1940a; Feng, 1941). Or possibly some other substance that inhibits the veratrine negative after-potential may be released during activity. In either case, the rest which is needed between stimuli under most experimental conditions, would represent the time needed for the released substance to diffuse away from the surface of the membrane. On the other hand, the veratrine at the surface may be used up during an after-discharge, and time would be needed for the veratrine concentration to again reach a sufficient level at the cell surface. Or some other substance may be lost from the surface of the membrane. In any event, it seems likely that the effect would be on the production of the veratrine negative after-potential rather than directly on the after-discharge since under the conditions where a rest between stimuli is needed, the negative after-potential (hence the potential gradient) is reduced, when the after-discharge is eliminated.

IV. THE EFFECTS OF VERATRINE TREATMENT ON THE FIBER MEMBRANE

A. Introduction

The two most striking features of the electrical activity of veratrine-treated nerve and muscle are the repetitive response and the delay in repolarization (or augmented negative after-potential) following an action potential. The tendency of most workers has been to consider them to be two expressions of the same fundamental process (Krayer and Acheson, 1946). When one is looking at an oscillograph tube, the sight of a series of discharges is much more inspiring than a slow drift in the base line, and, possibly for this reason, most workers have chosen to work with the repetitive response. Of course, the frequent occurrence of repetitive responses in normal nerves under both physiological and altered conditions also has had a part in determining this preference.

Here then, are two assumptions that have frequently been held at the start of investigations on the properties of veratrinized nerve and muscle. One, that the repetitive response and the delay in repolarization are expressions of one basic process, and the other that the mechanism of the repetitive response obtained with veratrine treatment is the same mechanism that produces all (or some) of the repetitive responses obtained under different conditions. There is yet a third assumption that has frequently been held. It is that the delay in repolarization following an action potential in veratrinized nerve and muscle is simply an increase or accentuation of the normally occurring negative after-potential. Frequently, the experi-

mental procedure is to compare the veratrine response with the normal or simply to study the veratrine response, and on the assumption that there is only one process involved, to use the results in trying to explain or understand the normal. Both these approaches leave one somewhat dissatisfied, the former because so little is usually known concerning the normal process, the latter because you can never feel sure just how true the initial assumption is and just how far you are justified in lumping the findings on the veratrine and the normal process.

The "differential repolarization mechanism" process proposed above clearly divorces the repetitive response from the delay in repolarization. That is to say, they are not expressions of the same basic process. Although the delay in repolarization is needed in setting up the conditions for the repetitive response, there need be nothing fundamentally the same in the two mechanisms involved. This relation is clearly shown by the fact mentioned above, that under appropriate conditions large "negative after-potentials" can be obtained with veratrine treatment without any after-discharge, but under no conditions can a repetitive response be obtained without the delay in repolarization. This does not mean that some other factors associated with the "negative after-potential", such as increased excitability, have no effect on the repetitive response, but it does mean that these factors have only a modifying influence and the basic mechanism can be studied without referring to these modifying factors. This concept has proved to be essentially correct and it has been of tremendous value in the study of the repetitive response.

At the conclusion of the experiments described in the previous section I had a fairly satisfactory explanation of the basic mechanism underlying the production of the repetitive response in veratrinized nerve and muscle, but I had no idea how the delay in repolarization (which I will call the "veratrine negative after-potential" for the sake of brevity) was caused. This section is concerned with a study of the veratrine negative after-potential. I must admit that my primary motivation for this study was curiosity, but there were also other reasons for this study. The information to be gained is essential for any intelligent examination of the other mechanisms proposed to explain the veratrine after-discharge, since almost all these mechanisms have considered the repetitive response and the veratrine negative after-potential as expressions of the same basic phenomenon. This information is also essential to a more detailed examination of the differential repolarization mechanism in veratrinized muscle.

At first, I had hoped that I could find a systematic study on the normal negative after-potential in the literature, which I could use as a basis or at least a starting point for the study of the veratrine negative after-potential. However, I soon realized that most other workers had approached this problem from the other direction and had used the veratrine negative after-potential as a model for the normal negative after-potential. Although the basic assumption in these experiments might be entirely incorrect, it is easy to understand why this approach was selected. It is far easier to study the O_2 consumption (Schmitt and Gasser, 1933), the heat production

(Hartree and Hill, 1922), and the effects of an altered environment (Graham, 1933, and 1934), on a process that lasts several seconds than on a process that lasts a few milliseconds.

Probably the most recent study specifically concerned with the negative after-potential of frog's skeletal muscle is that of Bremer (1955). Although little of the information contained in this paper is new or startling, it does contain a systematic review and study of the effects of various environmental factors on the normal negative after-potential. An interpretation of these results is limited by the techniques used. In this respect the results are similar to all previous work in which the response of a whole muscle or nerve is recorded with external electrodes.

I would like to briefly summarize the knowledge gained concerning the negative after-potential of nerve and muscle using this method. Most of this information can be found in the papers of Krayer and Acheson, (1946) or Bremer (1955). The normal negative after-potential of frog's skeletal muscle is independent of curare treatment and the tension on the muscle, it increases and decreases along with the temperature, it is decreased more than the spike by metabolic inhibitors, it is decreased by increasing the external concentration of either K^+ or Ca^{++} and it is increased in size and duration by veratrine treatment. The normal negative after-potential of frog's nerve on the other hand is increased by increasing the external Ca^{++} and decreased by increasing the external K^+ , and anesthetics.

Using more modern methods of study, such as recording potential changes with inside-the-cell microelectrodes (Graham

and Gerard, 1946; Nastuk and Hodgkin, 1950), determination of fiber membrane constants and changes (Hodgkin and Rushton, 1946; Katz, 1948; Fatt and Katz, 1951; Cole and Curtis, 1939) and the newer concepts of membrane activity (Hodgkin and Katz, 1949; Hodgkin and Huxley, 1952d), it should be able to determine the physical chemical processes producing both the normal and the veratrine negative after-potentials. A vigorous attach on the problem of the veratrine negative after-potential along these lines has been made by Shanes (1949 a,b, 1951, 1952a, b). His conclusions, based mainly on studies using invertebrate axons, may be summarized somewhat as follows (Shanes, et al., 1953): The veratrine negative after-potential may be divided into two phases, an initial rapid exponential decline and a much slower exponential decline (Shanes, 1952a). The slow decline is related to the increase of external K^+ following activity and its removal, and the rapid decline is associated with the liberation of the K^+ from the inside of the axon. As is admitted, there are several difficulties with this interpretation (Shanes, et al., 1953). In this theory, both the normal and veratrine negative after-potentials are considered to arise in the same manner. The more prolonged action with veratrine treatment is presumably due to a greater leakage of K^+ during activity. Recently, a more detailed analysis of the normal negative after-potential of the squid giant axon has appeared (Frankenhaeuser and Hodgkin, 1956). They propose that the normal negative after-potential is caused by the trapping of the K^+ that leaks out of the fiber during the passage of an impulse, in a space about the axon approximately 300Å thick, from which free diffusion into the surrounding fluid

is inhibited. They believe, however, that the enhanced negative after-potential obtained with veratrine treatment arises in a different manner than does the negative after-potential in the unpoisoned axon.

Aside from a few isolated observations, the study of the negative after-potential of skeletal muscle by modern techniques, has been relatively ignored. For example, Nastuk and Hodgkin (1950) mention that the negative after-potential seen with inside-the-cell microelectrodes was variable in size. Desmedt (1953) observed that treatment of frog's skeletal muscle with reduced external K^+ increased the negative after-potential when it was measured in a conventional manner, but it was unchanged when its size was measured with reference to the external potential.

The experiments to be described in this section were designed to study both the normal and the veratrine after-potentials of frog's skeletal muscle. Two assumptions were made at the start of this work. Not only were they a help in the design of the experiments, but they have proved to be essentially correct. One is that the veratrine and normal negative after-potentials were expressions of two fundamentally different processes or events. The other is that the action of veratrine on the muscle membrane is the same in the resting fiber as it is following an action potential, but, of course, the action potential accentuates this effect. It has been known for some time that strong veratrine concentrations block conduction in nerve and muscle (Krayer and Acheson, 1946) and since the block can be overcome by anodal polarization (Lorente de No, 1947; Fleckenstein,

1951), it is thought that the block is due to a depolarization of the cell membrane. In contrast to the depolarization produced by K^+ on frog nerve, that produced by veratrine is not a linear function of the log of the veratrine concentration (Feng and Liu, 1949). As mentioned in the previous section, I found that block was produced rapidly and completely with veratrine concentrations between 0.5×10^{-6} and 10^{-6} in frog muscle. It would seem that with moderate doses of veratrine the fibers are just able to overcome the depolarizing effect of veratrine, but with the passage of an impulse the balance is upset and a prolonged period is needed for the fiber to recover its resting potential. In line with this rather vague concept are the findings of Schmitt and Gasser (1933). They found that the O_2 consumption of resting nerve was increased by veratrine treatment, and that slight activity, which had no effect on the O_2 consumption of unpoisoned nerve, increased it even further.

The results of the experiments to be presented support the following description of the normal and the veratrine negative after-potential. In the unpoisoned fiber, the rapid falling phase of the spike, presumably due to the facilitated outward movement of K^+ (Fatt and Katz, 1951; Hodgkin and Huxley, 1952d), ends with the fiber slightly depolarized and the negative after-potential is simply the passive return of the membrane potential to resting level. In the veratrine-treated muscle, the membrane becomes more permeable to Na^+ following an action potential and the enhanced inward movement of Na^+ considerably delays repolarization.

B. Materials and Methods

1) Preparations. - In some of the initial experiments, muscle strips were prepared as previously described (pg. 26). In most of the experiments, however, the whole sartorius muscle was used. After isolation, the muscle was placed under a dissecting microscope for observation. Parasites (trematode larvae) generally were found (see pg. 17) and all seen were removed. The sartorius muscle from an uninfected frog has a thin, practically invisible connective tissue sheath on its inner surface, but when these parasites are present there is a proliferation of the connective tissue. As much of this connective tissue as possible was removed and the muscle was mounted in the bath with its inner surface exposed. When the whole muscle was to be used, it was removed from the frog with a silk thread about its distal tendon and its proximal end still attached to part of the bone. A piece of thread was tied to each side of this bone. The baths were provided with wires to which these threads were attached. The threads attached to the piece of bone were fastened first and the thread attached to the distal tendon was used to adjust the tension on the muscle.

2) Bath arrangements. - At first, the saline bath (Fig. 1) was used. Both the reduced size of the muscle and the petroleum jelly around the muscle in the slots restricted the movement of the muscle following an action potential and the microelectrode would generally remain in the fiber throughout the complete series of

events. Later it was realized that several of the experiments could be performed without recording the complete negative after-potential, and since the movement of the muscle did not occur until several milliseconds after the action potential, a more convenient bath arrangement was sought. A paraffin wax bath similar to that described by Fatt and Katz (1951, Fig. 1) was constructed. It was modified by having two chlorided silver wire electrodes permanently set into the part holding the muscle and acting as the bath electrodes. When indirect stimulation was used, it was found best to cover the nerve with mineral oil, rather than to use the small compartment as a moist chamber. The bath was cemented to the perspex top of s stage (see Fig. 22) which could be bolted to a myograph stand. A light source was placed beneath the stage and the preparation was viewed from above with a binocular disecting microscope. An eye-piece micrometer was placed in one of the oculars of the disecting microscope for measuring distances along the fibers. The outlines of the cells and the microelectrodes were best seen when the parallel lines of light were directly in line with the microscope objective. It was also found helpful to place a polaroid filter between the light source and the muscle.

Although the muscle movement was greater using this arrangement, the visibility was far superior to that obtained using the saline bath. It was particularly difficult to prevent twisting of the muscle strip when placing it in the saline bath. This, plus the distortion of the light due to the shape of the saline bath, and the frequent patches of petroleum jelly, made it practically impossible

to follow visually a single fiber for any distance when using the saline bath. Later, it was found that when the movement of the entire muscle was restricted by stimulating only a few of the fibers, the inside-the-cell microelectrode generally remained in the fiber and in this way it was possible to record complete after-discharges. This was done by stimulating with an external microelectrode having a relatively large tip diameter. The fibers stimulated were found visually and implants were made along the course of these fibers. Of course, difficulties due to movement of the fibers were at a minimum when single fibers were stimulated with internal electrodes.

Occasionally, the three compartment bath (Fig. 22) was used in place of the paraffin wax bath. In this case, the compartments were not isolated, the only difference being that larger quantities of solution were necessary to cover the muscle completely.

3) Solutions. - The composition of solutions used is shown in Table 1, and other modifications are described on pg. 19. The d-tubocurarine chloride was only added to the solutions in experiments with veratrine. All the experiments were performed at room temperature, and in long experiments the solution in the bath was changed at least once an hour.

4) Electrodes. - The glass microelectrodes were drawn by hand using fine pyrex tubing about 2 mm in diameter. The tips were examined with the high power magnification of an ordinary microscope with a dry objective. Only those electrodes having tips which could not be focused were selected for use. The electrodes selected were filled by a modified form of the method described by Tasaki, Polley

and Orrego (1954). They were mounted tip down in a beaker of methyl alcohol and placed in a vacuum dessicator. They were vigorously boiled for 15 min. by reducing the pressure in the dessicator. In this way, all the electrodes were completely filled with methyl alcohol. The electrodes were then placed in distilled water and left there at least overnight, although longer soaking was preferred. Then they were placed in 3 molar KCl and left there until used. The resistance of the electrodes was measured and only those having resistances between 10 and 40 megohms were used in the experiments.

At first, I attempted to use the soft solder electrodes for stimulating only a few fibers at a time. This proved unsatisfactory for several reasons. When used for stimulating, their resistances quickly increased to very high values, a single stimulus usually caused a series of action potentials, and when the muscle was examined large black blotches could be seen on the surface at the point of electrode contact. A much more satisfactory electrode for this purpose was constructed in the following manner. Electrodes having a tip diameter between 50 and 100μ were drawn from glass tubing about 1/4 inch in diameter. This was covered to within 5 mm of the tip with shielding braid and a fairly thick platinum wire was run down the tube. The platinum wire was connected to the output from the stimulator with shielded wire. The electrode was filled with Ringer's solution which made the contact between the muscle and the platinum wire. The shielding was joined and grounded.

5) Recording Systems. - For use with the high resistance microelectrodes a cathode follower similar to that of Krakauer

(1953) was employed. It was modified by replacing the electrometer tubes with EF 37a's. This circuit had an effective input capacitance of 3 $\mu\mu$f including the capacitance of the lead from the electrode. Thus with a 30 megohm input resistance on the recording grid it had a time constant of 90 μsec. In some experiments, it was necessary to stimulate the fiber with two pulses of different polarities, amplitudes and durations. For this purpose the output of two stimulators was fed through an anode follower. An anode follower is a circuit particularly well adapted for mixing pulses of different polarities and long durations.

The most difficult technique to master was the implanting of two electrodes inside a single cell. Several approaches to this problem were tried. The most successful method is illustrated in Fig, 10. With the switch in position 2 the stimulating electrode was inserted into a cell. Successful implantation was indicated by the usual sudden appearance of a resting potential. The switch was then returned to position 1 and the recording electrode was implanted. That both electrodes were in the same cell was checked by applying 5 msec hyperpolarizing pulses (tip negative) with the stimulating electrode and recording the response with the recording electrode. When necessary, the recording electrode was removed and inserted into the fiber at another point along its surface. Implantation into the same cell was again checked with the 5 msec. pulses. A more detailed discussion of the techniques employed, with inside-the-cell microelectrodes, can be found elsewhere (for example - Nastuk and Hodgkin, 1950; Fatt and Katz, 1951, 1953; Eyzaguirre and Kuffler, 1955a).

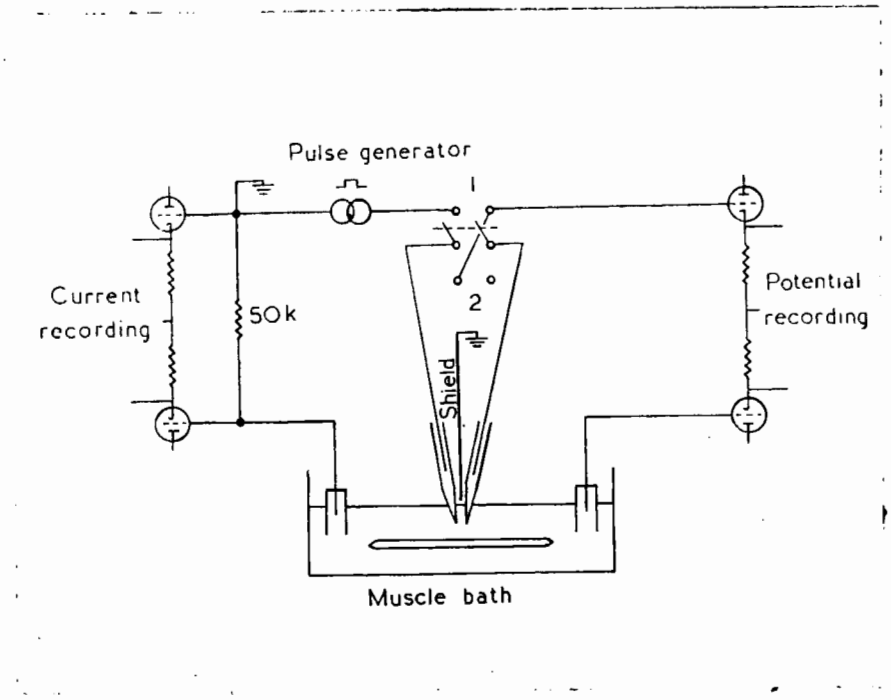

Figure 10. - Arrangement of electrodes for intracellular stimulating and recording. Note the double-pole, double-throw switch, position 2 for inserting stimulating electrode, position 1 for testing electrode insertion and for normal use.

C. Experiments and Results

1) Effect of veratrine on the electric constants of the resting fiber. - The subthreshold cable properties of several fibers were determined by the square pulse technique (Hodgkin and Rushton, 1946; Katz, 1948). The technique employed was identical to the method described by Fatt and Katz (1951). Two electrodes were inserted into a single cell. Square hyperpolarizing pulses, 70 msec in duration, were applied with one electrode, and the response of the membrane was recorded with the other at various distances from the stimulating electrode. In all these experiments the current was monitored on the upper beam of a two channel oscilloscope while the response of the membrane was recorded on the lower beam.

The calculations were the same as those described by Fatt and Katz (1951). However, for various reasons, I felt it necessary to make some additional checks. Generally, more than one response was recorded at each electrode separation, often with different current strengths. Occasionally the space constant (λ) was checked by plotting the $\log_{10}$ of the steady potential developed against the electrode distance. A straight line relation was found and the slope of this line was used to determine the space constant. (The slope = $\log_{10} e/\lambda$.- Hodgkin and Rushton, 1946).

It was usually difficult to obtain electrodes which would pass square pulses properly. Usually about 15 msec passed before a steady current level was obtained with each pulse. When the current has been steady for a sufficient length of time to allow

the membrane potential to reach a new steady value, the formula $V = V_o$ (1-erf t/τ_m)(Hodgkin and Rushton, 1946) describes the return of the membrane potential at the stimulating electrode to its resting level when the current is turned off. This is only true when the membrane capacitance is fully charged (Rushton - personal communication). Several of the experimental curves were compared with this curve (Fig. 17) and found to match it exactly, indicating that the poor shape of the start of the current pulse did not appreciably distort the results. However, only the maximum potential developed and the declining phase of the membrane response were used in making the calculations.

The constants determined in this manner are shown in Table 2. Most of the values found for the unpoisoned fibers (veratrine conc. = 0) were appreciably lower than those obtained by Fatt and Katz (1951) for muscle fibers from Rana temporaria using the same technique. However, Jenerick (1953), using a somewhat different technique for determining some of the cable constants of the sartorius muscle fibers of Rana pipiens, reported values very close to those reported here (see Table 5), and the difference, therefore, seems to represent a true species difference.

The changes produced by veratrine treatment are quite striking. In general, the changes increase with increasing veratrine concentration. It is obvious that veratrine in low concentration produces changes in the properties of the resting fiber membrane, even though such changes are not detected by the usual methods of studying the electrical activity of muscle fibers.

TABLE 2

Electric constants of resting sartorius muscle fibers.

(λ, length constant; τ_m, time constant; $1/2\sqrt{r_m r_i}$, effective resistance between inside and outside of fiber; "d", calculated fiber diameter; R_m and C_m, specific membrane resistance and capacitance; conc., veratrine sulphate concentration.)

Muscle	Fiber	conc.	λ (mm)	τ_m (msec)	$1/2\sqrt{r_m r_i}$ (Ω)	"d" (μ)	R_m (Ω cm^2)	C_m (μf/cm^2)
1	A	0	1.6	11.5	228000	105	2400	5
	B		1.8	12.5	204000	118	2700	5
	C		1.4	12.0	254000	93	2000	6
	Mean		1.6	12.0	229000	105	2400	5
	D	10^{-8}	1.1	12.5	330000	73	1700	8
	E		1.3	14.0	282000	84	1900	7
	Mean		1.2	13.0	306000	79	1800	8
	F	10^{-7}	1.2	18.5	180000	101	1300	14
2	G	0	1.3	14.0	240000	94	1900	8
	H		1.8	9.0	150000	139	2400	4
	I		1.4	11.0	199000	106	1800	6
	Mean		1.5	11.0	196000	113	2000	6
	J	10^{-7}	1.1	12.0	240000	86	1500	8
	K		.8	11.0	207000	78	800	14
	L		.8	19.0	251000	71	900	21
	Mean		.9	14.0	233000	78	1000	14
	M	2.5×10^{-7}	.4	7.0	217000	56	300	23
	N		1.0	10.5	250000	82	1400	8
	O		.7	12.0	238000	70	700	17
	Mean		.7	10.0	235000	69	800	16

2) Action potentials and resting potentials. - Figure 11 contains the action potentials recorded from two fibers of the same muscle before (A and C), and during (B and D) treatment with a veratrine concentration of 10^{-8} . The upper line represents the potential level outside the fiber, and the lower line the potential at the tip of the microelectrode inside the fiber. The distance between the lines is thus proportional to the potential difference across the membrane. The resting potential is the distance between the lines before the stimulus artifact and the start of the action potential. The reversal of the direction of the potential difference across the membrane at the height of the action potential (the "active membrane potential") is clearly shown in all four records. The slow phase of the recovery of the resting potential (or negative after-potential) is easy to distinguish from the rest of the action potential. In the unpoisoned fiber its decline is exponential and this is easiest seen with the slower sweep speed (Fig. 11C).

This figure (Fig. 11) illustrates several general observations that I have made in the course of the work to be described below. The resting potential is scarcely affected by low veratrine concentrations which have an appreciable effect on the electric constants of the fiber membrane (Table 2). The rapid rising and falling phases of the action potentials are also scarcely affected. The most dramatic effect is the change in the negative after-potential. In Fig. 11D, it seems as though the rapid decline of the action potential abruptly ended with the establishment of a new lower resting potential. Actually, the fiber membrane eventually recovers its initial resting potential, but it takes several seconds before this is accom-

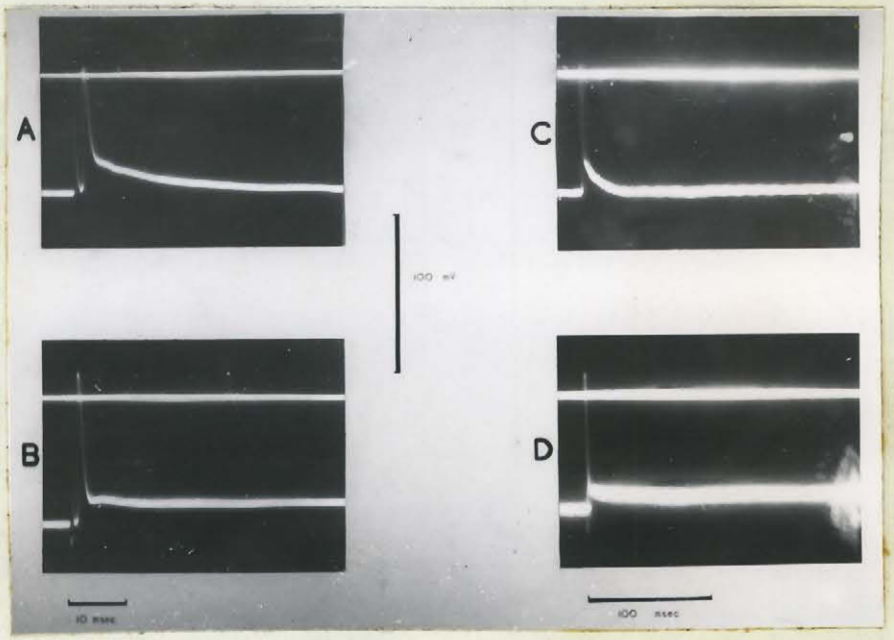

Figure 11. - Responses of single muscle fibers of the frog sartorius recorded with inside-the-cell electrodes. In A and C are two responses from the same fiber when the muscle was immersed in tubocurarine-Ringer solution. In B and D are two responses from the same fiber when the same muscle was immersed in a 10^{-8} veratrine sulphate solution. (Voltage calibration, 100 mV.; time calibration, 10 msec. in A and B, and 100 msec in C and D.).

plished.

Unfortunately, due to the parasite infection of almost all the frogs used in this work, it was difficult to obtain quantitative information concerning the normal resting potentials and active membrane potentials (Frank, 1956). The main effect of the infection was to increase the osmotic pressure of the frog's plasma. Both the Na^+ and K^+ concentrations were 1.5 X the normal values usually reported. The intracellular Na^+ was greatly increased while the intracellular K^+ was about the same as normally found. When these muscles were kept in Ringer's solution both the resting and the active membrane potentials increased with time. For example, one muscle in which a resting potential of 76.9 $\pm$ .78 (12) (Mean $\pm$ standard error of the mean followed by the number of observations in ( )) and an active membrane potential of 16.3 $\pm$ 2.27 (12) were initially observed, after about five hours in Ringer's solution at room temperature developed maximum potentials of 91.3$\pm$ 1.87 (7) and 34.6$\pm$ 2.13 (7) respectively. The maximum for the active membrane potential was recorded about 1/2 hour before the maximum for the resting potential. Both values declined after reaching their maxima. In the only frog in which a reasonable number of observations were made before the parasite infection was universal, the control resting potential was 114.3$\pm$ 2.5 (15) and the active membrane potential was 19.6 $\pm$ 4.0 (12). However, this was the only case in which resting potentials of this magnitude were recorded. Only one frog without parasite infection has since been obtained, and using the muscle obtained from it, a resting potential value of 83.2 $\pm$ 1.8 mV. (13) and an active membrane potential value of 34.3 $\pm$ 1.9 mV. (13) were obtained. These latter

values agree quite well with the values reported in the literature (Hodgkin, 1951; Desmedt, 1953; Jenerick, 1953) and are probably closer to the true values for the uninfected frog.

The lack of a steady base line made it unusually difficult to determine small changes in potential that might have been produced by veratrine in low concentrations. Larger changes produced by strong veratrine treatment were obscured by my method of selection of the responses to be used in calculations. Aside from the usual criterion of a sudden drop in the potential recorded when a fiber was penetrated (Nastuk and Hodgkin, 1950), I also considered it necessary for the fiber to have an action potential when it was stimulated and for the action potential to have a proper size and shape. This was necessary to prevent the inclusion of potentials reduced by injury, and of movement artifacts that occasionally looked somewhat like action potentials.

As mentioned above, weak veratrine concentrations (10^{-7} or lower) seemed to have no effect on either the resting potential or active membrane potential. With stronger veratrine concentrations, the resting potentials of the fibers on the surface of the muscle were considerably reduced, often to levels which blocked conduction. However, if the electrode was pushed into the muscle and fibers a couple of layers below the surface were impaired, it was usually possible to obtain resting and action potentials of normal size and shape. I never studied the effect of strong veratrine concentrations (0.5×10^{-6} or higher) which I had found to block conduction completely.

Occasionally preparations were obtained in which single stimuli were followed by a repetitive response. The records obtained looked very much like the records of the veratrine after-discharge with the electrode implanted in the vicinity of the site of origin of the after-discharge action potentials (Fig. 25). When veratrine was added to such a preparation, this type of after-discharge, which might be considered to be a type of injury discharge, occurred following each stimulus and the preparation was no longer of use for the experiments which I wished to perform. A very similar type of repetitive response was also a common occurrence when a soft solder type of microelectrode was used for stimulating only a few fibers at a time, except that in this case the action potentials of the repetitive response originated at the point of stimulation. This was so frequent an occurrence that I had to stop using this type of stimulating electrode.

3) The negative after-potential. - The most obvious change in the electrical activity of skeletal muscle fibers, produced by veratrine treatment, was the prolongation of the negative after-potential. With veratrine concentrations of 10^{-7} or less, it was prolonged without much increase in its maximum amplitude. With prolonged treatment in 10^{-7} veratrine sulphate or with stronger veratrine concentrations, its maximum amplitude was usually increased (Fig. 21) and a delayed rising phase was occasionally seen.

In order to study the veratrine negative after-potential it is extremely important to have some understanding of

the normal negative after-potential. If, as many assume, it is simply an increase or prolongation of the process that produces the normal negative after-potential, then what is the process that it increases and how does veratrine increase it? If it is an expression of some other phenomenon, in what way has the normal course of events been changed? Since little information could be obtained from the literature concerning the normal negative after-potential, I felt it necessary to study first the normal negative after-potential.

When one looks at a normal negative after-potential (Fig. 11A and C) it is seen that there are two properties that can be easily measured, and might possibly be modified by experimental procedures. They are the amplitude and the duration of the negative after-potential.

The question arises as to what is meant by the amplitude of the negative after-potential, and just how does one measure it. When the records obtained from unpoisoned fibers are inspected, four types of negative after-potential can be distinguished. These are shown in Fig. 12. The types are different only with respect to their shape at the beginning of the negative after-potential. In type a (Fig. 12a) the end of the rapid decline of the action potential is followed by a "plateau" lasting from 1/2 to several milliseconds, during which time the membrane potential neither declines nor increases. In type b (Fig. 12b) the rapid phase of repolarization at the end of the action potential is followed by a depolarization lasting a few msec. In

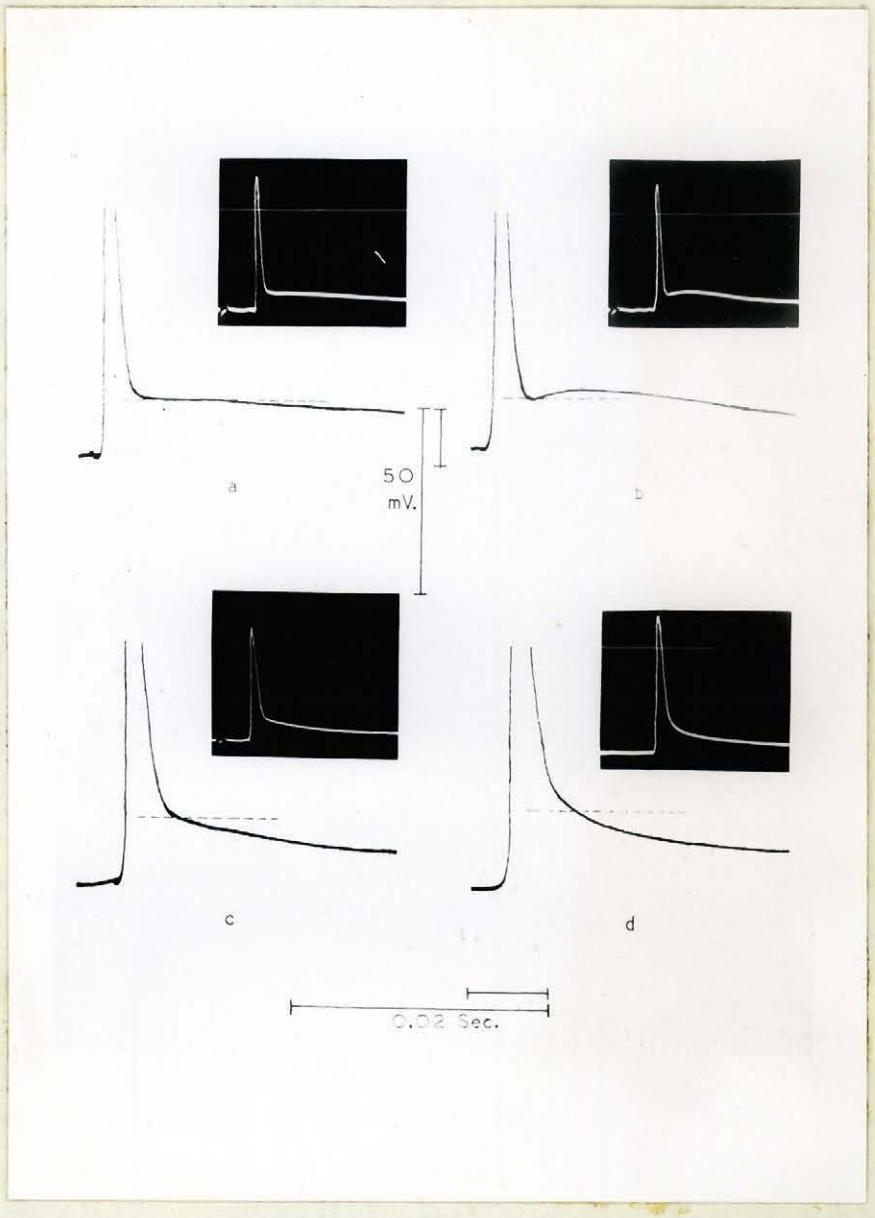

Figure 12. - Shapes of the normal negative after-potentials of the sartorius muscle recorded with inside-the-cell electrodes. The drawings were made from the records shown. All were recorded from fibers of a single muscle.

type c (Fig. 12c) there is a sharp distinction between the phase of rapid repolarization and the phase of slow repolarization (or negative after-potential), and in type d (Fig. 12d) there is a gradual transition from one phase to the next. Although in different muscles there was usually a tendency for one or two types to predominate, the different types provided little information on their own. The records shown in Fig. 12 were all obtained from fibers of a single muscle. In 321 cases under various types of experimental conditions the shape of the start of the negative after-potential was noted. Of these 46% were type a, 28% were type b, and 25% were either type c or d. The only experimental condition that seemed to have any effect on the shape of the start of the negative after-potential was the reduction of the external Na^+ concentration. When the external Na^+ was reduced to 50% of normal only type c or d negative after-potentials were obtained.

The dashed lines shown in Fig. 12, represent the voltage levels chosen as the maximum potential of the negative after-potential. In all the types except type d, they represent a definite start to the slow phase of repolarization at the end of the action potential. In the case of type d, the first phase gradually blended into the second. There was a region of about 5 mV. in size which might be considered to be a transitional region, since the two phases were easily distinguished outside this range. The dashed line is placed in the center of this region. Fortunately, this type rarely occurred.

Conventionally, the potential difference between the dashed line and the resting potential would be taken as a measure of the maximum potential of the negative after-potential. However, in keeping with the other potential measurements (resting potential and active membrane potential), the amplitude of the negative potential was taken as the difference between the dashed line and the line representing the potential outside the fiber. Another reason for choosing this way of measuring the amplitude of the negative after-potential, was the suggestion by Desmedt (1953), that the negative after-potential was independent of resting potential when measured in this manner. If one wishes to modify the amplitude of the negative after-potential experimentally, this method of measurement is the only way in which changes in the resting potential will not be reflected in the value given to the negative after-potential.

Probably the first observation I made when I started measuring the amplitude of the negative after-potential, was that it is not independent of the resting potential. The relation between the two is clearly shown in figure 13. The points were experimental observations while the line is the solution of the regression formula, $Y = a + bX$ where $b = .768$ and $a = 5.22$ (these figures were derived from a statistical analysis of the data and b was found to be significantly different from 0 at $P = 0.01$). The data was obtained from observations on fibers in ordinary Ringer's solution at room temperature before any experiments were performed using the muscles. The data represents observations on 141 individual fibers from 16 muscles. This result illustrates the importance of the

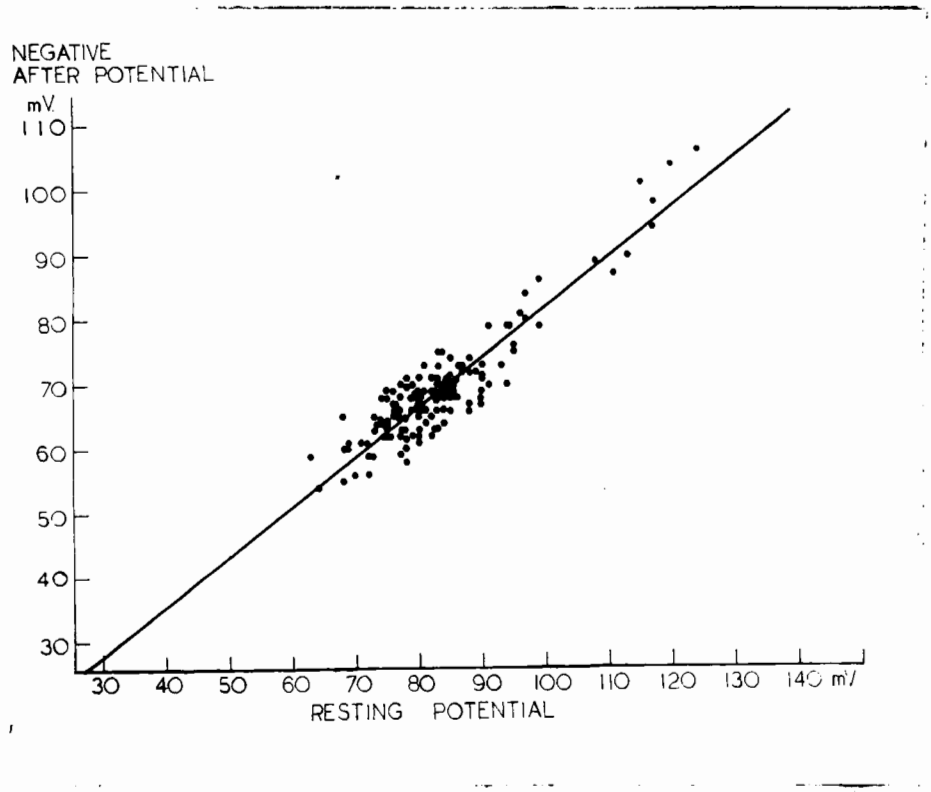

Figure 13. - Relation between the resting potential and the maximum amplitude of the negative after-potential of sartorius muscle fibers. I.Control curve. The points were experimentally obtained using inside-the-cell electrodes. The curve was obtained by a statistical analysis of the data.

measurement procedure. If the potentials were measured with respect to the resting potential, the two would vary together whether they were independent or not.

The next question of importance is, can the amplitude of the negative after-potential be modified by simple experimental procedures? From the above result (Fig. 13), we would expect to be able to change it by any procedure which changes the resting potential. But greater insight into the process involved would be gained if we could modify the amplitude of the negative after-potential independently from the resting potential. Since the resting potential is presumably dependent upon the ratio of the K^+ concentration between the external solution and the myoplasm (Hodgkin, 1951), I first tested the effect of modifying the external K^+ concentration. The results are shown in Fig. 14. As before, the points represent experimental observations. The muscles were kept in the altered solution for 15 min. before the records represented by the points were made. This was done to make sure that there was sufficient time for the ion concentration about the fibers to reach the same level as in the experimental solution (Keynes, 1954). Observations were made during the first 15 min. in all experiments but they have not been included in the graphs. The line was drawn from the constants determined in the control graph (Fig. 13).

It is obvious that most of the points do not deviate significantly from the line. Although a change in the external K^+ concentration does appreciably modify the resting

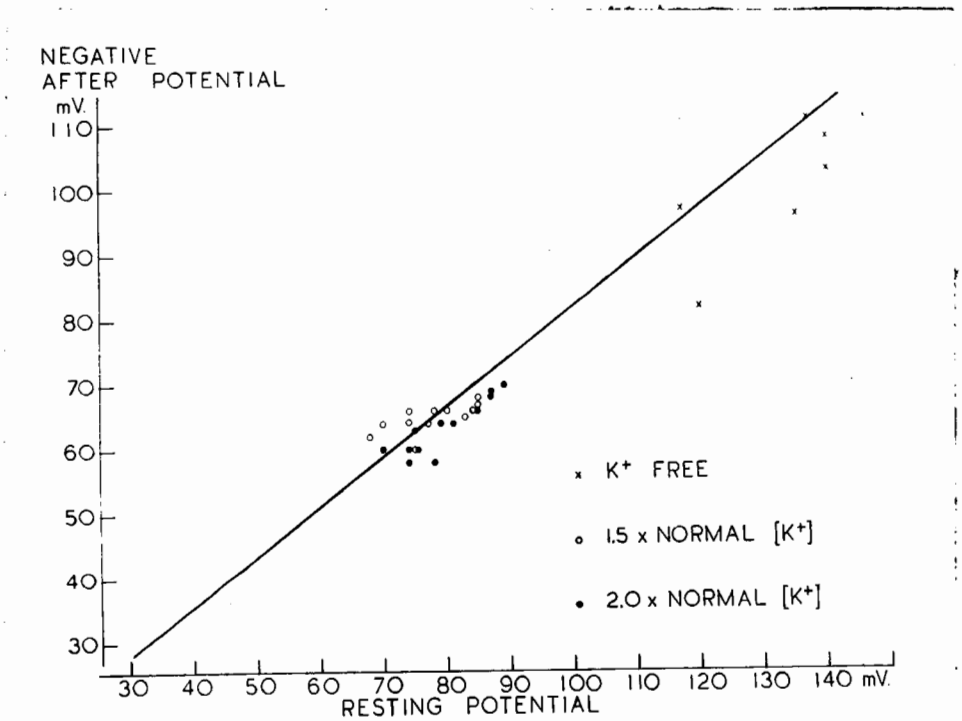

Figure 14. - Relation between the resting potential and the maximum amplitude of the negative after-potential of sartorius muscle fibers. II. Altered external K^+ concentration. Points experimentally determined. Line is the control curve (Fig. 13).

potential it does not change the relation between it and the amplitude of the negative after-potential. When the summed action potential of a bundle of fibers is recorded with external electrodes the amplitude of the negative after-potential will be changed by changes in the external K^+ concentration but as we can see the changes are primarily dependent upon the same factors that change the resting potential. The same effect, or rather lack of effect, can be seen when the external Na^+ concentration is altered (Fig. 15). Here again, the points were experimentally determined 15 min. and more after changing the solution, and the line was drawn from the constants of the control graph (fig. 13). The Na^+ concentration in the high Na^+ solution was 1.5 X normal, and in the low Na^+ solution it was 0.5 X normal. (The slight deviation seen in some of the K^+ free records is of some interest. However, it is hard to know if it is a true effect or caused by the harmful action of K^+ free solutions noted by Desmedt (1953). He also noted that in very low K^+ solutions the resting potential is rather independent of the internal K^+ concentration. Although interesting, the effect is small and of doubtful significance).

Fig. 16 shows the results obtained with universal veratrine treatment. The points were recorded only after a definite veratrine effect was noted. The observations with 10^{-8} lasted over an hour, while those with 10^{-7} lasted for only 1/2 hour. As before, the line was drawn from the control values. Under these experimental conditions, veratrine treatment does not upset the relation between the resting potential and the amplitude of the

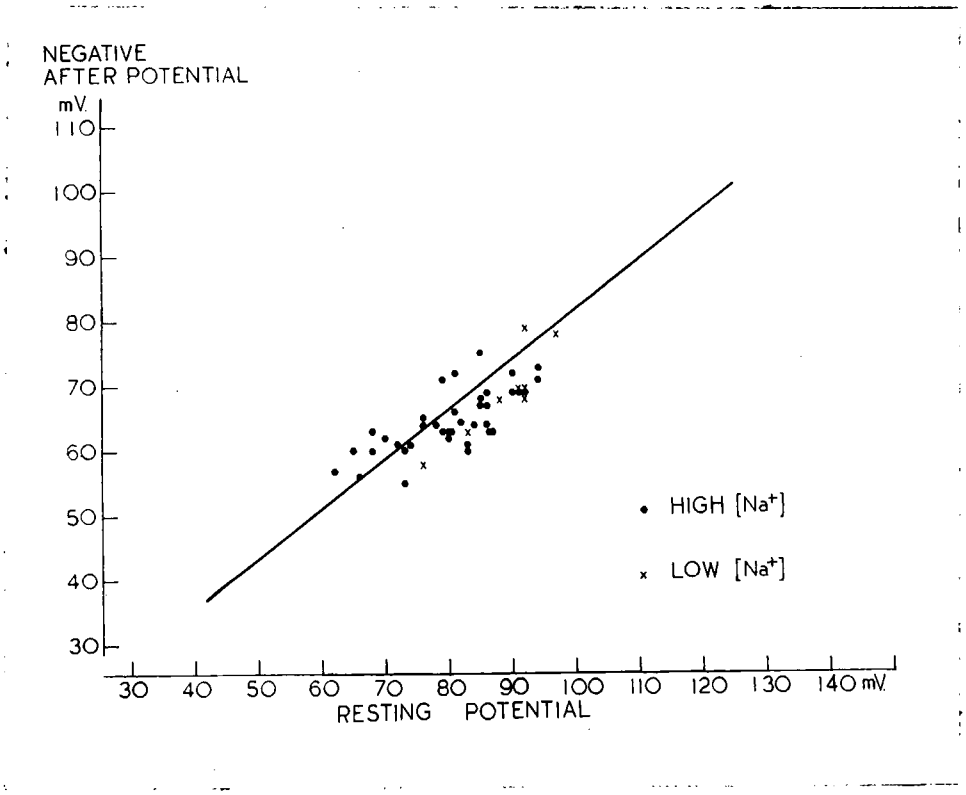

Figure 15. - Relation between the resting potential and the maximum amplitude of the negative after-potential of sartorius muscle fibers. III. Altered external Na^+ concentration. Points experimentally determined. Line is the control curve (Fig. 13).

negative after-potential. However, with stronger veratrine concentrations or with longer treatment with 10^{-7} the amplitude of the negative after-potential increases (in the way that I measure it, its potential decreases) and the experimental points would be well below the control line (see for example Fig. 20a and Fig. 21).

Till now, I have discussed the negative after-potential as if it were an independent phenomenon associated with the action potential. I have considered its two measurable quantities, its amplitude and duration, to be a measure of the outcome of a process unique to the negative after-potential. This point of view has been difficult to avoid, since almost all previous workers have found that the negative after-potential seemed to be more affected by certain experimental conditions than the other measurable electrical phenomena. My inability to alter the relation between the amplitude of the negative after-potential and the resting potential, has led me to the belief that this is not the case, that the negative after-potential is not the expression of a process unique to itself. The occurrence of a rather inflexible relation between the resting potential and the amplitude of the negative after-potential strongly indicates that they are determined by the same factors. There is considerable evidence that the resting potential is determined by the ratio of internal K^+ to external K^+ concentrations (Boyle and Conway, 1941; Hodgkin, 1951; Jenerick, 1953). There is also considerable evidence that the rapid declining phase of the action potential is produced by a rapid outward movement of K^+ ions (Hodgkin, 1951; Hodgkin and Huxley,

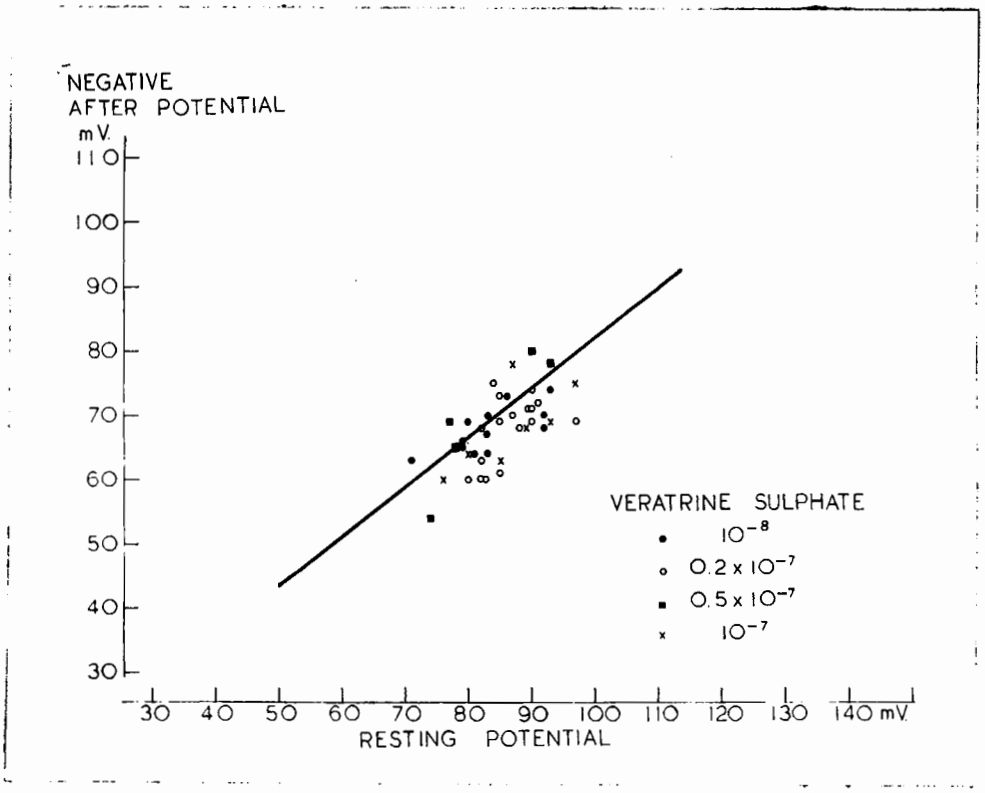

Figure 16. - Relation between the resting potential and the maximum amplitude of the negative after-potential of sartorius muscle fibers. IV. Veratrine treatment. Points experimentally determined. Line is the control curve (Fig. 13).

1952d; Fatt and Katz, 1951; Desmedt, 1953) and so, it too is dependent upon the ratio of K^+ concentrations and particularly upon the internal K^+ concentration. Thus the maximum amplitude of the negative after-potential might be a measure of the end of the rapid phase of outward K^+ movement, rather than the maximum potential developed by a process responsible for the formation of a negative after-potential. Viewed in this way, not only is the relation between the amplitude of the negative after-potential and the resting potential not surprising, but it is a relation to be expected.

What about the remainder of the negative after-potential? In the Squid giant axon the rapid declining phase of the action potential ends with the membrane slightly hyperpolarized (Frankenhaeuser and Hodgkin, 1956). If, in the frog skeletal muscle fiber, this process ends with the membrane slightly depolarized, then the simplest explanation of the rest of the negative after-potential would be that it is simply a passive return of the membrane potential to its resting level.

If this is the case, the time constant for the decay of the negative after-potential should be the same as the time constant for the decay of an impressed hyperpolarization. Table 2 contains some of the time constants that I have found in my studies on the unpoisoned fiber. In the two muscles studied, the time constant (τ_m) ranged between 9 and 14 msec. In some other muscles the values ranged between 9 and 15 msec with an average of about 11.5 msec. If a fiber is hyperpolarized or slightly depolarized at a single point along its surface, with a constant current lasting a

sufficient time to establish a steady potential difference across the fiber membrane, when the current is turned off the return of the membrane potential to its resting level at the polarizing electrode will be an error function (Hodgkin and Rushton, 1946). Under these conditions, the steady potential change established will decline to 1/e at a distance of one space constant (λ) from the polarizing electrode. However, if the steady potential across the membrane is developed by universal application of a constant current, then the return of membrane potential to resting levels, after the current is turned off, will be an exponential function (Hodgkin et al., 1952).

Fig. 17 contains both these theoretical curves. The dashed lines show the errors that will occur if the wrong theoretical curve is assumed to be the correct one when the time constant of the experimental curve is determined. As can be seen, the errors would be considerable. It can easily be shown that the decay of the normal negative after-potential is exponential in nature, either by matching with the theoretical curves or by plotting the log of its potential against time. In the latter case, a straight line relation is found. It is easy to visualize the actual physical conditions that occur, and why the decline of the negative after-potential is an exponential function. Martin (1954) has reported the conduction velocity of frog sartorius muscle fibers to be about 1.88 to 2.30 m/sec (or mm/msec). Referring back to Table 2, we find that this is of the order of 1 space constant (λ) /msec. If we now look at the potential at a

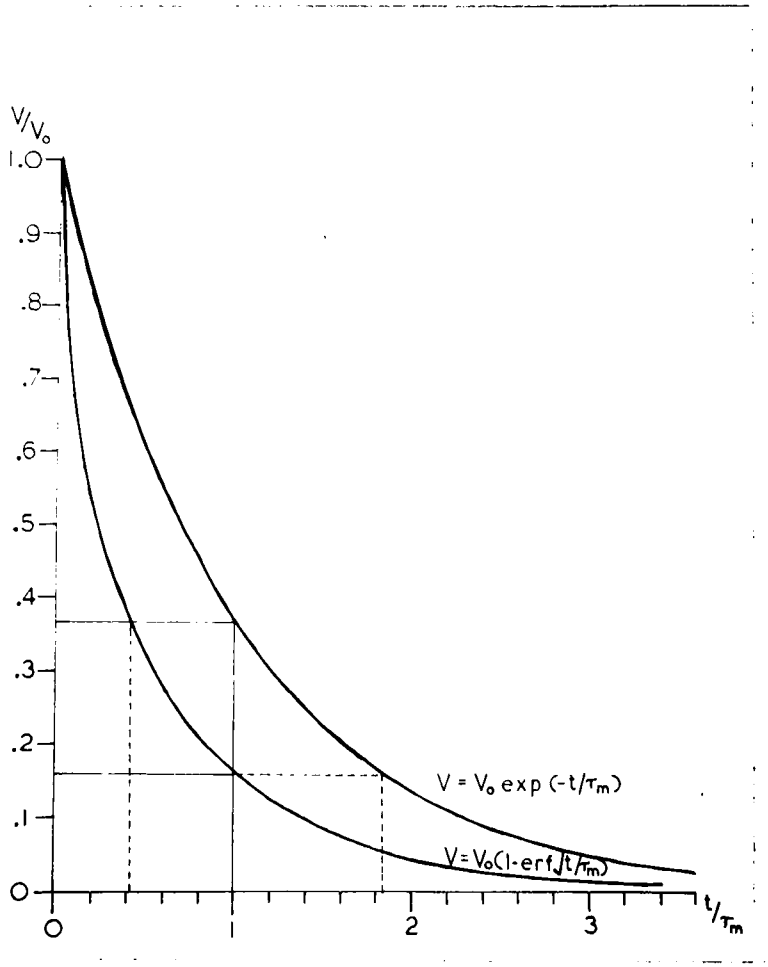

Figure 17. - Theoretical curves for the return of membrane potential (V) to resting levels following universal ($V_o \exp(-t/\tau_m)$) and local ($V_o(1-\mathrm{erf}\sqrt{t/\tau_m})$) polarizations.

point (P) along the surface of a fiber and at the points 1 space constant on each side of P, 4 msec after the peak of the action potential, at which time the membrane resistance (R_m) has presumably returned to resting level, we find that the membrane potential at these points differ by less than one mV. This figure is obtained by looking at the potentials recorded during a single action potential with an inside-the-cell microelectrode, at 3, 4 and 5 msec after the peak of the spike. The conditions are certainly closer to those obtained with universal polarization.

Unfortunately, it is not easy to obtain reliable records of the complete negative after-potential. Aside from the more obvious movement artifacts, such as the electrode hopping out of the fiber (eg. see Nastuk and Hodgkin, 1950, Fig. 7), there is often a slight damage of the fiber at the point of electrode insertion during the movement of the muscle. The only indication that this has occurred is that the fiber membrane potential fails to return to its previous resting level. For these reasons, I was only able to obtain 33 reliable determinations of the time constant for the decay of the negative after-potential. These were obtained from fibers in 7 muscles, during the initial control periods of several experiments. The mean thus determined was 13.5$\pm$ .9 msec. which is well within the range found during the experiments to determine the electric constants of the fiber membrane (Table 2), and it is consistent with the view that the negative after-potential is simply a passive decay of the membrane potential back to its resting level. Only one change in the external ion environment seemed to affect the

constant, that was reduction of the external Na^+ concentration. In one experiment, the initial τ_m averaged 15.1 ± 1.27 (4), when the muscle was bathed with a 50% Na^+ solution τ_m averaged 12.6 ± .95 (11) and when the normal Ringer's solution was returned to the bath the τ_m averaged 15.0 ± .71 (5).

The veratrine negative after-potential lasts several seconds. As can be seen in many of the figures (Fig. 11D, 19c, 20a and 21) for the first few tenths of a second it does not appear to decline at all, and it may even increase in amplitude during this period. Since the longest sweep duration that I had available lasted only 1.5 sec. I had to resort to the method illustrated in Fig. 21 in order to measure the decay of the veratrine negative after-potential. The fiber was stimulated once a sec. for several secs., only the first and last stimulus was of sufficient strength to produce an action potential. The sweep was triggered at the same time as the stimulus, and several successive sweeps were superimposed. The results obtained from 8 fibers of a muscle bathed with 2.0×10^{-7} veratrine are shown in Fig. 18. It is obvious that only the latter part of the decay is exponential in nature. Two values for the decay time were determined. One was the time for the veratrine negative after-potential to decay to 1/e of its initial maximum ($t_{1/e}$) and the time for the exponential part of the decay to decline to 1/e (τm). These values determined from the data contained in Fig. 18, are given in Table 3. The veratrine negative after-potential may be divided into two phases, an initial, slow declining phase with a highly variable duration, and a

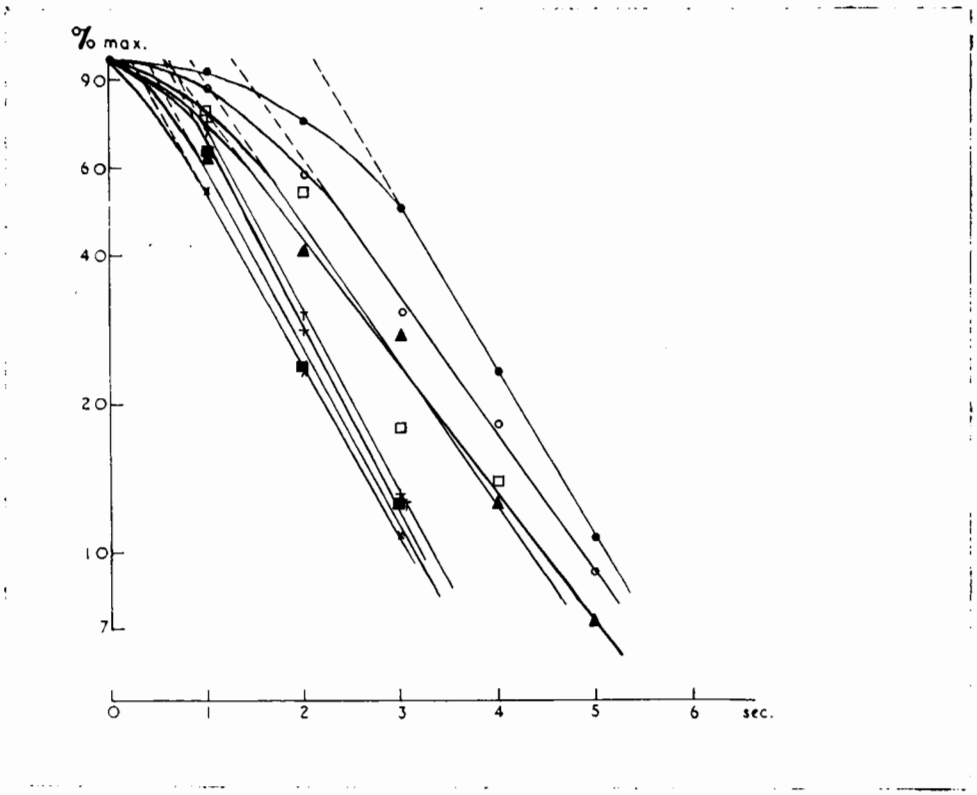

Figure 18. - Decline of the veratrine negative after-potential of sartorius muscle. Muscle immersed in 2.0 X 10^{-7} veratrine sulphate. 8 fibers represented by different symbols. Maximum potential developed soon after the action potential.

TABLE 3

Decay constants for the veratrine negative after-potential (see text for further description).

Fiber	$t_{1/e}$ (sec)	τ_m (sec)
A	3.4	1.3
B	1.5	1.2
C	1.8	1.2
D	2.7	1.6
E	2.3	1.4
F	1.6	1.4
G	2.3	1.7
H	1.8	1.1
Mean	2.2	1.4

TABLE 4

Comparison of the membrane electric constants in the resting fiber with those obtained during the veratrine negative after-potential. Veratrine concentration = 10^{-8}. (see TABLE 2 for a description of the symbols).

Fiber	Conditions	λ (mm)	τ_m (msec)	"d" * (μ)	R_m ($\Omega \cdot cm^2$)	C_m ($\mu f/cm^2$)
1	A. Resting	1.2	10.6	68.2	2020	5.3
	B. Veratr. after-potent.	1.1	12.1	68.2	1900	6.4
2	A. Resting	1.1	9.3	67.8	1700	5.5
	B. Veratr. after-potent.	1.0	10.8	67.8	1590	6.8

* The values given are the averages calculated for the two conditions. In Fiber 1 they were within 2% of each other, and in Fiber 2 they were within 5% of each other.

later, rapid declining phase, which is exponential in nature, and relatively fixed in duration. Although the decline during the exponential phase of the veratrine negative after-potential is rapid when compared to the initial phase, it is still 100 times slower than the exponential decline of the normal negative after-potential.

In order to understand what is occurring during the veratrine negative after-potential, it is of interest to compare the electric constants during the veratrine negative after-potential with those of the resting fiber in the same veratrine concentration. Since the membrane potential is rather steady during the first few tenths of a second during the veratrine negative after-potential, it is possible to determine these constants by the square pulse technique described on pg. 69. In this experiment, the anode follower was used in order to stimulate and polarize the fiber membrane with the same electrode. The procedure adopted was to hyperpolarize with a constant current for 70 msec. Several records were obtained at each electrode separation, both with and without a prior stimulus. The values determined in this manner for two fibers which were kept in 10^{-8} veratrine are shown in Table 4. Although the difference in membrane potential between the resting fiber and the veratrine negative after-potential is large, the changes in the electric constants are small. The increase in C_m and the decrease in R_m are the same changes that are produced by increasing the veratrine concentration (Table 2). However, larger changes are produced by simply increasing the veratrine concentration which has the smaller effect on the membrane potential.

Since the membrane time constant during the veratrine negative after-potential is essentially the same as in the resting fiber, which is not much bigger than in the unpoisoned fiber, the decline of the veratrine negative after-potential, cannot be considered a passive process. The fact that the membrane potential is considerably depressed during the veratrine negative after-potential, at a time when the membrane conductivity is increased by about 6.5%, indicated that the decrease in the membrane potential might be due to a specific increase in the Na^+ permeability of the membrane. Since the effects of veratrine on the muscle increase with the duration of treatment, this is a very simple idea to test. All one has to do is to soak a muscle in a veratrine solution until an obvious effect is produced, and then replace the solution bathing the muscle with one containing the same concentration of veratrine, but with a reduced Na^+ concentration. This experiment is shown in Fig. 19 and 20. Fig. 19a shows the response with the muscle in normal Ringer's solution. Fig. 19b and c show the development of the veratrine negative after-potential with the muscle in normal Ringer's solution containing 2.0×10^{-8} veratrine sulphate. Several minutes (19 min. in this case) after the muscle is placed in a 50% Na^+ solution containing the same concentration of veratrine, the veratrine negative after-potential is eliminated (Fig. 19d). Fig. 19e shows a response with the muscle in a 30% Na^+ solution containing the same concentration of veratrine. The latter has been included mainly for comparison with Fig. 20d, but it also shows that the duration of the negative after-potential is less in 30% Na^+ solution containing veratrine than in the un-

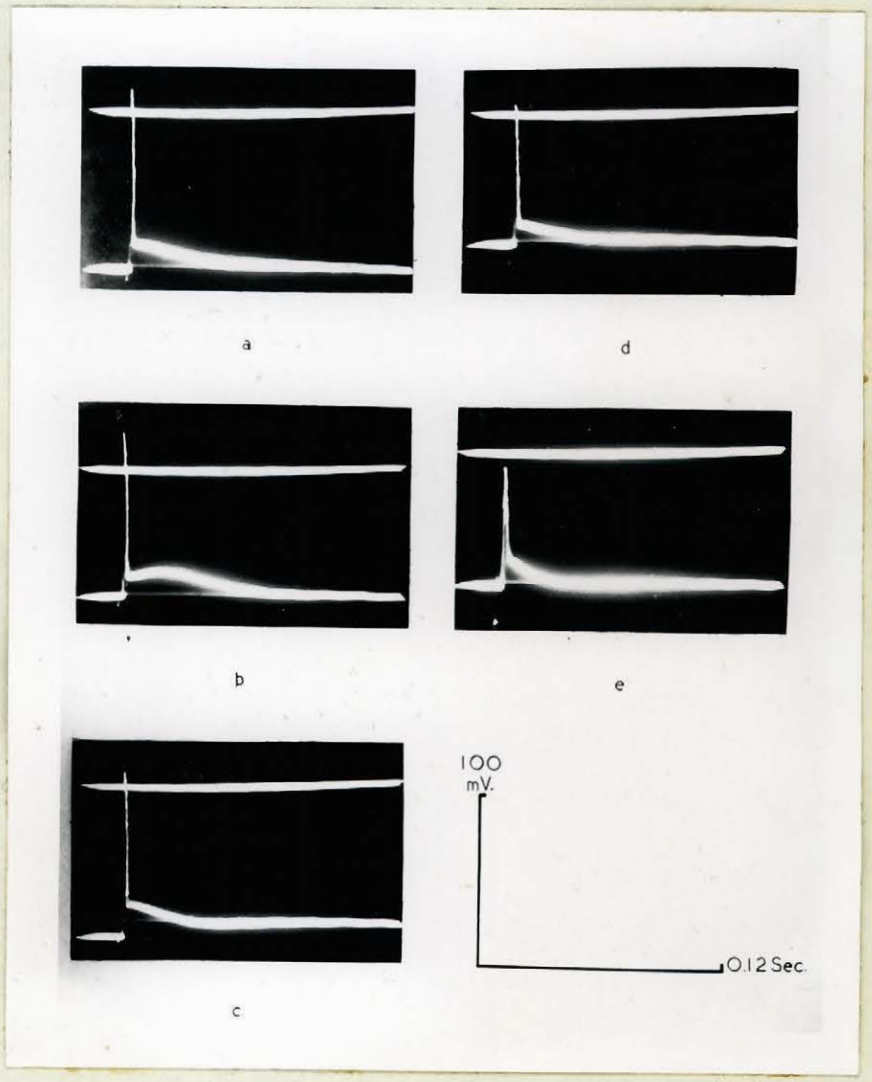

Figure 19. - Effect of reducing the external Na^+ concentration on the veratrine negative after-potential. - I. (a) Control. (b) After immersion of the sartorius muscle in 2.0×10^{-8} veratrine sulphate for 8 min. (c) 20 min. later. After a further 11 min. the muscle was placed in a solution contaning 50% of the normal Na^+ concentration plus the same veratrine sulphate concentration as above. (d) 19 min after the muscle placed in the 50% Na^+ solution. After a further 4 min. the muscle was placed in a 30% normal Na^+ solution containing the same veratrine sulphate concentration. (e) After 25 min. in the 30% Na^+ solution.

poisoned fiber in normal Ringer's solution. The back sweeps, which are just visible, show the membrane potential at the end of the sweep which lasted approximately 150 msec. Fig. 20 shows the same effect of external Na^+ reduction on the veratrine negative after-potential, except in a more dramatic fashion. Fig. 20a shows the response of a fiber after soaking for 39 min. in normal Ringer's solution containing 2.0×10^{-7} veratrine sulphate. Not only is repolarization delayed but the negative after-potential is considerably increased in size. Note also the lack of effect on the rate of repolarization during the rapid falling phase of the action potential. Fig. 20b and c show the reduction in the veratrine negative after-potential produced by bathing the muscle in a 50% Na^+ solution containing the same concentration of veratrine. Fig. 20d shows the effect of a 30% Na^+ solution. Although the veratrine negative after-potential is not completely eliminated, it is reduced to a greater extent than is the spike. I was unable to use lower Na^+ concentrations for I found that when the Na^+ concentration was reduced to 25% of normal, the muscle fibers quickly became inexcitable, and conduction was blocked.

It is believed by many workers that the normal negative after-potential, and in particular the veratrine negative after-potential, is associated with a period of "supernormality" or increased excitability (Krayer and Acheson, 1946). Bremer (1955) has shown that when the frog's muscle is stimulated during the veratrine negative after-potential, with a stimulus strength just sufficient to stimulate a few fibers in the resting muscle, a con-

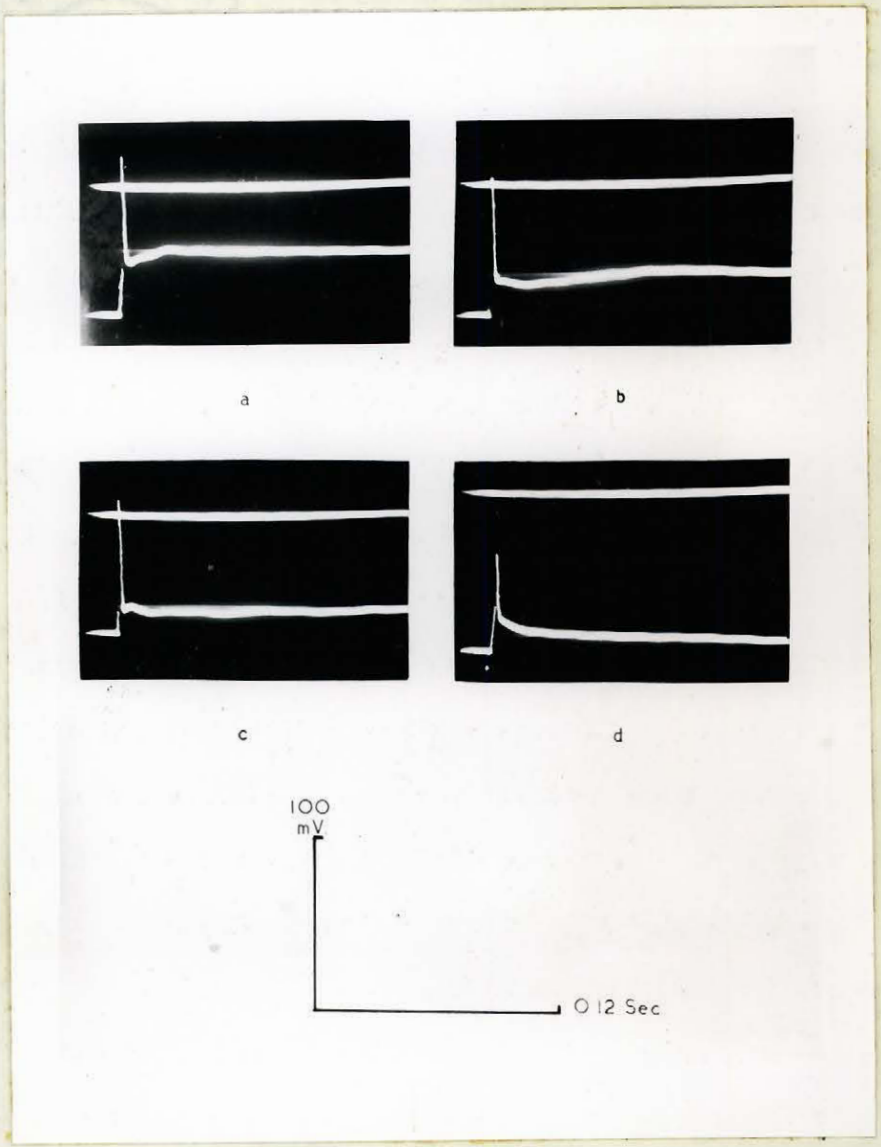

Figure 20. - Effect of reducing the external Na^+ concentration on the veratrine negative after-potential. - II. (a) 39 min. after immersion of the sartorius muscle in 2.0 X 10^{-7} veratrine sulphate. After a further 14 min. the muscle was placed in a solution containing 50% of the normal Na^+ concentration plus the same veratrine sulphate concentration as above. (b) After 16 min. in the 50% Na^+ solution. (c) 5 min. later. After a further 20 min. the muscle was placed in a 30% normal Na^+ solution containing the same veratrine sulphate concentration as above. (d) After 3 min. in the 30% Na^+ solution.

siderably larger number of fibers are excited. The experiment illustrated in Fig. 21 shows that as far as the response of the individual fiber is concerned, the situation seems to be just the opposite, and the veratrine negative after-potential is associated with a phase of decreased excitability. In this experiment, a few fibers were stimulated by means of an external saline electrode. The stimulus strength and duration was adjusted to be above threshold when the impaled fiber was in a resting state. The fiber was stimulated once a second, with the same stimulus strength and duration, until another response was obtained. Successive sweeps were superimposed. In the case shown in Fig. 21, another response was not obtained until after the membrane had fully recovered its resting potential. This was at least 4 and possibly 5 sec after the initial action potential. This experiment was tried several times. With this concentration of veratrine (2.0×10^{-7}), the soonest a second response was obtained was 2 sec, after the initial spike, at which time the veratrine negative after-potential had declined to 24% of its initial height. Unfortunately, no attempt was made to obtain a just threshold stimulus, and it seems likely that in this latter case the stimulus strength and duration was considerably above threshold level.

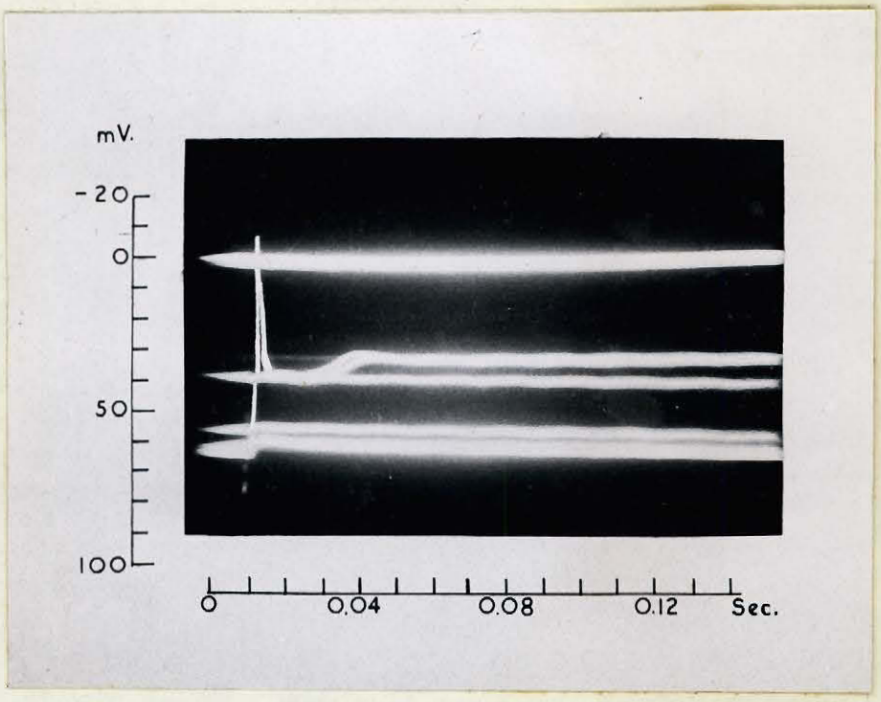

Figure 21. - Threshold increase during the veratrine negative after-potential of a single fiber of the sartorius muscle. Muscle immersed in 2.0 X 10^{-7} veratrine sulphate. Superimposed sweeps 1 sec. apart. At the beginning of each sweep the fiber was stimulated with a pulse of the same strength and duration by means of an external saline electrode. Only the first and last stimuli produced an action potential.

D. Discussion of Results

Table 5 presents some values for the electric constants of resting frog muscle fibers obtained from the literature. The values given by Katz (1948) and Nicholls (1956) were determined using external electrodes. Those given by Fatt and Katz (1951), Castillo and Machne (1953) and found in my work, were determined with internal electrodes using the method described by Fatt and Katz (1951). The values given by Jenerick (1953) were determined by a rather different method, and although the basic assumptions were the same, the calculations were different. In the method given by Fatt and Katz (1951), "d" was calculated from r_i and by assuming that the specific internal resistance (R_i) equaled 250Ωcm. In the other methods "d" was visually determined. Despite the different method employed, my results agree quite well with those of Jenerick (1953) and the difference between these results, and those given by the other authors, for the sartorius muscle seem to represent a true species difference. This is not surprising when one considers the larger difference observed when using different muscles from the same species (Table 5).

For several reasons, the general impression that veratrine in low or moderate concentrations has little effect on the resting fiber membrane, cannot be held. Of particular interest in this respect are the findings reported by Shanes (1952b). He found that treatment of resting frog sciatic nerve with veratrine (1.7×10^{-6} to 1×10^{-5}), appreciably increased the net loss

TABLE 5

Electric constants of fibers from a few frog muscles.

Muscle	λ (mm)	τ_m (msec)	$1/2\sqrt{r_m r_i}$ (Ω)	"d" (μ)	R_m (Ωcm^2)	C_m ($\mu f/cm^2$)	References
Rana temporaria							
adductor magnus	0.65	9	------	75	1500	6.1	Katz (1948)
extensor longus dig. IV	1.1	18.5	------	43	4300	4.4	Katz (1948)
extensor longus dig. IV	1.06	17.2	------		4970	4.1	Nicholls (1956)
sartorius	2.4	34.5	204000	137	4100	8	Fatt & Katz (1951)
sartorius	1.59	22	167000	122	2064	10.6	Castillo & Machne (1953)
Rana pipiens							
sartorius	1.54		304000	90.4	2500		Jenerick (1953)
sartorius	1.55	11.7	213000	109	2200	6	-(TABLE 2)-

of K^+ and uptake of Na^+ in the presence of oxygen. From the work of Feng (1941), we can estimate that this is equivalent to treating frog muscle with veratrine concentrations of 10^{-8} to 10^{-7}, or slightly greater (but certainly less than 10^{-6}).

It is generally believed that the resting potential of nerve and muscle fibers is dependent upon the distribution of ions across the cell membrane, and that the ion distribution in turn is dependent upon the metabolic activity of the cell (Hodgkin, 1951). There are several reasons for believing that the resting potential is primarily dependent upon the K^+ distribution (Boyle and Conway, 1941; Hodgkin, 1951). At first it was felt that this state of affairs could be explained by assuming that the fiber membrane was impermeable to Na^+ (Boyle and Conway, 1941) and that the distribution was simply a case of a Donnan equilibrium. However, recently it has been found by several workers, primarily by use of radioactive tracer techniques, that the fiber membrane has an appreciable Na^+ permeability, and so other explanations of the ion distribution had to be sought (see Hodgkin, 1951 for references). Thus, it has been suggested that the fiber actively secretes Na^+ (Hodgkin, 1951), that it actively absorbs K^+ (Shanes, 1952b) or that there is an active process involving both these ions (Hodgkin and Keynes, 1953 and 1954). In any event, probably the simplest explanation of the veratrine effect on resting nerve and muscle, would be to assume that the veratrine simply blocks the metabolic processes that maintain the ionic distribution. However, such an explanation could hardly explain the increase in resting O_2 consumption

produced by veratrine treatment of nerve (Schmitt and Gasser, 1933) or the great increase in resting membrane conductivity (membrane conductivity = 1/membrane resistance) that I have found. For example, it might be argued that the increase in conductivity might be due to the increase in the external K^+ concentration (Shanes, 1952b; Shanes, et al., 1953). Jenerick (1953) studied the effect of external K^+ concentration on the membrane resistance (or conductivity) of frog sartorius muscle fibers. From his data, we find that for the membrane resistance to go from 2400 cm^2 to 1300 cm^2 (Table 2, Muscle 1) the external K^+ concentration would have to rise from 2.5 mmole/l to 10 mmole/l. Under the conditions of my experiments (a small volume of muscle in a large volume of external solution) this would be an impossibly great increase. But even if the rise in external K^+ were limited to a thin sheath of fluid about the cell membrane (similar to that demonstrated by Frankenhaeuser and Hodgkin, (1956) in the squid axon), this increase in external K^+ would also be associated with a fall of the resting potential from 82 mV to about 63mV. Such a large fall in resting potential has never been observed with this concentration of veratrine (10^{-7}).

A much more reasonable explanation of the results would be to assume that the veratrine, in some manner, increases the permeability of the cell membrane. Keynes (1954), using radioactive tracers, has found that K^+ enters the frog muscle fiber some 20 times more easily than Na^+, and Jenerick (1953) by applying his data to the "constant field theory" equations of Goldman (1943;

Hodgkin and Katz, 1949) has calculated that the sartorius muscle fiber membrane is some 37 times more permeable to K^+ than to Na^+ ions. Since this difference in permeability is primarily dependent upon membrane structure, it is not inconceivable that a non-specific increase in membrane permeability (eg. an increase in the average size of membrane pores) might result in a relatively greater increase in Na^+ permeability. Thus for example, if the relative Na^+ permeability (P_{Na}/P_K) were to double, the resting potential would drop about 10mV (using Jenerick's (1953) figures) according to Goldman's constant field theory. A fall in the resting potential of this magnitude is not inconsistent with my findings (see also Feng and Liu, 1949). On the other hand, if the relative permeabilities were not to change, then there would be no fall in the resting potential. The changes in the internal ion concentration would be of little consequence. It was found, (again using Jenerick's figures) that if the internal Na^+ were to increase by 10% and the internal K^+ were to decrease by the same amount, there would be practically no change in the resting potential. This is quite a large estimate of the changes in internal ion concentration as compared to the actual changes found by Shanes (1952b). With the order of veratrine concentrations under consideration, they were in the vicinity of 1 mmole/liter of tissue/hr.

The relative ineffectiveness of decreasing the internal K^+ on the resting potential is to be expected even on the simple assumption that the resting membrane is impermeable to Na^+, and acts roughly like a potassium electrode (Boyle and Conway,

1941; Nastuk and Hodgkin, 1950). In this case, the resting potential (E_r) would be related to the K^+ concentrations in the following manner: $E_r = 59 \log K^+_i / K^+_o$, where E_r is in mV., and K^+_i and K^+_o are the internal and external potassium concentrations respectively. Accordingly, to reduce the resting potential from 99 mV. to 59 mV. without changing the external K^+ concentration (of 2.5 mmole/l) the internal K^+ concentration would have to be lowered from about 120 mmole/l to 25 mmole/l. Thus for a reduction of less than half the original resting potential, the internal K^+ concentration would have to be lowered to about 1/5 of its initial level. This relation between the resting potential and K^+ concentration has been experimentally verified by several authors (see Hodgkin, 1951). Desmedt (1953) found that the resting potential was quite steady when frog sartorius muscles were kept for long periods of time in very low K^+ solutions, under which conditions considerable losses of the internal K^+ occurred.

It would seem that veratrine treatment reduces the membrane potential by altering the membrane permeability in such a way as to increase the relative Na^+ permeability, rather than by changing the distribution of ions across the fiber membrane. This concept is particularly well supported by the experiments on the veratrine negative after-potential. In this case, the rather large, long-lasting depolarization is either completely eliminated, or severely reduced by reduction of the external Na^+ concentration (Fig. 19 and 20). It is hard to envision the external Na^+ concentration having such a profound effect on the membrane potential during the vera-

trine negative after-potential, unless it makes the membrane relatively more permeable to Na^+. It is of some interest to see how the experimental results compare with the theoretical results that would be obtained using Goldman's constant field theory (1943). The equations, based on this theory, were derived mainly by Hodgkin and Katz (1949), who applied the equations to the results obtained in studying the electrical activity of the squid giant axon. The constants I used in my calculations are those obtained by Jenerick (1953) in studying the electrical properties of the sartorius muscle of Rana pipiens. Since the membrane potential is rather constant in the veratrinized muscle fiber, for the first few hundred msec. following the action potential (Fig. 11D, 19c, 20, and 21), I shall limit the discussion to this period of time. When the fiber is poisoned with a veratrine concentration of about 10^{-8} (Fig. 11B and D, Fig. 19), the steady membrane potential developed following an action potential, usually equaled the maximum amplitude of the normal negative after-potential of an unpoisoned fiber having the same resting potential. If we assume that a veratrinized fiber had a resting potential of 83 mV., then from Fig. 13, we find that the steady potential following an action potential will be about 68 mV. Assuming that the only change following the action potential, is a relative increase in the Na^+ permeability of the membrane, the calculations show that this new membrane potential would be established, if the relative Na^+ permeability (P_{Na}/P_K) increased from a resting value of 0.027 to

a new value of 0.064. Using this new value for the Na^+ permeability following the action potential, we find that when the external Na^+ concentration is reduced by 50% the steady membrane potential would equal 80 mV., or 3 mV less than the resting potential. The experimental results are somewhat better than predicted, since the veratrine negative after-potential seems to be completely eliminated (Fig. 19d). However, the steady potential in normal Ringer's solution (Fig. 19c) was less than the assumed value, the resting potential of the fiber shown in Fig. 19d was less than assumed and it would be rather difficult to see a potential change of only 3 mV. on the voltage scale used in Fig. 19. When a veratrine concentration of 2.0×10^{-7} is used (Fig. 20) the steady potential following the action potential is about 1/2 the resting potential. Thus for a fiber with a resting potential of 83 mV. the steady potential would be about 41 mV., and a relative Na^+ permeability of 0.235 is found by calculation. When the external Na^+ concentration is reduced to 50% of normal, the steady potential following the action potential would be 57 mV., or still about 26 mV less than the resting potential. When it is reduced to 30% of normal, the steady potential equals 62 mV which is only a slight improvement over the 50% reduction of external Na^+ concentration. Although necessarily only an approximation of the true case, the calculations, based on the theory of the production of the veratrine negative after-potential, which I have been discussing, agree quite well with the experimental results.

As I mentioned above, the membrane conductivity

($1/R_m$) increased only about 6.3% during the veratrine negative after-potential. This had the effect of more than doubling the relative Na^+ permeability as indicated by the calculations using the constant field theory. According to the equations given by Hodgkin (1951), if the movement of Na^+ in the membrane was due to thermal agitation and the electric potential gradient, and the ions moved independently of one another (which is almost certainly not the case), then that part of the total membrane conductivity due to the movement of Na^+ ions would be proportional to the Na^+ flux. Since, as Keynes (1954) has shown, the Na^+ and K^+ fluxes are approximately equal, their contribution to membrane conductivity would also be approximately equal. As Keynes (1954) has said, "It is difficult to predict the contribution of sodium to the membrane conductance without knowing more about the mechanisms by which it crosses the cell membrane, ...". The results with veratrine indicate that its contribution is relatively small.

The increase in the membrane capacity produced by veratrine treatment is an interesting effect. This effect has previously been reported by Machine (1950), who surmised its presence by a rather indirect approach. Larger membrane capacitances have been reported for crustacian muscle fibers (Fatt and Katz, 1953), but usually only smaller values are reported in the literature. Changes in membrane capacity produced by experimental procedures are rarely found. The increase might be produced by an increase of the dielectric constant of the membrane, or by a folding of the membrane which would cause an error in the estimate of the surface area per

unit volume of fiber. There is some evidence that the membrane of frog sartorius muscle fibers is normally folded (Martin, 1954). All that can be said is that this effect is somewhat independent of the decrease in membrane resistance, and it tends to reach a maximum with increasing veratrine concentration (Table 2).

It is quite clear that the veratrine negative after-potential cannot be used as a model for the normal negative after-potential. The normal negative after-potential is essentially a passive decay of membrane potential back to resting levels, whereas even the exponential phase of the veratrine negative after-potential is far too slow to be considered a passive phenomenon. However, in the case of the normal negative after-potential, there is some indication that the external Na^+ has a modifying effect. Thus, at the start of a negative after-potential having a shape such as shown in Fig. 12a, the inward and outward movement of (positive) charge must be equal, since the membrane potential is steady for a few msec., and in the case shown in Fig. 12b, the inward movement of charge actually surpasses the outward movement for a few msec. Since Na^+ is the predominant cation in the external solution, it would seem reasonable to suppose that it can account for a large part of the inward movement of positive charge. The fact that these shapes (Fig. 12a and b), which were the types usually found (74% of the observations), are completely eliminated by reducing

the external Na^+ concentration, indicates that this supposition is correct. Reduction of the external Na^+ concentration also decreases the time constant for the decay of the normal negative after-potential, and it seems possible that, under normal conditions, there is a slight leakage of Na^+ during the negative after-potential which slightly retards repolarization. On the other hand, reduction of the external Na^+ concentration had no effect on the maximum amplitude of the normal negative after-potential. This would be expected if, as I have previously suggested, the latter potential is determined by the end of the facilitated outward movement of K^+ ions during the latter part of the action potential, or if in some manner this level ended the facilitated outward movement of K^+ ions.

V. SITE OF ORIGIN AND PRODUCTION OF THE REPETITIVE RESPONSES

A. Introduction

The location of the site of origin of the action potentials belonging to the repetitive response is of importance both from a theoretical and an experimental point of view. According to the "differential repolarization mechanism", the repetitive responses should arise in the junctional region between the slowly and rapidly repolarizing portions of the fiber membrane. Wible (1924a and b) has shown that when the end of the frog sciatic nerve is placed in veratrine solution, the after-discharge, as indicated by the mechanical response of the gastrocnemus muscle, is independent of the point of stimulation along the length of the nerve, and originates in the vicinity of the veratrine application. This finding is important for it shows that the veratrine after-discharge is independent of the stimulating current and any local change in the fiber membrane that may be produced by the stimulating current. The stimulus is necessary only for the production of a propagated action potential. When the entire sciatic nerve is soaked in a veratrine solution the after-discharge originates in the fine motor nerve fiber endings, and if these are removed, it originates in the smaller branches of the nerve which are more sensitive to the veratrine than the main nerve trunk (Dun and Feng, 1940a; Feng, 1941). Since a sufficient gradient of membrane potential along the fiber membrane is necessary for the production of the veratrine after-discharge (pg 45), it is to be expected that the

repetitive responses would arise in the vicinity of the greatest veratrine sensitivity, since this is the region where the membrane most slowly repolarizes following an action potential, and where, therefore, the largest gradient of membrane potential would occur. However, this need not be the case, and under certain conditions the largest gradient of membrane potential would not be at or near the point of maximum depolarization following an action potential. In such cases the repetitive responses should not originate at the point of slowest repolarization (or maximum depolarization), but in the vicinity of the maximum gradient of membrane potential. This latter expectation has been confirmed by the experiments described below.

In order to gain a more intimate understanding of the "differential repolarization mechanism" and to compare it with other forms of after-discharge and repetitive activity, it is important to record the potential changes along the fiber during the repetitive activity. The ideal method for recording such potential changes is by the use of inside-the-cell electrodes. However, these electrodes record the potential changes at only one point on the membrane, and for records obtained in this manner to have any meaning, the relation of the recording point to the point of origin of the repetitive responses must be known. Using the three compartment bath (Fig. 22), it has been found that the first action potential of the repetitive response originates approximately 1.5 to 2.0 space constants (λ) from the petroleum jelly junction in the veratrinized region of the fibers. The site of origin sub-

sequently moves further into the veratrinized region during the repetitive response.

B. Materials and Methods

1) Preparations, solutions and electrodes. - These three subjects have been dealt with in detail above (pp 17-21, 26-29, and 63-66). In the experiments contained in this section only normal Ringer's solution, containing appropriate amounts of d-tubocurarine and veratrine sulphate, was used.

2) Bath arrangements. - The saline bath (Fig. 1) and the perfusion arrangement (Fig. 9) were used in a few of the experiments. Since accurate observations of distance, and a good view of the muscle fibers were difficult to obtain using these arrangements, a specially designed bath was used in most of the experiments (Fig. 22). The bath and slides (S) were constructed from perspex (lucite) and cemented to a stand with a perspex top. Before mounting a muscle, the grooves (G) were filled with petroleum jelly, and the small platforms (P) were also covered. The threads attached to the ends of the muscle were tied to the silver wires (W). The muscle was placed against the bottom of the bath by pushing the threads along these wires. A bit of petroleum jelly was placed on the top of the muscle in the region of the grooves. The slides were inserted and pushed down as far as they could go, the petroleum jelly was smoothed, and saline placed in the compartments. In this way, the muscle was divided into three sections which could be bathed with different solutions. The holes in the sides of the

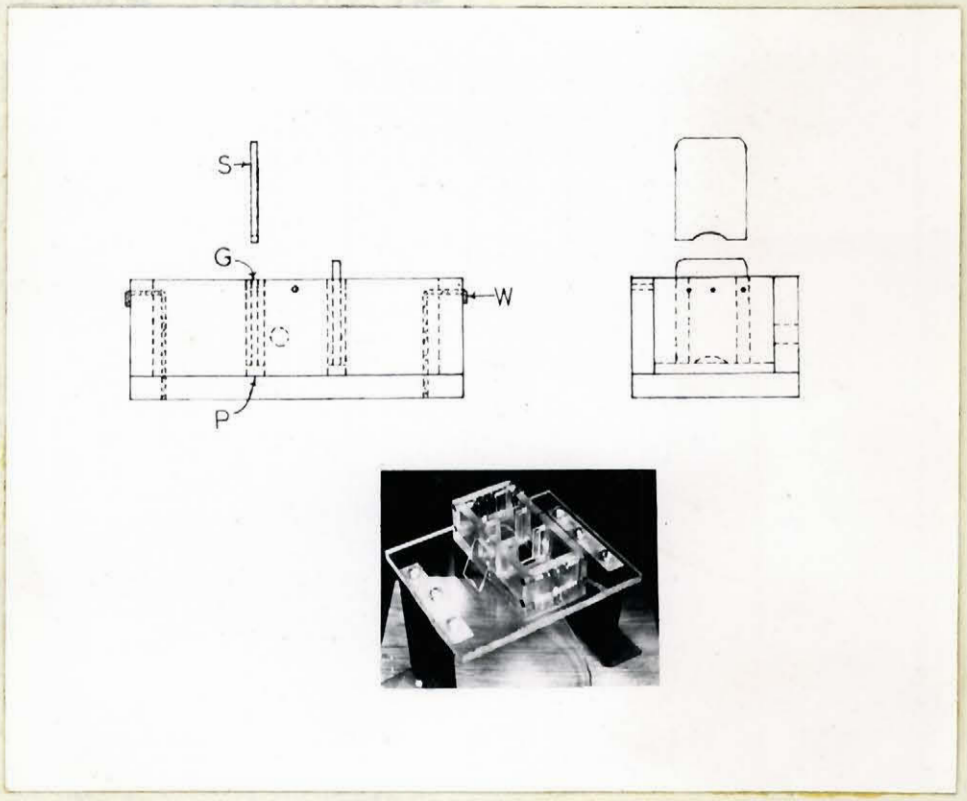

Figure 22. - Three compartment bath. For description see text.

center bath were provided as a means of circulating the center bath with Ringer's solution. This was necessary when using the saline bath for local veratrine application. However, the isolation of the compartments, as indicated by the maintenance of different solution levels, was excellent and it was never necessary to circulate Ringer's solution in the central compartment. The three compartments were electrically connected by means of a bath electrode constructed from a single piece of chlorided silver wire (see photo, Fig. 22). This electrode was generally grounded. The muscle was viewed from above with a disecting microscope, and distance measurements were made with an eye-piece micrometer contained in one of the oculars.

3) Recording systems. - Most of the recording arrangements used in these experiments have been described above (pp 21, 27, and 66). The bath shown in Fig. 22 was used in some experiments in which the exact location of the site of origin of the repetitive responses was determined. The recording arrangement used in this type of experiment is shown in Fig. 23. First the microelectrode attached to channel 1 (Y_1) was inserted into a fiber. Then, with the switch in position 2, the second microelectrode was inserted into the same fiber about 1.5 mm away. To check that the electrodes were actually in the same fiber, the switch was placed in position 1, and hyperpolarizing pulses (tip negative), of short duration and low current, were applied by means of the second electrode. The response recorded by the other electrode showed if the implantation was successful. The switch was then returned to position 2, and the fiber stimulated by means of an

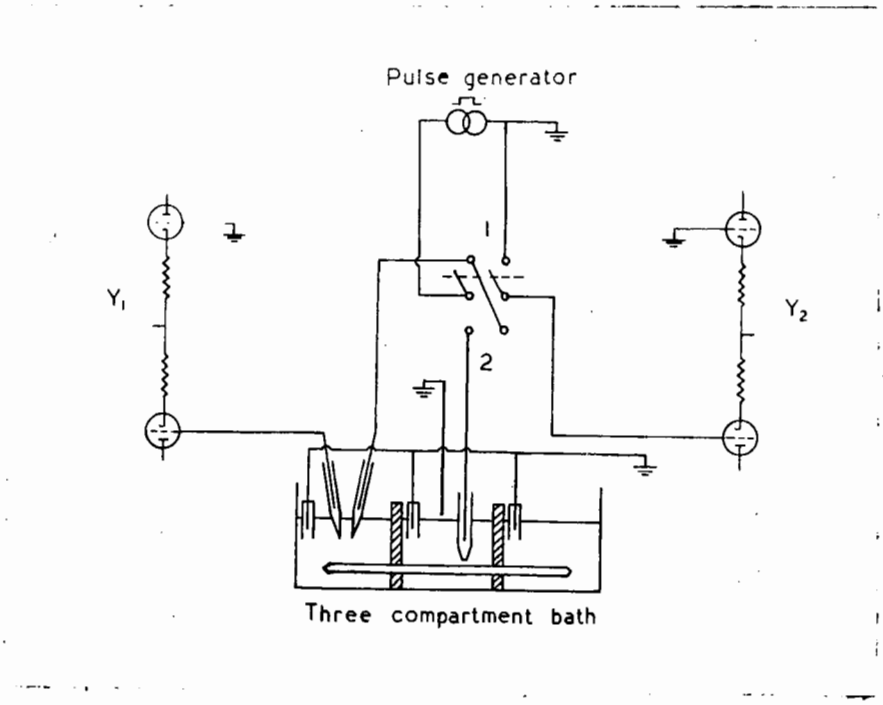

Figure 23. - Arrangement of electrodes for recording the after-discharge at two points along the same fiber. Note: double-pole, double-throw switch; position 1 for testing electrode insertion, position 2 for normal use. Veratrine sulphate put in end compartment containing the proximal end of muscle and the two microelectrodes.

external saline electrode in the central compartment. The veratrine solution was always placed in the compartment which contained the proximal end of the sartorius muscle.

C. Experiments and Results

1) Site of origin. - Using the saline bath, an approximate location of the site of origin of the repetitive responses can be obtained by the recording arrangement shown in Fig. 24. In Fig. 24a, the response recorded before veratrine application is shown. When the action potential travels from right to left, the diphasic response is first phase up on both beams, and appears first in channel 2. Since the electrical resistance of the saline is low compared to that of the petroleum jelly junctions the amplifiers record only the potential difference across these junctions. Fig. 24b shows the repetitive response obtained after placing veratrine sulphate, 0.5×10^{-6}, in baths 1 and 2. Since the repetitive responses are first phase up in channel 1, they are traveling from bath 2 to bath 1. In channel 2, they are first phase down and, therefore, traveling from bath 2 to bath 3, or in the opposite direction to that of the initial driven response. Since the repetitive responses appear first in channel 2, their site of origin must be closer to the junction between baths 2 and 3, than to the one between baths 1 and 2. The diameter of the baths was 6 mm and that of the junctions 0.5 mm. Therefore, under the conditions of this experiment, the site of origin of the repetitive responses is limited to a 3.25 mm length of muscle fiber, starting in the center of the first veratrine bath adjacent to a bath containing no vera-

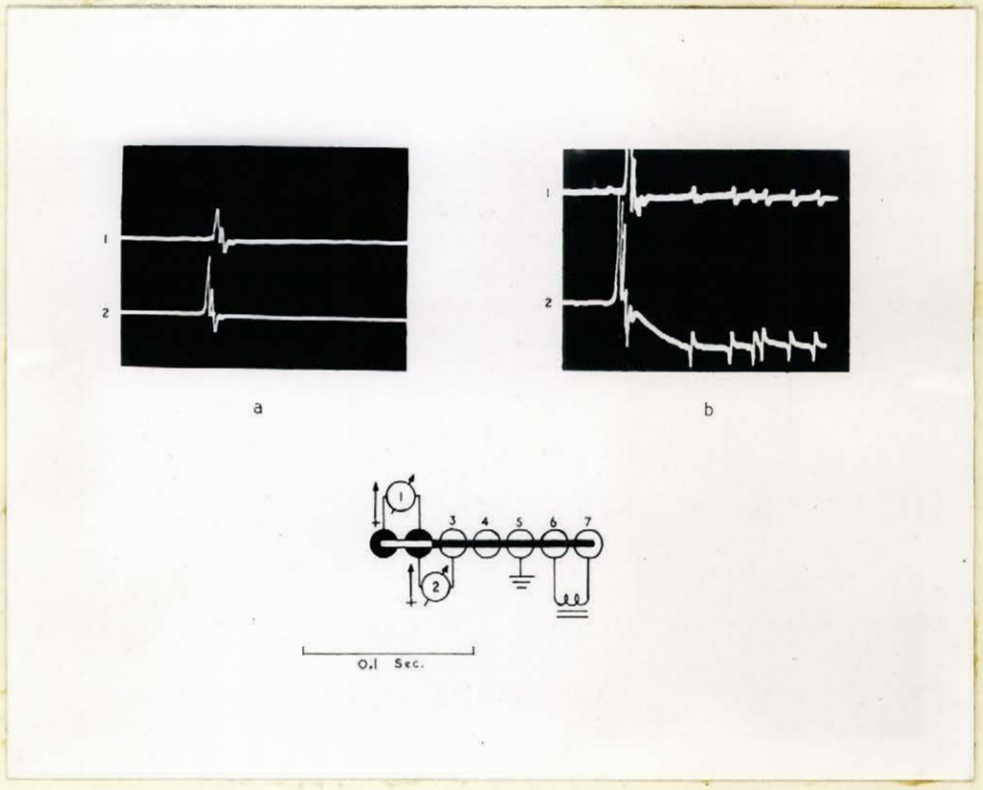

Figure 24. - Location of the site of origin of the repetitive responses using the saline bath. (a) Control response. (b) Repetitive response following a single stimulus with 0.5×10^{-6} veratrine sulphate in baths 1 and 2.

trine, and ending in the center of the junction of these two baths. The slight depression of the base line in channel 1 following the initial action potential indicates that the depolarization following the action potential is slightly greater in bath 1 than in bath 2.

In experiments such as those illustrated in Fig. 3 and 4, where only a small portion of the fiber surface is not treated with veratrine, and the stimulating electrodes are in baths containing veratrine, the repetitive responses always originated in one or the other of the two baths next to the bath which contained no veratrine. Using the experimental arrangement shown in Fig. 9, it was found that the repetitive responses originated in a region slightly below the point where the veratrine solution was run onto the muscle strip, but the point of maximum depolarization following an action potential was always considerably below this point.

A method for a more exact determination of the point of origin of the repetitive responses is shown in Fig. 25. The three compartment bath (Fig. 22) was used. The diagram in the lower left hand corner shows the end bath which contained 10^{-7} veratrine sulphate, and a part of the center bath which contained normal tubocurarine-Ringer's solution. The two microelectrodes were inserted into the same fiber, and the fiber was stimulated by an external saline electrode in the center bath. The photo in Fig. 25 shows the type of oscillograph record obtained when the point of origin of the repetitive responses is between the two internal electrodes. Assuming that the conduction velocity is the same in the resting fiber and during

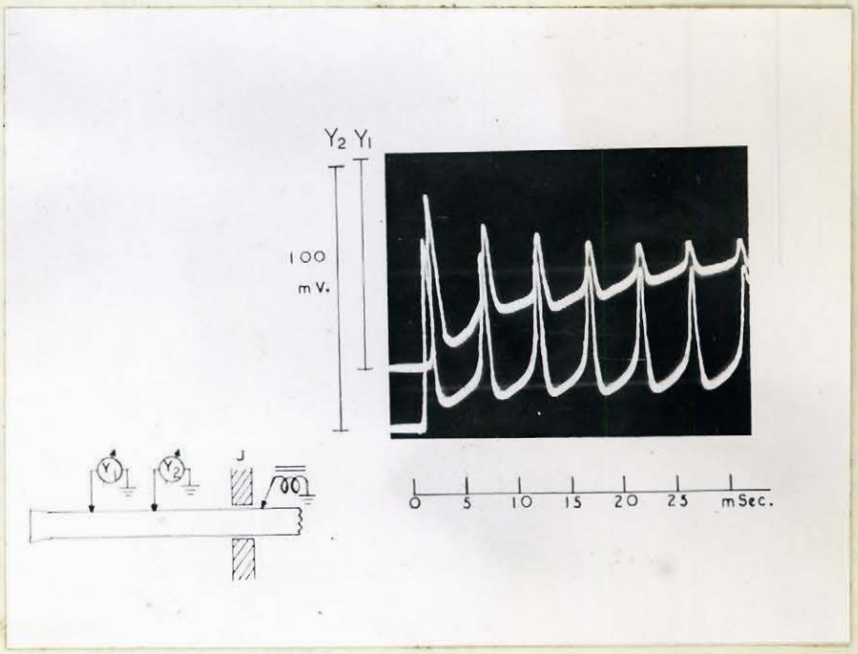

Figure 25. - Veratrine after-discharge recorded at two points along the surface of a single fiber of the sartorius muscle. The site of origin of the repetitive responses was between the two internal electrodes. 10^{-7} veratrine sulphate was only in the compartment containing the proximal end of the muscle. The electrode joined to Y_2 was 1.52 mm. from the veratrine bath side of the junction. The distance between the electrodes was 1.22 mm.

the veratrine negative after-potential, it is clear that if the site of origin is on other side of the two internal electrodes, then the time between the appearance of each repetitive response on the two sweeps will be the same as the time between the appearance of the initial response. The sweep on which the repetitive response first appears will tell the direction in which it is traveling. If the site of origin is between the two internal electrodes it can be determined from the following formula:

$$Z_n = C.V. \ (t_1 - t_n)/ 2 \qquad (1)$$

C.V. = conduction velocity = X/t_1

X = distance between the two internal electrodes

Z_n = distance between the site of origin of the n th repetitive response and the point of insertion of the internal electrode leading into amplifier Y_2

t_1 = time of appearance of initial action potential on Y_1 - the time of appearance on Y_2

t_n = time of appearance of the n th repetitive response on Y_1 - time of appearance on Y_2

This formula assumes that the point of origin of the repetitive responses can be considered to be an infinitely small point along the surface of the fiber, and that the conduction velocity during the veratrine negative after-potential is the same as in the initially resting fiber. According to the formula given by Fatt and Katz (1953) the conduction velocity would be equal to (Radius/specific internal resistance) $^{1/2}$ X C_m^{-1}. Since C_m increases about 20 to 25% during the veratrine negative after-

potential (Table 4), the conduction velocity would decrease about 15 -20% according to this formula. Table 6 lists the distance of the site of origin of the first few repetitive responses from the veratrine bath side of the petroleum jelly junction, determined by use of equation (1). Values are also given which were determined by assuming that the conduction velocity had decreased by 20%. This correction is small. The site of origin of the initial repetitive response is 1.5 to 2.0 space constants (λ) from the veratrine bath side of the junction, and it moves further into the veratrine region during the after-discharge (see Table 2 for λ in 10^{-7} veratrine sulphate).

2) Membrane potential changes during the veratrine after-discharge. - Trace Y_2 in Fig. 25 shows the typical membrane changes observed during the veratrine after-discharge near the site of origin of the repetitive response. The rapid declining phase of the initial action potential ends with the membrane repolarized to about the same extent as in the unpoisoned fiber. As the after-discharge continues, there is a slight decline in the membrane potential at the end of the rapid declining phase of each action potential. In trace Y_1, which was recorded from a point approximately 1.00 mm. further into the veratrinized region, the initial action potential ends with the membrane a few mV. more depolarized, the rate of depolarization following the initial action potential is much greater, and the decrease in the amount of repolarization following each repetitive response is also much greater. The amplitude of the veratrine negative after-potential increases as the

TABLE 6

Site of origin of the repetitive responses during the veratrine after-discharge. (Distance from the veratrine-bath side of the junction).

Response	Fiber 1		Fiber 2		Fiber 3	
	uncorr. (mm)	corr.* (mm)	uncorr. (mm)	corr.* (mm)	uncorr. (mm)	corr.* (mm)
1	1.36	1.58	1.73	1.82	1.72	1.81
2	1.69	1.84	1.87	1.93	1.93	1.97
3	1.77	1.91	1.87	1.93	1.97	2.00
4	1.90	2.02			2.05	2.07

* Corrected by assuming that the conduction velocity decreased 20% during the veratrine negative after-potential.

recording point moves away from the junction of the poisoned and unpoisoned lengths of the fiber membrane. Since the veratrine concentration is uniform throughout the length of the poisoned stretch of fiber, the gradation in its effect on the negative after-potential is most probably due to the spread of current from the adjacent stretch of unpoisoned fiber. Fig. 26b is a record made during a veratrine after-discharge on the border of the junction in the non-veratrinized region of the bath. The membrane potential during the initial response and until the appearance of the first repetitive response is quite normal (eg. Fig. 12b, Fig. 19a) and it is the spread of current from this region that tends to repolarize the adjacent veratrinized stretch of fiber. The reverse effect is seen in Fig. 26a which was also recorded from a point just inside the compartment containing normal Ringer's solution. The conditions were just subthreshold for the production of a veratrine after-discharge, but the spread of current into the adjacent veratrinized region during the veratrine negative after-potential, prevents full repolarization at this point on the unpoisoned stretch of fiber. As would be expected, records obtained from points further away from the veratrinized stretch of fiber show normal recovery of membrane potential following an action potential.

Fig. 27 shows the membrane response in the veratrine region when the conditions were just threshold for the production of a repetitive response. The picture obtained for the initiation of the first repetition is not very different than that obtained in normal nerve and muscle with just threshold stimulations (eg Hodgkin,

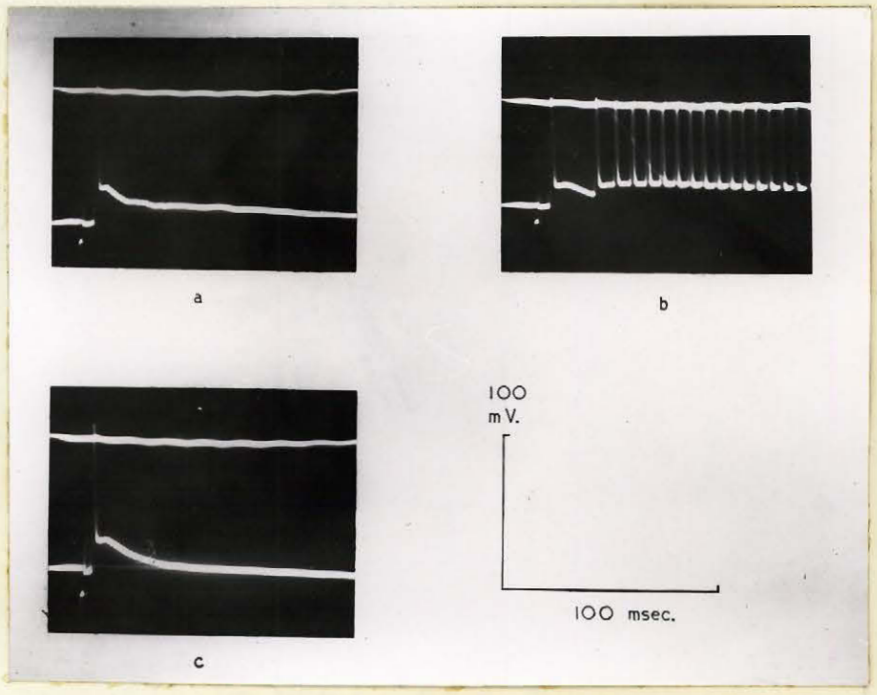

Figure 26. - The responses to a single stimulus of the sartorius muscle recorded with an internal electrode in the nonveratrinized region of fiber. 10^{-7} veratrine sulphate in end compartment only. Electrode implanted 0.1 mm. from the junction. (a) Response of a fiber when conditions just subthreshold for a repetitive response. (b) Repetitive response. (c) Control response before veratrine application.

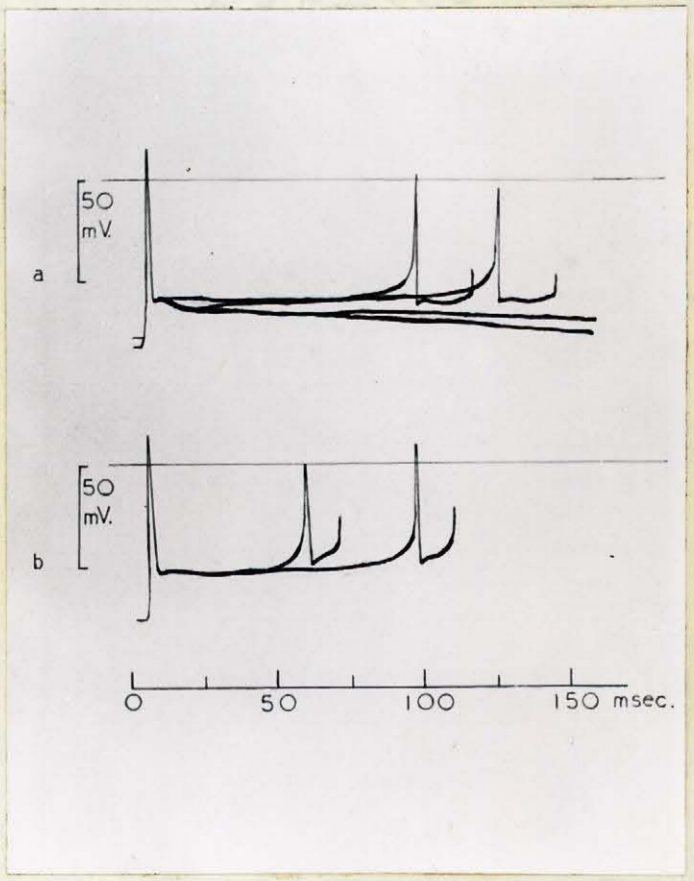

Figure 27. - Responses of single fibers from the sartorius muscle recorded with inside-the-cell electrodes when the conditions were just threshold for the production of an after-discharge. 10^{-7} veratrine sulphate in end bath. (a) Responses of a single fiber recorded 2.58 mm. from the junction in the veratrine compartment. (b) Responses of another fiber recorded 0.1 mm. from the junction in the veratrine compartment.

1938; Fatt and Katz, 1951) and it would seem that, as Kuffler (1945) has said, the veratrine negative after-potential acts in much the same manner as an externally applied cathodal current. However, this would not explain why universal veratrine application fails to produce after-discharging. Part of the explanation must be that the veratrine negative after-potential prevents the fiber membrane from recovering sufficiently to be able to develop another action potential. If this is the case, we should be able to produce a repetitive response, with universal veratrine application, by forcing part of the membrane to repolarize following an action potential. Fig. 28 shows this effect. The muscle from which this photo was obtained was kept in 2.0×10^{-8} veratrine sulphate. Unless the fiber is injured this concentration never leads to a repetitive response of the muscle fibers, but in this case the hyperpolarization, which was produced by a second microelectrode inserted into the same fiber a short distance away, established the conditions for an after-discharge. The abrupt start of the repetitive action potentials during the hyperpolarization indicates that their site of origin is a considerable distance from the recording electrode. The repetition following the end of the hyperpolarization is due, in part, to injury of the fiber membrane at the point of electrode insertion. This injury is also indicated by the fact that the hyperpolarization declined during the time of current application, although the polarizing current was actually increasing slightly.

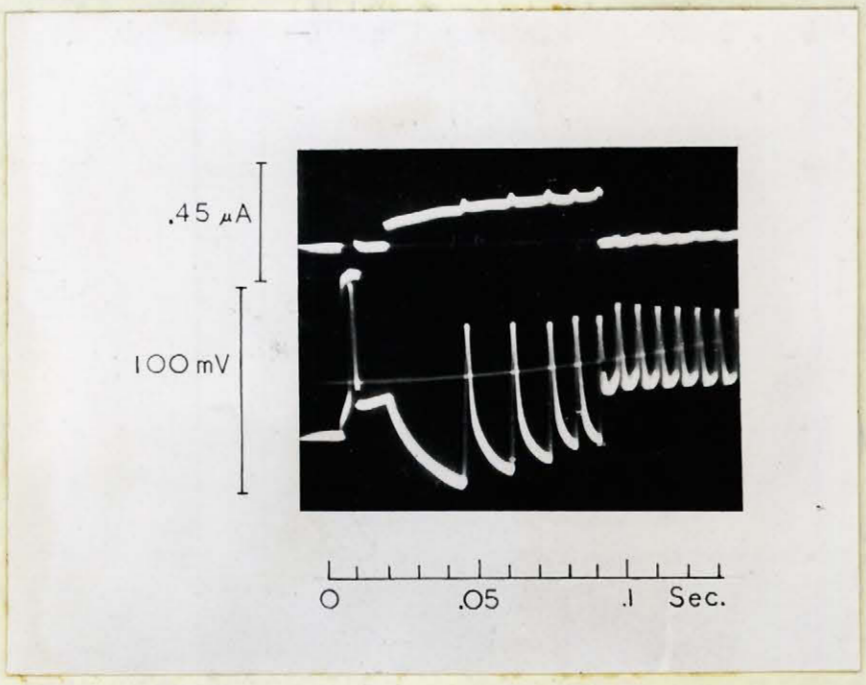

Figure 28. - Production of a repetitive response by hyperpolarizing the membrane of a single sartorius muscle fiber during the veratrine negative after-potential. Muscle completely immersed in 2.0 X 10^{-8} veratrine sulphate. Note: the poor shape of the current pulse (upper trace) was the property of the stimulating electrode. With most stimulating microelectrodes a steady current level was established within 30 msec.

D. Discussion of Results

All the evidence that I have presented supports the view that the decrease in membrane potential during the veratrine negative after-potential acts in much the same manner as a depolarization produced by an externally applied current. I have found nothing that indicated an increase in excitability, a failure to accommodate, an oscillation in membrane potential, or any other change during the veratrine negative after-potential that might, in some other manner produce the after-discharge. How then does this depolarization differ from membrane depolarizations produced by other means? In particular, why does a universal depolarization of large amplitude during a veratrine negative after-potential fail to produce an after-discharge, and how does local veratrine application overcome this difficulty?

With low veratrine concentrations (around 10^{-8} veratrine sulphate) the membrane depolarization following an action potential is undoubtedly too small to produce an action potential, for I was not able to obtain after-discharging using these concentrations, even with optimum conditions. With larger veratrine concentrations, the depolarization was sufficient, for with the proper conditions after-discharging could be obtained. With universal veratrine application, the conditions following the initial action potential are quite similar to those obtained with a voltage clamp that universally depolarizes the fiber membrane. Hodgkin et al. (1952) found that when the voltage clamp was set so

that it produced a large depolarization, there was an initial inward movement of Na^+ ions (or more exactly, an increase in Na^+ conductance), which corresponded to the rising phase of the action potential in the normal fiber, and this was followed by a maintained outward movement of K^+ ions. As long as the membrane was kept at this potential, the increase in membrane K^+ conductivity remained. Another pulse of increased Na^+ conductivity could only be obtained by first repolarizing the membrane and then depolarizing it again. With large veratrine negative after-potentials, it would seem that the membrane has not repolarized sufficiently following the action potential to permit recovery of the mechanism responsible for the increase in membrane Na^+ conductivity which is necessary for the production of another action potential. The question now arises as to whether there is some critical membrane potential at which the process responsible for the increase in membrane Na^+ conductivity is sufficiently recovered to permit the development of another action potential, and which at the same time will initiate another action potential? If such a membrane potential exists it would be rather easy to explain the ease of obtaining after-discharge with local veratrine application, for with local veratrine application there is an increase in the depolarization along the fiber as it gets further away from the junctional region. However, the inability of a gradual gradient of membrane potential to produce an after-discharge (Fig. 6) seems to rule out this possibility.

As can be seen in Fig. 27, the membrane potential in the veratrine treated region goes through two distinct phases follow-

ing the initial action potential. There is an initial slow repolarization which is probably caused by the spread of current from the unpoisoned stretch of fiber. This is followed by a slow depolarization which, after a variable length of time, leads to a more rapid depolarization, and finally to the production of an action potential. A comparison of Fig. 27a with Fig. 27b, shows that the slow rising phase starts sooner in the length of membrane further away from the junction, and a much greater depolarization is produced before the rapid depolarization leading to the action potential occurs. It would seem that the slow increase in depolarization starts in a region rather distant from the junction. It then spreads slowly towards the junction, and as it spreads, it comes in contact with muscle membrane which is less refractory because of the repolarization forced on it by the spread of current from the unpoisoned length of fiber. In the less refractory region the slight additional depolarization initiates the process that produces an action potential. A similar spread of depolarization from a refractory region of fiber membrane into a less refractory region, where an action potential was initiated, was observed by Hodgkin (1938) in single isolated Carcinus axons.

The experiment illustrated in Fig. 21 clearly shows that the muscle fiber membrane has a decreased excitability during the veratrine negative after-potential. Such a finding is hard to correlate with the simple assumption that an action potential is initiated by any process which reduces the membrane potential to a fixed critical level (Jenerick and Gerard, 1953). It is however, in

keeping with the findings of Hodgkin and Huxley (1952a) who observed that persistent depolarizations reduced the ability of the fiber membrane to respond to a subsequent larger depolarization. They refer to this process as "inactivation". The ability of a local hyperpolarization during the veratrine negative after-potential, to produce an after-discharge (Fig. 28) also agrees with their finding that hyperpolarization decreases "inactivation". A similar effect was observed by Hagiwara and Watanabe (1955) who found that a brief hyperpolarization during the negative after-potential of a muscle treated with veratrine in TEA-Ringer solution, was followed by an action potential. On the other hand, a depolarization of the membrane by an outward directed current failed to initiate an action potential.

Hodgkin and Huxley (1952a, b, and c) found that the initial increase in Na^+ conductivity which occurred when the fiber membrane was depolarized, always declined and disappeared when the depolarization persisted. If the membrane is repolarized during the time that the Na^+ conductivity is large, its decline is accelerated. They refer to all the factors that decrease the change in membrane Na^+ conductivity associated with depolarization as a single process of "inactivation" (Hodgkin and Huxley, 1952c). It was shown above that veratrine treatment of frog skeletal muscle increases the Na^+ permeability (or conductivity) of the fiber membrane. I suggested that this might be produced by a change in the membrane structure. It is also possible that veratrine prevents complete "inactivation" of the increased Na^+ conductivity mechanism. There is some evidence that the increased Na^+ conductivity outlasts

the spike of the action potential (see pg 111) in the unpoisoned muscle fiber and veratrine might simply prolong this effect. Both the decline of the Na^+ conductivity, and an increase in the K^+ conductivity are necessary for a quick recovery of resting potential during an action potential (Hodgkin and Huxley, 1952d). If veratrine specifically inhibits "inactivation", then the reason why the dramatic effect of veratrine follows an action potential is easier to explain. Certainly the action of veratrine on the electric constants of the resting fiber membrane is greater than its effect following an action potential, whereas its effect on membrane potential is just the opposite. This problem cannot be resolved until more is known concerning the mechanisms involved in the movement of Na^+ across both the resting and active fiber membrane. Veratrine itself might serve as a useful tool in such studies.

VI. GENERAL DISCUSSION

Probably the most fascinating aspect of the response of nerves and muscles treated with veratrine is the repetitive activity that occurs following a single stimulus, or, as I have chosen to call it, the veratrine after-discharge. Several theories have been proposed to explain this phenomenon. Most of these have been described above. I would now like to discuss in greater detail some of the currently held theories.

The idea that the veratrine negative after-potential is directly involved in the production of the repetitive response is a part of the "differential repolarization mechanism" proposed in this work, and is supported by the experimental results. However, the frequently held view that the veratrine negative after-potential is associated with, or causes an increase in membrane excitability (Krayer and Acheson, 1946; Kuffler, 1945; Bremer, 1955) and in this way produces the repetitive response, is not supported by the results of any of the experiments described above. It is quite likely that the leakage of K^+ ions during a veratrine after-discharge would cause a decrease in resting potential which is independent of any direct effect of the drug itself. Fig. 29b shows such an effect. There were 14 action potentials in the after-discharge which was recorded in an unpoisoned region of fiber. After an initial rapid phase of repolarization there was a considerably slower phase which was never present following a single response. The slow phase of repolarization is probably caused by the reabsorption of some of the external K^+, and the diffusion of the excess K^+ out of the

muscle, which is known to be a relatively slow process (Keynes, 1954). In one case a veratrine after-discharge which contained 32 action potentials ended with the resting potential temporarily depressed by 20 mV. The decrease in resting potential produced by increasing the external K^+ concentration would reduce the threshold, as measured by the amount of current necessary to stimulate the muscle (Jenerick and Gerard, 1953). Therefore, one would expect that a submaximal stimulus applied during a veratrine after-discharge would excite a larger number of fibers than in the resting condition. This would be particularly true for the fibers in the muscle that are relatively less affected by the veratrine treatment. This is the generalway in which supernormality is demonstrated during the response of a veratrinized muscle (Bremer, 1955). Viewed in this manner the supernormality can hardly be called an effect of veratrine, and it is probable that the effect would be greater in the unpoisoned stretch of muscle, where the fibers are not refractory, as they are during the veratrine negative after-potential.

Ever since the "humoral transmission theory" of veratrine action was first proposed (Szent-Gyorgyi et al., 1939), the feeling has prevailed that the action of veratrine on nerve and muscle was related to, and dependent upon the K^+ ions released by the fibers during activity. Most of the experimental evidence presented in this work either directly, or indirectly refutes this hypothesis. There are, however, several similarities in the action of veratrine and K^+ on muscle and nerve. For example, both reduce the membrane potential of nerve and muscle fibers. However, the

reduction of membrane potential produced by increasing the external K^+ is a continuous function of the log of its concentration (Boyle and Conway, 1941; Jenerick, 1953; Feng and Liu, 1949), whereas the reduction in resting potential produced by veratrine treatment is not (Feng and Liu, 1949). Both treatments increase membrane conductance. However, the increase in membrane conductance produced by K^+ treatment is proportional to the change in membrane potential produced at the same time (Jenerick, 1953), whereas the effect of veratrine treatment on membrane conductance is greatest in the resting fiber, while its effect on membrane potential is greatest following an action potential. Also, the increase in membrane conductance produced by veratrine treatment is associated with an increase in membrane capacitance (Tables 2 and 4) while K^+ treatment has little or no effect on membrane capacitance (Hodgkin, 1947). Another interesting similarity of action is contained in an observation of Hodgkin (1947). He found that local, but not universal, application of high K^+ along the surface of an isolated Carcinus axon produced a repetitive response and the impulses arose, not at the point of K^+ application but in an adjacent region due to local circuit action.

The fact that the conditions that best favor the removal of K^+ ions that leak out of the fibers during activity (Fig. 9), are the optimal conditions for the production of the veratrine after-discharge, makes it difficult to accept the "humoral transmission theory", which proposes that the main or sole action of

veratrine treatment is to make the muscle more sensitive to the action of external K^+ ions, and that the reason the action of veratrine is most prominent following an initial response, is because this response liberates K^+ ions to which the muscle fibers are more sensitive due to the action of veratrine (Bacq, 1939b). An equally serious difficulty is the relation found between the external Na^+ concentration and the effect of veratrine (Fig. 19 and 20). The membrane potential changes associated with veratrine treatment are dependent upon the external Na^+ and it would seem that the veratrine after-discharge is also dependent upon this factor. This agrees with the findings of Feng (1941), and most other workers (see Krayer and Acheson, 1946), that increasing the external K^+ concentration decreases the effects of veratrine treatment, and increasing the external Na^+ concentration increases its effects. It would seem, therefore, that both veratrine treatment and increasing the external K^+ concentration produce changes in nerve and muscle fibers that are of a similar nature but these similar effects are produced by entirely different means. It is quite likely that under certain circumstances they might augment the activity of each other, but it would be wrong to infer that veratrine simply potentiates the activity of external K^+ ions.

The theory proposed by Gordon and Welsh (1948), that veratrine binds Ca^{++} ions that are released from the fiber membrane during the passage of an impulse, is a bit harder to evaluate. Their finding that high veratrine concentrations can cause spontaneous activity in the perfused crayfish chela-motor

axon preparation (Welsh and Gordon, 1947) is the only case in which veratrine treatment has been reported to produce such an effect (Krayer and Acheson, 1946). Although increasing the external Ca^{++} concentration will tend to inhibit the veratrine after-discharge, and decreasing the external Ca^{++} concentration will tend to favor the veratrine after-discharge (Welsh and Gordon, 1947; Krayer and Acheson, 1946), these effects are not limited to the veratrine after-discharge. Thus increasing the external Ca^{++} concentration will increase the threshold of most nerves and muscles (Brink, 1954; Jenerick and Gerard, 1953), and lowering it will have the opposite effect. Dun and Feng (1940a and b) found that barium and guanidine, like veratrine, caused the production of retrograde discharges in frog motor axons following a single stimulus of the nerve when the attached muscle was bathed in solutions containing these chemicals. Unlike veratrine, however, they also caused spontaneous activity of the motor nerve fibers, and the nature of the repetitive response caused by treatment with these agents was very different from the veratrine after-discharge. The activity of these three agents was inhibited by increasing the external Ca^{++} concentration. It would seem that Ca^{++} in high concentration reduces the ease with which action potentials can be produced, and in low concentration increases the ease of obtaining action potentials and repetitive activity. The effect of external Ca^{++} on the veratrine after-discharge is probably similar to the effect produced by quinine (Harvey, 1939), in that both these chemicals seem to inhibit all forms of repetitive activity, rather than to specifically

inhibit the action of veratrine treatment.

Some other difficulties in accepting this hypothesis are the facts that the effects of Ca^{++} on excitability and on repetitive activity seem to be relatively independent of any effect on membrane potential (Jenerick and Gerard, 1953; Brink, 1954), and that increasing the external Ca^{++} concentration increases the amplitude and duration of the negative after-potential (Brink, 1954). The latter effect is just the opposite of what would be expected if veratrine simply acted like a decrease in external Ca^{++} concentration. On the other hand, it has been suggested that Ca^{++}, and Ca^{++} compounds are involved in the transport of Na^{+} ions across the fiber membrane, either by acting as carrier molecules (Hodgkin et al., 1949) or by acting on the membrane structure (Brink, 1954). If this should prove to be the case, then there might well be a specific relation between the veratrine and Ca^{++} effects on the fiber membrane. At the present time, however, the experimental evidence does not seem to favor such a view.

As mentioned above, the initial interest in this problem was not in the effects of veratrine itself, but in the veratrine after-discharge as an example of the more general type of after-discharge mechanism called the "differential repolarization mechanism" (Burns, 1955). The electrical activity of nerves and muscles can be classified in several ways. The type of classification found in the literature generally depends on the interest and the prejudice of the individual author. The actual types of activity found in nature generally tend to fall in between the various classes proposed,

and are, therefore, often difficult to fit into the proposed scheme. With this difficulty in mind, I would like to present a simple scheme for the classification of repetitive discharges as follows: When the repetitive discharge outlasts the change in the environment of the cell that initiated it, it can be called an after-discharge. A repetitive discharge produced by a prolonged or steady change in the environment, might be considered to be the result of a failure or lack of accommodation in the cells involved. Only when action potentials arise without any change in the environment of the cells involved, should they be classed as "spontaneous activity".

Viewed in this manner, many types of repetitive activity which are commonly called forms of "spontaneous activity", such as the "spontaneous activity" produced by the removal of Ca^{++} from the external environment of nerve cells, should be considered a result of the failure of the cells to accommodate to a change in their environment. Physiologists probably began referring to the activity produced by various forms of chemical stimulation as "spontaneous activity" at the time when they felt that most cells were stimulated by electric currents. Today, it appears that chemical stimulation is the usual way in which cells within the body are stimulated (Loewi, 1954; Dale, 1954) and it no longer seems reasonable to refer to activity produced by chemical stimulation as "spontaneous activity". The classic example of spontaneous activity is, of course, the rhythmic activity of cardiac muscle. However, there is the possibility that there might be some form of spontaneous activity of cells in the cerebral cortex (Bremer, 1949; Burns, 1956)

or elsewhere in the central nervous system.

The responses seen in sensory axons (Adrian, 1928) are typical of the form of repetitive activity which is produced by a maintained change in the external environment. The frequency of the response might reach a maximum when the stimulus is first applied and then decrease until the activity stops (Katz, 1950 a and b; Eyzaguirre and Kuffler, 1955a) or it might remain constant as long as the stimulus remains constant (Eyzaguirre and Kuffler, 1955a). This form of activity has been duplicated by constant current stimulation of Carcinus axons (Hodgkin, 1948) and many other tissues show a repetitive response to constant current stimulation.

In order to distinguish after-discharges produced by a "differential repolarization mechanism" from other forms of after-discharge, it would be proper to list some of the more important features of this type of after-discharge. Thus, when the conditions are adequate for the production of this form of after-discharge, it is found that:

(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 for an after-discharge consisting of many action potentials and it is extremely hard to

obtain conditions for the production of a single after-discharge.

(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. 29b).

(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. 29b).

(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 stop suddenly (Fig. 29b).

(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. Part of this gradient must involve a portion of membrane that has repolarized sufficiently to permit the development of another action potential (reactivation of the Na^+ carrier mechanism).

Fig. 29 contains records of two types of after-discharge that can be obtained using frog's skeletal muscle. The after-discharge in Fig. 29a was initiated by a single stimulus applied to the motor nerve while the muscle was immersed in a solution containing 10^{-6} eserine sulphate. This after-discharge is produced by the persistence of the humoral transmitter. It differs from the after-discharge illustrated in Fig. 29b in several respects. The most important differences that can be found by simply looking at the records are that the after-discharge has its maximum frequency

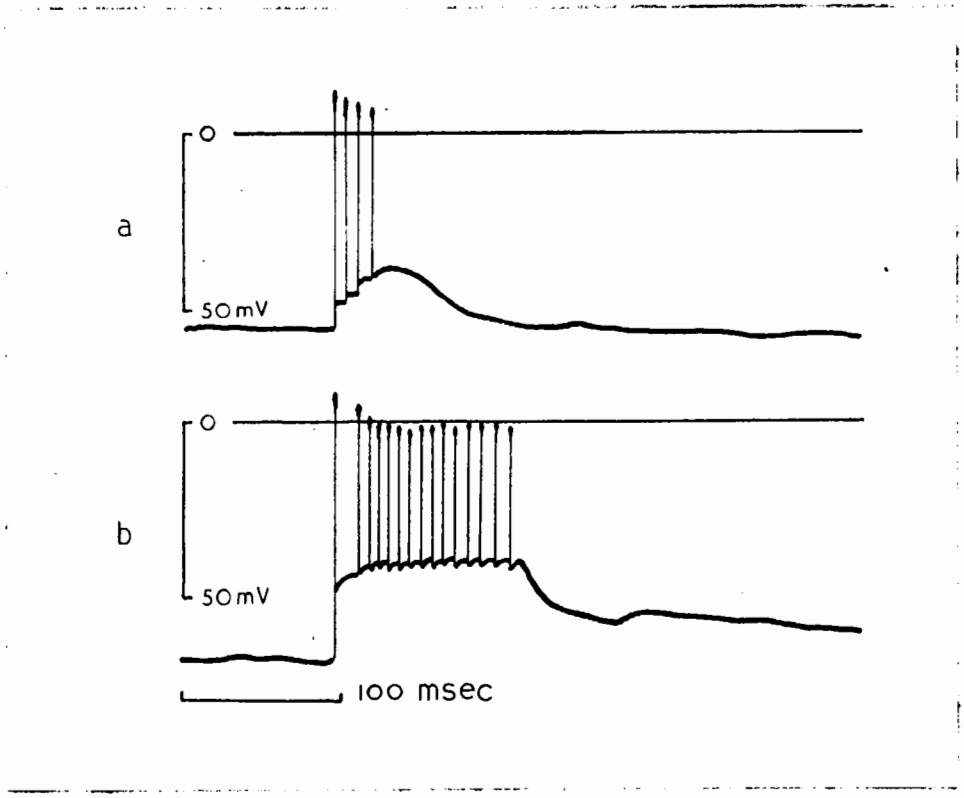

Figure 29. - After-discharges in frog sartorius muscle fibers caused by drug application. (a) Response of a single fiber to an indirect stimulus when the muscle was immersed in 10^{-6} eserine sulphate. Recorded with an inside-the-cell electrode. (b) Repetitive response obtained with 10^{-7} veratrine sulphate in end compartment of bath (Fig. 22). Recorded with an inside-the-cell electrode 6.5 mm. from the junction in the nonveratrinized region of fiber.

at the beginning and that the latent period before the start of the after-discharge is shorter than the time interval between the first two action potentials of the repetitive response.

The response of the Limulus eye to brief flashes of light (Hartline et al., 1952) shows all the characteristics of an after-discharge produced by a "differential repolarization mechanism". Their records, obtained from single optic nerve fibers, bear a striking resemblance to veratrine after-discharge records, and it would seem that the mechanisms involved are quite similar. Modified forms of this "differential repolarization" type of after-discharge have occasionally been reported. For example, there is the repetitive response of crayfish sensory cells (Eyzaguirre and Kuffler, 1955b), or of catfish Mauthner cell to antidromic stimulation (Tasaki, Hagiwara, and Watanabe, 1954).

The "differential repolarization mechanism" was first proposed to explain the "after-bursts" that can be produced in isolated slabs of cat's cerebral cortex (Burns, 1954, 1955). It has also been suggested as a possible explanation for the production of epileptiform after-discharges (Burns, 1953). Although it has already served as the catalyst for an investigation on a rather different subject, its ultimate value depends on just how great a help it will be in explaining the after-discharges that occur in nature.

SUMMARY

1. The literature concerning the use of veratrum alkaloids in scientific studies has been reviewed. In this review the effects of veratrine on the electrical properties of nerves and muscles have been stressed.

2. Direct excitation of fully curarized sartorius muscle fibers gave rise to an after-discharge only when veratrine was locally applied to the length of the fibers. Veratrine delays repolarization of the muscle fiber's membrane after an action potential has passed through the veratrinized region and the difference in repolarization rates of veratrinized and untreated lengths of muscle fiber is the immediate cause of the after-discharge.

3.. Factors which modify this difference in repolarization rates (or gradient of membrane potential) modify the after-discharge in the predicted manner.

4. The site of origin of the repetitive responses was located 1.5 to 2.0 space constants from the junction between treated and untreated fiber in the veratrinized region of fiber. The membrane potential changes along the fiber's surface during the veratrine after-discharge was investigated with inside-the-cell microelectrodes.

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

6. The electric constants of resting sartorius muscle fibers were investigated and it was found that veratrine treatment increased the membrane capacitance (C_m) and decreased the membrane resistance (R_m).

7. The negative after-potentials of normal and veratrinized sartorius muscle fibers were investigated. A linear relation between the resting potential and the maximum amplitude of the negative after-potential was

found. This relation was undisturbed by changing the external Na^+ or K^+ concentrations, or by moderate veratrine treatment. Strong veratrine concentrations or prolonged treatment with moderate concentrations increased the maximum amplitude of the negative after-potential.

8. The time constant for the decay of the normal negative after-potential was similar to that for the decay of an impressed hyperpolarization. In the unpoisoned fiber the evidence supports the view that the rapid phase of repolarization following an action potential ends with the fiber membrane slightly depolarized and the rest of the negative after-potential is a passive return of the membrane potential to its resting level.

9. In the veratrinized fiber there is an increased Na^+ conductivity following an action potential which considerably delays repolarization. The findings that there is a slight increase in membrane conductivity during the veratrine negative after-potential, and that decreasing the external Na^+ concentration can severely reduce or completely eliminate the veratrine negative after-potential, support this hypothesis.

10. The other theories proposed to explain the veratrine after-discharge and the veratrine negative after-potential have been discussed in detail.

The claims to original research are included in the above summary.

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