Graded and All-or-None Electrogenesis in Arthropod Muscle

II. *The effects of alkali-earth and onium ions on lobster muscle fibers*

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ABSTRACT Conversion of graded responsiveness of lobster muscle fibers to all-or-none activity by alkali-earth and tetraethylammonium (TEA) ions appears to be due to a combination of effects. The membrane is hyperpolarized, its resistance is increased, and its sensitivity to external K^+ is diminished, all effects which indicate diminished K^+ conductance. While the spikes are prolonged, the conductance is higher throughout the response than it is in the resting membrane. Repetitive activity becomes prominent. These effects indicate maintained high conductance for an ion which causes depolarization. This is normally Na^+, since its presence in low concentrations potentiates the effects of Ba^{++}, but the alkali-earth ions and TEA can also carry inward charge. Ba^{++}, Sr^{++}, and TEA appear to be more effective than is Ca^{++} in its normal role, which is probably to depress K^+ conductance and Na inactivation. Thus, conversion of graded to all-or-none responsiveness appears to occur because of the relative increase of depolarizing inward ion flux and decrease of repolarizing outward flux.

INTRODUCTION

The preceding paper (72) reported that applications of alkali-earth ions converted the gradedly responsive electrically excitable membrane of *Romalea* muscle fibers (8) to one which responds with all-or-none spikes. The insect muscle fibers thus resemble in that respect the muscle fibers of crayfish (13), which normally also produce only small, graded responses. The data of the preceding paper also indicated that the processes involved in the conversion

might be rather complex. Further studies were therefore desirable, as a possible means for analyzing the different components of bioelectrogenic activity.

However, the muscle fibers of *Romalea* respond with vigorous twitches and these either tend to break intracellular microelectrodes or to damage the impaled fibers. This technical problem is particularly acute in experiments with the alkali-earth cations, since *Romalea* muscle fibers tend to develop "spontaneous" myogenic or neurogenic contractions which persist for considerable time. Lobster muscle fibers respond to stimuli with much less vigorous contractions. Preliminary experiments (31) had shown that Ba^{++} increases resting membrane resistance in lobster muscle fibers and converts their graded responsiveness to the all-or-none variety. The present paper accordingly reports work which took advantage of these findings in seeking to analyze the nature of the action of the alkali-earth cations and also of tetraethylammonium ion (TEA), in converting graded to all-or-none activity.

METHODS

The stretcher muscle of the walking legs of the lobster, *Homarus americanus*, was used throughout this study. The exoskeleton over the bender muscle was removed, then the bender muscle itself. The nerves and membrane overlying the stretcher muscle were then carefully dissected free of the muscle. The exoskeleton remaining served as a convenient bath for the muscle. Only small amounts of test solution (less than 0.5 ml.) were usually required to effect the changes described.

The experiments were performed at temperatures of 15–20°C. The standard bathing solution used was that developed by Cole (10) but with the $SO^{=}_{4}$ replaced by Cl^{-} to avoid precipitation of $BaSO_4$. Ba^{++} was used in various concentrations to substitute for equivalent amounts of Na^{+} in the Ringer's solution. In some experiments all the chloride was replaced with acetate ions.

Solutions low in Na^{+} were made by reducing NaCl or by substituting choline chloride. Occasionally, up to 50 per cent of the NaCl was replaced by sucrose, but higher concentrations of sucrose caused depolarization and spontaneous contractions of the muscles. Soaking was carried out in large volumes at 5°C., and up to 1 hour of soaking in markedly hypotonic solutions caused no change in size of fibers, membrane resistance, or resting potential. After 5 hours the fibers were appreciably swollen, but again without significant change in resting potential or effective membrane resistance. All experiments in hypotonic low NaCl solutions were repeated with choline chloride substitution of the Na^{+} and some with sucrose substitution as well. No qualitative differences were observed in the results.

Solutions lacking K^{+} were made by omitting KCl or by substituting NaCl, CsCl, or NH_4Cl. Experiments with low Ca^{++} were carried out by addition of 90 mM/liter sodium citrate or by omission of $CaCl_2$. The latter method appeared to work more quickly and was preferred. In contrast to the effects of removal of K^{+}, those with Ca^{++} removal were quite slow, the maximum effect usually requiring at least 10 min. TEA, as the chloride or iodide, was substituted for Na^{+} on a milliequivalents per liter basis or was added in place of Ca^{++} as described in the Results.

One to three KCl-filled microelectrodes were inserted into a single muscle fiber for recording and stimulating as required by the design of the various experiments. Amplifying and stimulating equipment were standard for the laboratory. A four-trace cathode ray oscillograph permitted simultaneous recording of the different functions under study. The current employed in intracellular stimulation or for measurement of the fiber resistance was monitored as the voltage drop across a precision series resistor. Relative changes in the muscle fiber resistance were adequately significant for the purposes of the present work, and the resistance values employed are of the effective resistance or slope given by the applied current-membrane voltage data. Calculations of specific membrane resistance (R_M) of crustacean muscle fibers from the effective resistance give considerable scatter of values (14). This is in part due to the fact that lobster muscle fibers and those of other decapod crustaceans are cross-linked by ephaptic connections (58). Because of the graded responsiveness of the lobster muscle fibers, a criterion for anatomical continuity in the form of decrementless propagation was thereby excluded. It was therefore not always clear whether current and voltage electrodes were in the same fiber or in adjacent ones. Calculations of the specific membrane resistance are further complicated by the fact that the length constant (λ) of lobster muscle fibers is as great as or greater than the length of the fibers (60), as has also been observed in crayfish muscle fibers (13). Calculated by approximation equations (*cf.* reference 13), R_M ranged between 2000 and 4000 Ω cm^2 in lobster muscle fibers as compared with values of up to 15,000 Ω cm.^{2} in crayfish (20).

RESULTS

A. Conversion of Graded to All-or-None Responses

Muscle fibers bathed in *Homarus* Ringer's solution responded to direct stimulation with small diphasic graded potentials (Fig. 1). Occasional fibers developed a train of two or three highly damped pulses, but this type of response was less frequent than in crayfish (14) or in *Romalea* muscle fibers (*cf.* reference 72, Fig. 1). Within a few seconds after applying a Ba^{++}-containing medium the response developed into an overshooting all-or-none spike. The change was produced by substituting as little as 3 per cent of the Na^+ with Ba^{++}, but somewhat more slowly than when higher concentrations of Ba^{++} were applied. The essential features underlying this change are shown in Fig. 1. After applying Ba^{++} (*B*, *C*), the membrane resistance increased. A depolarizing pulse which prior to treatment had been well below threshold for a graded response (*A*) now evoked an overshooting spike (*C*, *D*). The critical level for this response was at less depolarization than the membrane potentials at which the graded responses were evoked by stronger currents prior to treatment of the muscle fibers (*A*). Thus, the apparent "threshold" for responsiveness, as measured by the applied current, had fallen. The response had also changed and was now a prolonged spike (*D*).

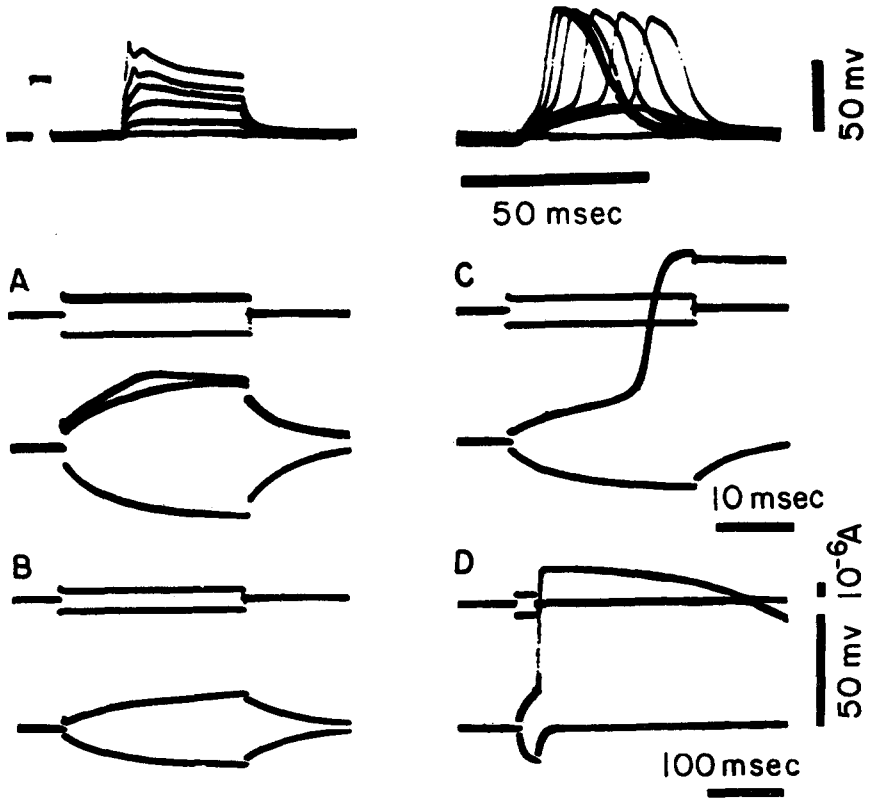

FIGURE 1. Conversion of graded responses to all-or-none spikes by Ba^{++} in lobster muscle fibers. *Upper left*, before applying Ba^{++}. Sequence of superimposed records of membrane potential in one fiber on applying progressively increasing current pulses of 60 msec. duration through an intracellular microelectrode. Calibrating pulse at beginning of traces is 50 mv. and 10 msec. A threshold response is seen in the third trace from the top, and a maximal response is shown in the highest trace. Note the decreasing latency as well as the increased amplitude of the response. The latter shows an undershoot indicative of delayed rectification. The rectification is also indicated by the fall of the membrane depolarization from its initial peak. *Upper right*, after applying 115 m.eq. Ba^{++}/liter to another muscle fiber. The superimposed traces show the effects of increasing strengths of intracellularly applied 30 msec. depolarizing pulses. The weakest stimulus failed to evoke a spike. The response evoked by the next stronger pulse occurred immediately after the end of the stimulus. The increasingly stronger stimuli evoked responses with briefer latencies, but of the same amplitude and form. *Below*, sequence of records from another preparation, showing the changes associated with conversion of the response. Upper trace in each set is of the intracellularly applied current (depolarizing stimulus upward). Lower trace in each set is of the membrane potential recorded with another microelectrode. *A*, before applying Ba^{++}. The effects of a subthreshold and a just threshold depolarizing stimulus and of a hyperpolarizing current are shown in the three superimposed records. *B*, a few seconds after applying 115 m.eq./Ba^{++}/liter. Note increased membrane resistance to the weak applied currents. *C*, the currents were increased slightly. The depolarizing stimulus now evoked a spike. *D*, the early part of the latter is shown on a slower time base.

The effective resistance, resting potential, and response height at first increased together as the Ba^{++} was raised to about 100 m.eq./liter (Fig. 2). The resistance then tended to remain at the new maximum value, while the resting potential when it changed, rose or fell only slightly. The response, which by this time had become all-or-none, continued to grow in height, but less rapidly than at the low concentrations of Ba^{++}. In individual fibers the resistance increased from 225 to 1000 per cent, the hyperpolarization by as much as 10 to 15 mv., and the responses attained amplitudes of up to 100 mv. These effects

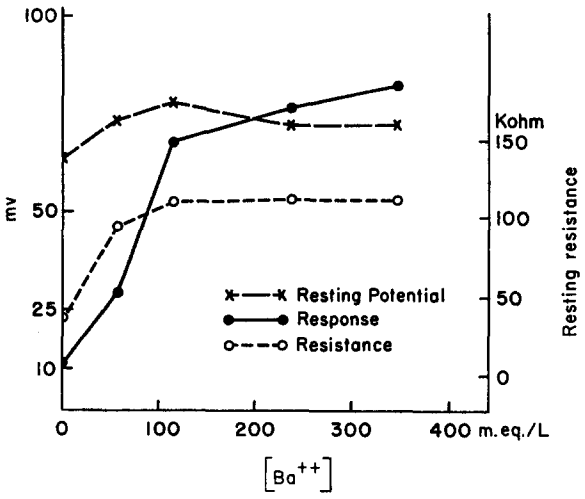

FIGURE 2. The effect of increasing concentrations of Ba^{++} on the resting potential, resting effective resistance, and maximum response amplitude. Effective resistance scale on the right ordinate. The potential scale on the left applies to response amplitude as well as resting potentials. In this preparation the all-or-none response did not develop an overshoot until 30 per cent of the Na^{+} had been replaced with Ba^{++}.

all developed within about 10 sec. after changing the solutions, and the data reported here are limited to experiments during the first 2 or 3 hours after the preparation was first treated with Ba^{++}. On prolonged soaking of the preparations in Ba^{++}-containing solutions (especially those with 50 per cent or more of the Na^{+} replaced) the resistance and resting potential decreased to below their values in the normal bathing medium, and the fibers lost responsiveness. The long term effect of Ba^{++} on lobster muscle fibers thus differs from the action of alkali-earth ions on crayfish muscle fibers which retained all-or-none responsiveness after soaking for up to 20 hours (13).

Details of the early stages in the development of the responses of Ba^{++}-treated lobster muscle fibers are shown in Fig. 3. The increased membrane resistance that developed relatively slowly after applying a low concentration of Ba^{++} is seen most clearly in the records of the experiment B_1–B_4. The same

current pulse was used throughout, but in the course of time after application of Ba^{++}, it evoked large terminal depolarization both at the "near" and "far" electrodes. The activity, recorded at 4 times gain differentially between these two sites (N-F), increased from a small response (B_1) to a large diphasic spike (B_4), the diphasicity indicating that there was propagation of the impulse. The diphasicity was approximately symmetrical, denoting the all-or-

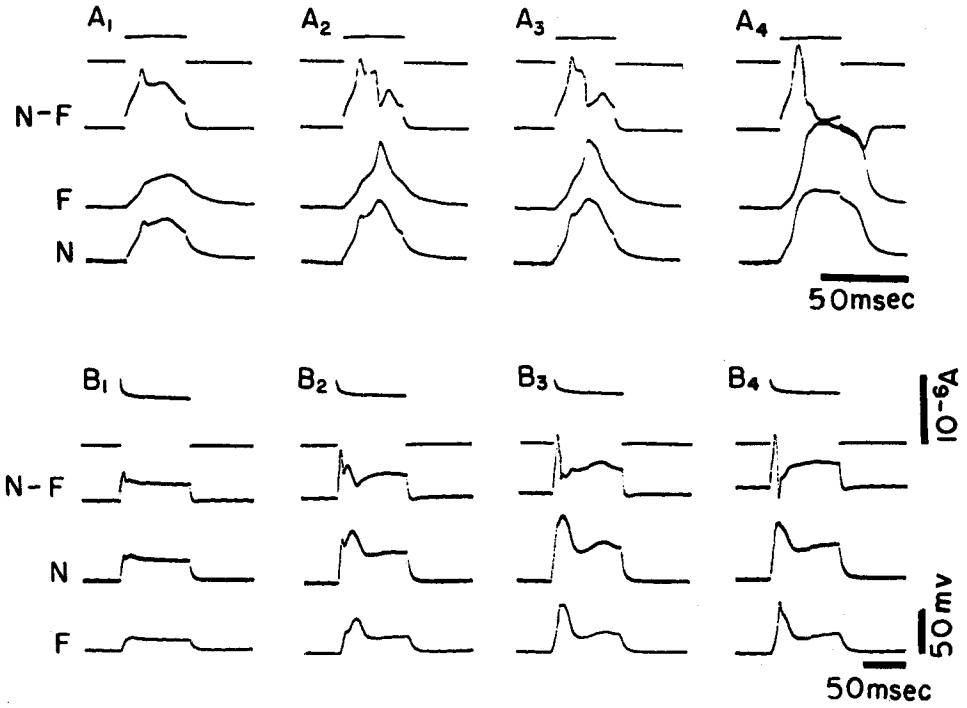

FIGURE 3. Two experiments (on different muscle fibers) showing development of all-or-none responses after treatment of the fiber with 50 m.eq. Ba^{++}/liter. Sequences of records A_1-A_4, B_1-B_4 made at about half-minute intervals. Two recording microelectrodes were inserted at different distances from a third, stimulating microelectrode. The differential recordings between the two microelectrodes (N-F) were made at 4 times the amplification of the near (N) and far (F) monopolar records. Note that in the experiment of A_1-A_4 the third trace represents the potential of the far electrode. Further explanation in text.

none character of the propagated activity. More complicated, repetitive, and prolonged activity developed in the experiment of records A_1–A_4. The prolonged response (A_4) on differential recording gave rise to an early large peak, indicating the earlier onset of the response at the near electrode site. A plateau then ensued, which denotes that both electrode sites were nearly isopotential and therefore equally active. The later subsidence of the activity at the far site resulted in a smaller potential of opposite sign. The smaller

deflection of this phase also reflects the slower rate of termination of the responses as compared with their rise. Conduction velocities, calculated from the differential recordings, were of the order of 0.4 m./sec.

The spikes evoked after adding Ba^{++} were always of longer duration than the graded responses of the untreated muscle fibers. However, the increase was never as marked as that seen in *Romalea* muscle fibers (72), the spike rarely

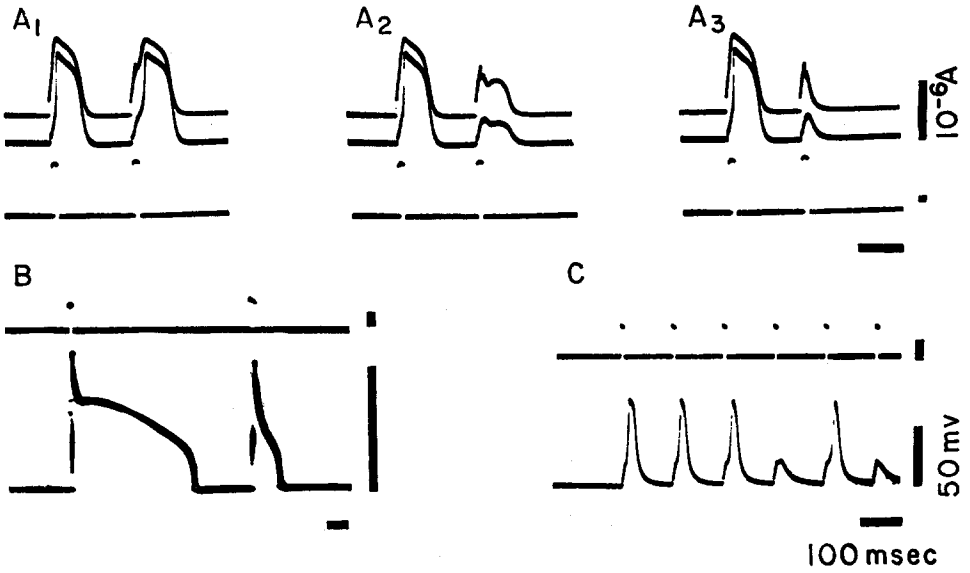

FIGURE 4. Changes in responses of Ba^{++}-treated muscle fibers during relative refractoriness. Three experiments. A_1-A_3, simultaneously recorded responses near (*upper trace*) and far (*middle trace*) from an intracellular stimulating electrode. Paired stimuli of equal strength; currents monitored on lowest trace. Testing response converted from spike to graded activity in A_2. Responses were probably absent in A_3. *B*, this Ba^{++}-treated muscle fiber produced a response lasting about 600 msec. Duration of second response was greatly diminished. *C*, in this muscle fiber refractoriness was evidenced by delayed onset and dropping out of responses during a train of stimulating pulses.

persisting longer than 3 sec. The longest duration recorded was 6 sec. The prolonged spikes were associated with refractoriness (Fig. 4) which frequently lasted for many seconds. The conditioning response recorded at the far electrode (*middle trace*) in Fig. $4A_1$ was larger than that at the near electrode (*upper trace*). When the testing response manifested refractoriness (A_2, A_3) the potential at the far electrode became the smaller. This indicates that the initially all-or-none responses had become converted to graded activity during relative refractoriness, as is also the case in several other electrically excitable tissues (1, 25). Even when all-or-none responses did occur after conditioning activity, relative refractoriness could manifest itself by shortening of the spike

(Fig. 4*B*). Another manifestation was the dropping out of spikes during repetitive stimulation (Fig. 4*C*).

B. Mode of Action of Ba^{++} on the Resting Membrane

Removal of half the Na^+ and half the Cl^- from the bathing solution did not lead to changes in resting potential or resting conductance of the membrane. Removal of up to 75 per cent of the Ca^{++}, likewise, did not change these values. Complete removal of Mg^{++} or $SO_4^{=}$ was also without apparent effect. Removal of K^+, however, caused a 10 to 15 mv. increase in resting potential, which was also seen when K^+ was replaced by equivalent amounts of Na^+, NH_4^+, or Cs^+ (56, 57). The removal of K^+ was also characterized by an increase in resting resistance, though never exceeding 40 per cent. Addition of

TABLE I

PREVENTION OF DEPOLARIZATION BY HIGH K^+ IN BA^{++}-TREATED MUSCLE FIBERS

	Resting resistance	Resting potential
	× 10^4 *ohms*	*mv*
1. Control	5.1	56.5
2. 25 m.eq./liter K^+ 16 m.eq./liter Ba^{++}	5.1	56.5
3. 25 m.eq./liter K^+ 0 Ba^{++}	3.6	49.0

twice the normal K^+ produced 8 to 15 mv. depolarization. This was invariably accompanied by increased resting conductance and reduced response height. These data indicate that the resting membrane of the fibers acts as a K^+ electrode and has a high K^+ conductance. The membrane no longer acted as a high conductance K^+ electrode in Ba^{++}-treated muscle fibers since it was not as readily depolarized by increased external K^+. It therefore seems likely that the increased membrane resistance and hyperpolarization of muscle fibers in Ba^{++} resulted from a profound reduction of the resting K^+ conductance.

Table I illustrates a typical experiment demonstrating the protection of Ba^{++} against depolarization produced by high K^+. In the absence of Ba^{++}, raising the external K^+ concentration to 25 m.eq./liter caused a depolarization of 7.5 mv. and a decrease in resistance of 30 per cent. However, the addition of 16 m.eq./liter Ba^{++} prevented both of these changes. Indeed, the resting potential increased to 60 mv. and the resistance also increased by more than 70 per cent above the initial value.

By increasing the external K^+ considerably, the effect of the Ba^{++} could be overcome and the membrane potential then again became K^+-dependent.

The higher the concentration of Ba^{++} in the medium the higher was the K^+ concentration required to reinstate K^+ dependence of the membrane. In the K^+-dependent range, the slope was no longer about 58 mv. for a tenfold increase of K^+, but became as low as 30 mv. (*cf.* Fig. 17). Difficulties arose in carrying out measurements on the same preparation with and without Ba^{++} as in the experiment of Table I. Reproducible results were not always obtained if the muscle fibers were first subjected to depolarization by high K^+ before applying Ba^{++}. Whether this was due to the changes in internal K^+, or to loading of the membrane, or of some space around it with K^+, is not yet clear. In this respect, also, lobster muscle fibers differ from those of crayfish (20).

The hyperpolarization caused by Ba^{++} was usually slightly less than the change caused by removal of K^+ from the medium. However, it occurred in

TABLE II

EFFECT OF pH ON RESTING RESISTANCE AND RESTING POTENTIAL OF LOBSTER MUSCLE FIBER TREATED WITH 50 M.EQ./LITER BA^{++}

pH	7.24	6.95	4.32	3.45	1.52
Resting resistance, $\times 10^4$ *ohms*	8.2	8.2	8.2	8.2	3.9
Resting potential, *mv.*	57	57	57	55	39.5

the presence of high as well as of normal K^+, under conditions indicating that the membrane had ceased to act as a K^+ electrode. In this state, therefore, the resting potential might be expected to represent the electrochemical potential of another, or several other, ion system(s). The role of H^+ is ruled out since large decreases in pH of the medium do not change the resting potential, nor the membrane resistance. Table II presents the data of one of three experiments of this type.

C. Effects of Ba^{++} on Membrane Conductances during Activity

While removal of K^+ from the bathing medium increased the resistance of the muscle fibers, the removal of K^+ never converted graded to all-or-none responsiveness. The graded responses merely increased somewhat in amplitude and duration in keeping with the increased resistance. Thus, the conversion of graded to all-or-none responsiveness must be related to other actions of the alkali-earth ions which are analyzed in the experiments of Figs. 5 and 6.

In the experiment of Fig. 5 all the external cations had been replaced with Ba^{++} and all the Cl^- with acetate ion. The muscle fibers nevertheless produced all-or-none spikes (*A*) though these were not as prolonged as the spikes in a medium containing NaCl as well as Ba^{++}. Increasing the applied depolarizing

currents evoked repetitive activity, which was also composed of large spikes. Higher currents caused repetitive discharges at higher frequencies, the discharges lasting throughout the applied pulses (*B–E*). However, on increasing the current still more there was alternation and progressive diminution in the size of the responses (*E, F*), but a perceptible oscillatory activity persisted for almost 1 sec. (*F*), whereas in the normal bathing medium lobster muscle fibers usually responded with only one pulse (Fig. 1).

The preparation of Fig. 5 also exhibited delayed rectification. The steady level of membrane potential for the strongest applied current (*F*) was only about double that for the weakest (*A*), although the applied current was

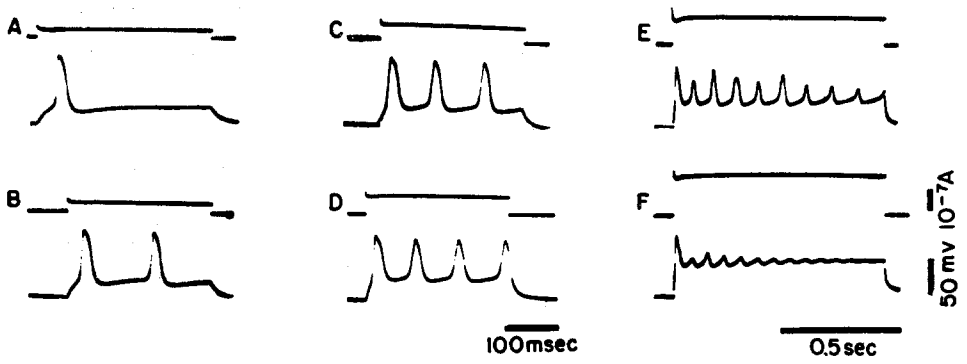

FIGURE 5. Repetitive responses during sustained intracellularly applied depolarization. Muscle fiber in a preparation bathed in isotonic barium acetate. Sequence of changes produced by increasing currents (current monitored on upper trace). Note change in time scale for records *E* and *F*. Further discussion in text.

about three times higher. Particularly noticeable in the initial records (*A–C*) and also in Fig. 6, is the undershoot after the spikes. Since the two experiments were performed with the muscle in a Cl^--free medium the repolarizing conductance change denoted by the undershoot and by the delayed rectification was probably due to K^+ efflux. The major portion of this increased conductance lasted somewhat more than 100 msec. (as the undershoot), but a small component persisted throughout the applied current (as the delayed rectification; Fig. 6*E, F*).

The capacity for repetitive activity of the Ba^{++}-treated muscle fibers, despite occurrence of an enhanced repolarizing conductance change, indicates concomitant development of a persistent depolarizing conductance component. According to the ionic theory (39) this would have to be one permitting entry of Ba^{++} in the present experiments. However, an "inactivation" process (39) is also evidenced by the progressive diminution and virtual subsidence of the repetitive responses during strong depolarization (Fig. 5*E, F*). The occurrence

of inactivation is indicated further in the experiment of Fig. 6. Early in the relatively refractory period the responses which were evoked by strong testing stimuli were smaller in amplitude (*A–D*), becoming smaller still as the latency of the testing response was diminished by increasing the stimulus (*D*). Thus, the "activation" process (39) leading to entry of positively charged ions into the fiber was operative, but impaired by inactivation. Persistence of inactivation during the long applied depolarizing pulse is also indicated by the spike caused on break excitation after a brief hyperpolarizing pulse (*E*, *F*).

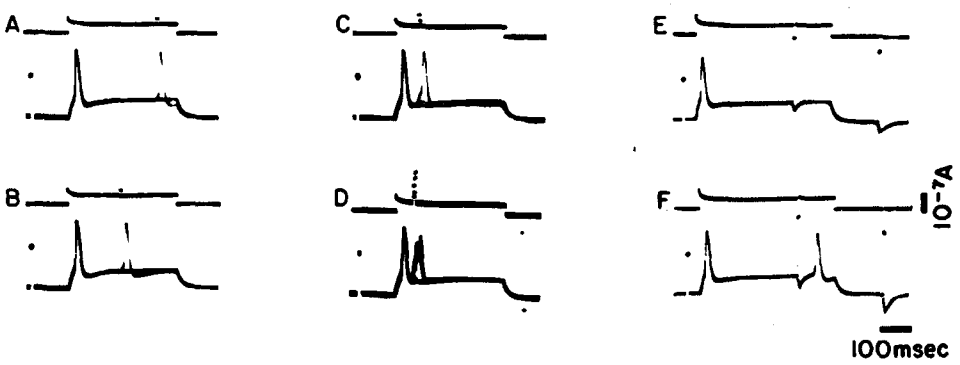

FIGURE 6. Further data on membrane properties of muscle fiber bathed in isotonic barium acetate. The spike evoked by a long depolarizing current shows an undershoot. After accommodation during the applied current a weak brief pulse evoked a response which had a somewhat smaller peak amplitude and a marked undershoot (*A*, *B*). At shorter intervals after the initial spike there was relative refractoriness, stronger stimuli being required to evoke responses (*C*, *D*). The testing responses became graded (*D*) and did not contribute an undershoot. The resistance during the applied current was lower than at rest as measured by hyperpolarizing pulses (*E*, *F*), and the stronger pulse caused an anodal break response (*F*). Calibrating pulses at beginning of voltage traces represent 50 mv.

The data of Figs. 5 and 6 lead to the following conclusions: (*a*) Lobster muscle fibers, like those of crayfish (13) and of *Romalea* (72), can produce spikes in the absence of Na^+ in the external medium when that ion is replaced by an alkali-earth ion. (*b*) Cl^- is also unnecessary for production of spikes. (*c*) Persistence of refractoriness and of depolarizing block as well as occurrence of anodal break excitation in the absence of Na^+ indicates that a process of depolarizing inactivation of the pathway carrying inward positive charge, whatever the latter may be, goes on in a Ba^{++} medium. (*d*) However, inactivation in the presence of Ba^{++} must be less than that which occurs in its absence. (*e*) An undershoot after the spike and delayed rectification indicate the occurrence also of conductance for a repolarizing ion, probably signifying K efflux.

D. Membrane Events during the Prolonged Spike

The relative conductance during the prolonged spike was measured by applying single brief anodal and cathodal pulses at various phases during responses, or by applying trains of pulses. Each record of Fig. 7 (insets) shows two responses, one with depolarizing, the other with hyperpolarizing pulses superimposed. The envelopes of the voltage displacements were smallest at the peaks of the spikes, and became progressively larger, showing also that the membrane resistance was smaller throughout the spike than at rest. The

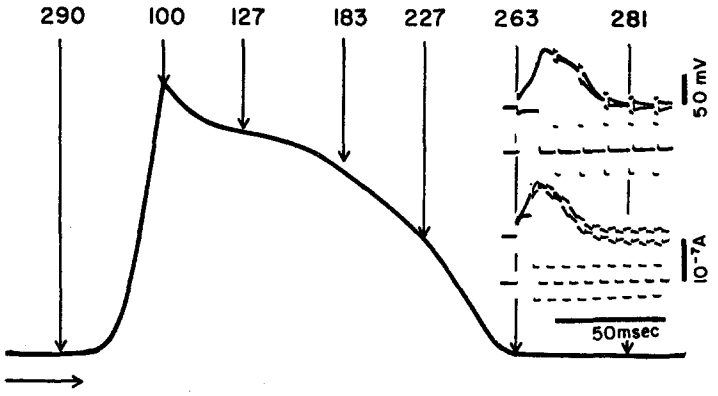

FIGURE 7. Relative effective resistance at rest and during spike in Ba^{++}-treated muscle fiber. Data calculated as average of values for ten fibers, all of which gave spikes between 50 and 100 msec. in duration and of similar form. Resistance at peak of response taken as 100. Insets show records from two experiments with fibers that had produced still shorter spikes. Each shows two superimposed responses with trains of cathodal and anodal measuring pulses applied in the successive sweeps. Lower traces monitored the currents applied through an intracellular stimulating electrode.

averaged relative changes in effective resistance during the spike as obtained on ten muscle fibers are also indicated in Fig. 7. The effective resistance at the peak of the spike was only decreased to about one-third of the resting value of the Ba^{++}-treated fibers. However, persistence of an increased membrane conductance during the prolonged depolarization represented by the falling phase of the spike indicates persistent inward flux of positively charged ions to overcome the repolarizing influence of any outward flux. This inward flux of positive charge could be abolished by strong hyperpolarizing pulses (Fig. 8). When the resting membrane potential was restored by the anodal pulse, the effective resistance rose abruptly from the low value obtaining in the falling phase to the higher value at rest. The curves resulting from such measurements were similar to those already shown for *Romalea* muscle fibers (*cf.* reference 72, Fig. 14).

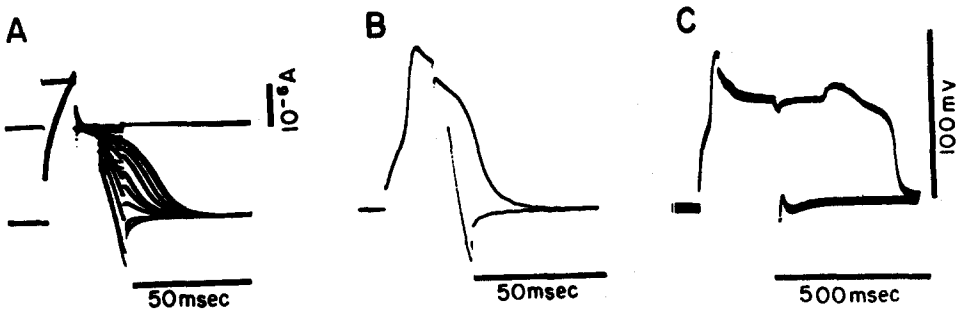

FIGURE 8. Abolition of spikes of Ba^{++}-treated muscle fibers by anodal pulses. *A-C*, three different experiments. Intracellularly applied current moditored only in *A* (upper trace). *A*, graded hyperpolarizing pulses diminished the spike to various degrees, the two strongest pulses causing abolition. *B*, *C*, abolition of relatively short (*B*) and long spikes (*C*) by anodal pulses.

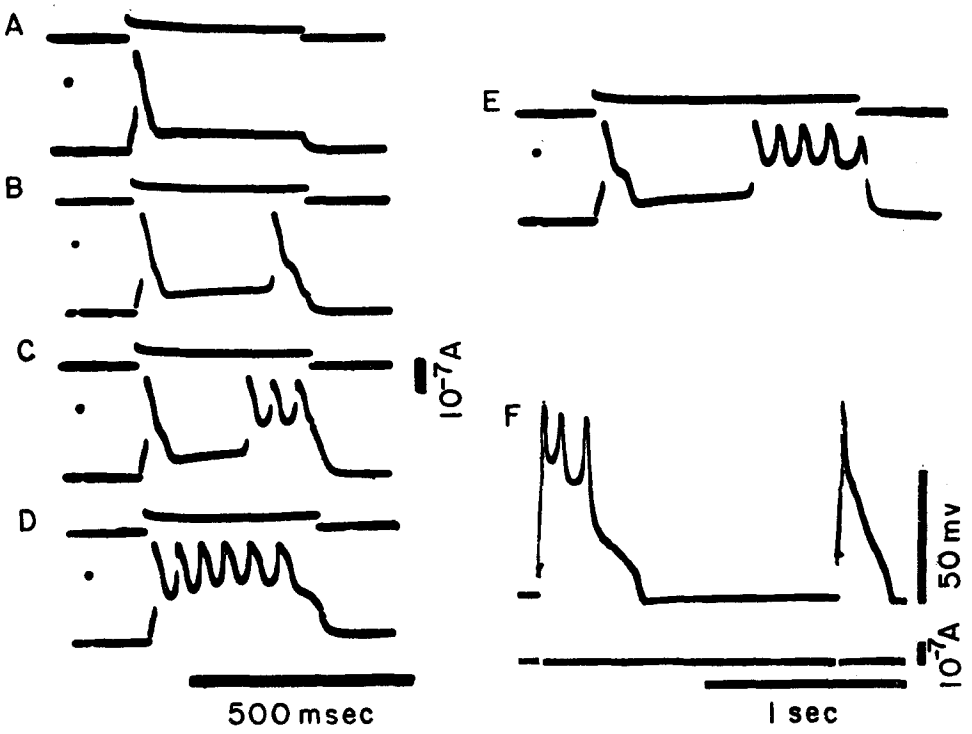

FIGURE 9. *A-E*, different patterns of responses to prolonged intracellular depolarizing stimuli. Calibrating pulse at beginning of each voltage record (*lower trace*) represents 50 mv. Current monitor on upper traces. *F*, a prolonged response with three peaks was evoked by a brief pulse. A second pulse delivered 1.5 sec. later evoked a shorter spike with only one peak. Sweep speed reduced. Currents on lower trace.

Complex interplays between depolarizing and repolarizing activities are indicated by various types of sequences of repetitive activity that could be evoked in a single muscle fiber by stimuli of constant intensity (Fig. 9*A–E*). Under certain experimental conditions (61) that will be detailed elsewhere, the oscillatory changes in membrane potential were even more frequent and could occur also "spontaneously" during a prolonged spike elicited by a brief stimulus (Fig. 9*F*).

E. The Roles of Na^+ and Ba^{++} in the Spike

The occurrence of spikes even after replacement of all Na^+ by Ba^{++} (Figs. 5 and 6) does not necessarily signify that in the standard Ringer's solution the inward positive charge is carried by the normally occurring alkali-earth cation, Ca^{++}. Experiments designed to evaluate the role of Na^+ are detailed in this section.

Replacement of Na^+ by Choline When choline chloride was used to replace all the Na^+ of the bathing solution, the muscles were rendered inexcitable, but there were no significant changes in membrane resistance or resting potential. Replacement of the choline chloride solution with the standard Na^+-containing Ringer's solution restored the excitability of the fibers. The effect of choline chloride on lobster muscle fibers thus is different from that seen in crab muscle (14). In the latter case choline augmented the size and duration of the response. In lobster muscle fibers choline appears to be relatively inert.

When less than 75 per cent of the Na^+-free choline Ringer's solution was replaced by Ba^{++} the membrane resistance of the muscle fibers increased, but the fibers still remained inexcitable. Prolonged graded responses were elicited in more than 50 per cent of the trials upon replacement of 75 to 95 per cent of the choline with Ba^{++}. Replacement of 95 to 100 per cent of the choline with Ba^{++} led to production of all-or-none spikes in about 25 per cent of the trials. These spikes were never longer than 150 msec. Thus, while high concentrations of Ba^{++} could effect all-or-none responsiveness of muscle fibers in the absence of Na^+, the presence of the latter ion appeared to potentiate the conversion of graded to all-or-none responsiveness by Ba^{++}. This conclusion was confirmed in two series of experiments.

Interplay of Mixtures of Na^+ and Ba^{++} When a muscle was soaked for ½ hour in a solution lacking, or low in, sodium (0 to 115 m.eq. Na^+/liter) its fibers usually became inexcitable (Fig. 10*A*). The subsequent records of Fig. 10 show the effects of successive additions of Ba^{++}. A small response, about the size of that seen in the normal Na^+ medium was produced when the Ba^{++} concentration reached 250 m.eq./liter (*E*). A relatively brief all-or-none response, developing an overshoot, was produced at a Ba^{++} concentration of 400 m.eq./liter (*F*).

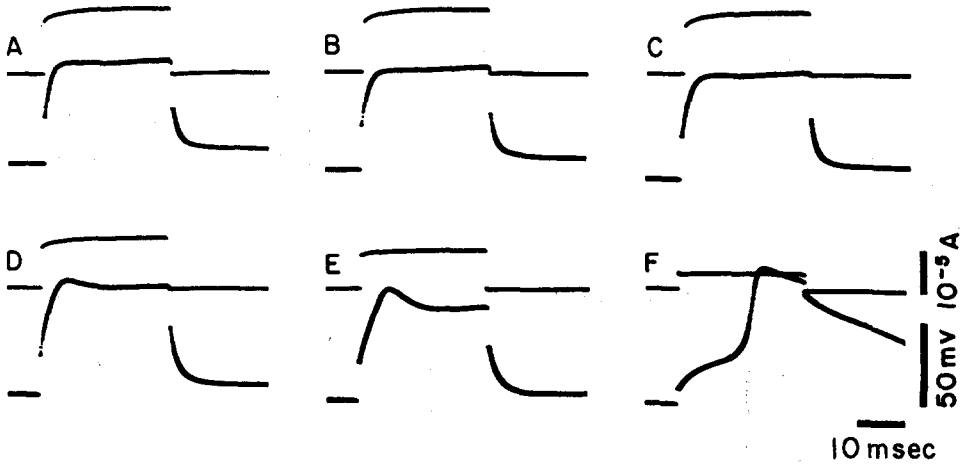

FIGURE 10. Interaction of Ba^{++} and Na^{+}. The preparation had been soaked in a solution containing 115 m.eq. Na^{+}/liter and 335 m.eq. choline/liter for ½ hour. *A*, strong depolarizing current failed to evoke a response. *B*, *C*, addition of Ba^{++} (50 and 100 m.eq./liter respectively) did not cause a response. Note that the resting potential increased markedly but that the time constant and membrane resistance increased much less. *D*, *E*, further addition of Ba^{++} (200 and 250 m.eq./liter, respectively) produced small responses. There were further increases in resting potential and the time constant of the membrane became somewhat larger in *E*. *F*, response became an all-or-none spike in 400 m.eq. Ba^{++}/liter. Note lower stimulating current and large time constant of the membrane depolarization, indicating further increase in membrane resistance. The increase in resting potential between records *A* and *F* was about 15 mv.

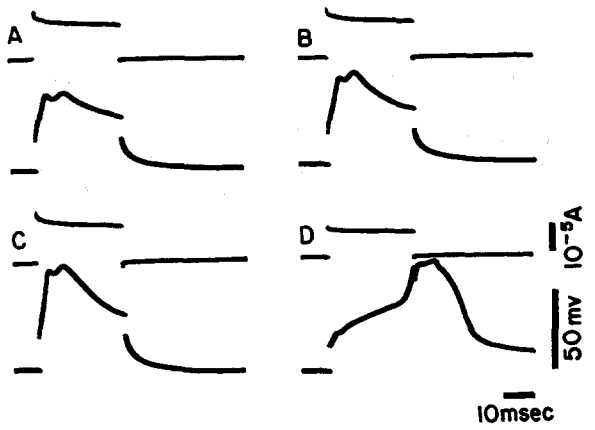

FIGURE 11. Potentiation of Ba^{++}-induced all-or-none responsiveness by Na^{+}. Preparation was soaked in sodium-choline Ringer's solution (115 m.eq. Na^{+}/liter and 335 m.eq. choline/liter). *A*, intracellular depolarizing pulse produced a small response. *B*, response augmented somewhat on substituting 50 m.eq. Ba^{++}/liter for an equivalent amount of choline. *C*, a further increase in response amplitude was caused by increasing Na^{+} to 230 m.eq./liter with removal of equivalent amount of choline, Ba^{++} remaining at 50 m.eq./liter. *D*, Na^{+} increased to 345 m.eq./liter. A weaker depolarizing stimulus now produced a large, all-or-none response.

The converse type of experiment is shown in Fig. 11. The muscle had been soaked for 10 min. in a solution containing 115 m.eq.Na^+/liter and 345 m.eq.choline/liter. The response to a maximal stimulus (*A*) was a small graded potential. A somewhat larger response was produced after addition of 50 m.eq.Ba^{++}/liter (*B*). The response increase when the Na^+ was raised to

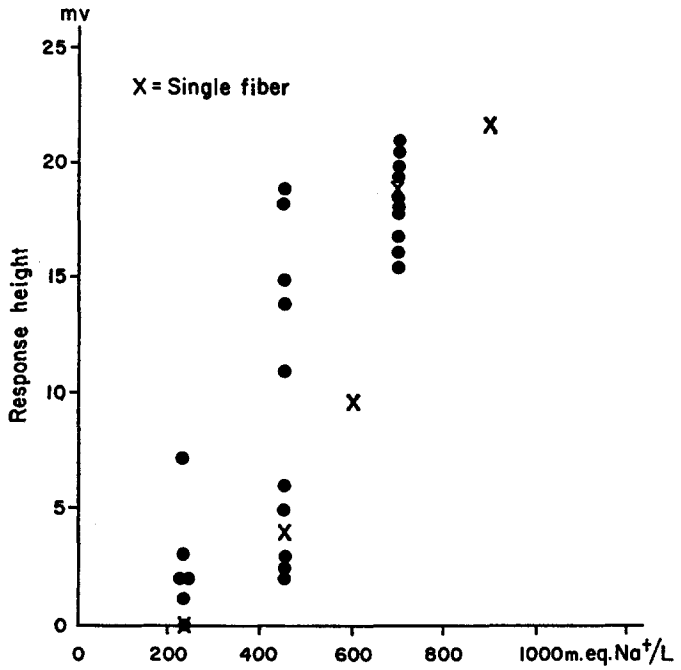

FIGURE 12. Effects of changing Na^+ concentrations on maximal heights of graded response of lobster muscle fibers. Six preparations were used for the data represented by the filled circles. Two were soaked for 1 hour or more in a sodium-poor solution (225 m.eq.Na^+/liter); two were soaked in a sodium-rich solution (700 m.eq. Na^+/liter); and the other two were freshly made in *Homarus* Ringer's solution (450 m.eq. Na^+/liter). The filled circles represent measurements of maximum responses recorded from five muscle fibers from each preparation. The dot at 0 mv., 225 m.eq./liter Na^+, represents five fibers which proved to be unresponsive in the Na^+-poor solution. Crosses represent another experiment on a single muscle fiber soaked for 5 min. in each of five solutions (225, 450, 600, 700, and 900 m.eq. Na^+/liter, respectively). See text for discussion of scatter of values in the normal Ringer's solution.

230 m.eq./liter (*C*) and when the Na^+ was increased to 345 m.eq./liter the response to a weaker stimulus was an all-or-none spike (*D*). Thus, the positive inward charge can be carried by Na^+, as well as by Ba^{++}.

Dependence of Response Amplitude on Na^+ Concentration Fig. 12 summarizes measurements of the effect on the height of the maximal graded responses produced by changing the Na^+ concentration in the absence of Ba^{++}. Six

preparations, all from the same animal, were used in the measurements represented by filled circles. Two preparations were in a low sodium medium (225 m.eq.Na^+/liter); two in a high sodium medium (700 m.eq.Na^+/liter); and two in the standard medium (450 m.eq.Na^+/liter). Five muscle fibers in each preparation were sampled for the experiment. In another experiment (*crosses*), also using a leg from this animal, the changes were followed in a single muscle fiber on which five different solutions were allowed to act for 5 min. each. The dependence of response height on external Na^+ is clear, although the data for the fibers in the normal Na concentration (450 m.eq./liter) showed a large scatter. The muscles in the Na^+-poor and Na^+-rich solutions had been soaked for at least 1 hour, while the muscles in the normal Ringer's solution had been used soon after dissection. Local variations in Na^+ concentration were possibly larger in these fresh preparations. Experiments in which the muscles were soaked in normal Ringer's solution for prolonged periods after dissection showed much less variation in response height. For example, in one series of measurements the range of variation was limited to values between 14 and 19 mv.

The foregoing data indicate that, whether in the absence or presence of Ba^{++}, at least part of the inward positive charge which according to the ionic theory (39) causes the response, can be carried by Na^+ in lobster muscle fibers. Indeed, from the fact that the response of the muscle fiber vanishes in low Na^+ solutions, it seems likely that Na^+ must be the major, or the sole carrier of inward charge in the normal state of the preparation in which Ca^{++} is the available alkali-earth ion.

F. Role of Ca^{++}

This view is contrary to that of Fatt and Katz (14) and Fatt and Ginsborg (13), who regard Ca^{++} as the ion chiefly carrying inward positive charge during the response. In part, their conclusion was based on the finding that responses of crustacean muscle fibers "were greatly reduced or abolished" on removal of Ca^{++} (14, p. 192). This finding has been confirmed in the present work, but analysis of the data on lobster muscle fibers leads to a different interpretation of the role of Ca^{++}.

The effects of adding 90 m.eq./liter sodium citrate to the bathing solution are illustrated in Fig. 13. After 10 min. in this solution there was no longer any response (*B*). However, the conductance of the membrane had increased almost fourfold and depolarization was produced. Fatt and Ginsborg (13) also observed decreased membrane resistance and depolarization on removal of Ca^{++} from preparations of crayfish muscle fibers.

Fig. 14 demonstrates the time course of the change associated with exposure of a muscle fiber to a Ca^{++}-free solution. Records made at 1 minute

intervals (*B–E*) show progressive depolarization, decrease in resistance, and reduction in response height. At the time that record *E* was made the muscle fiber had not actually become unresponsive, as was shown by increasing the intracellularly applied current (*E'*).

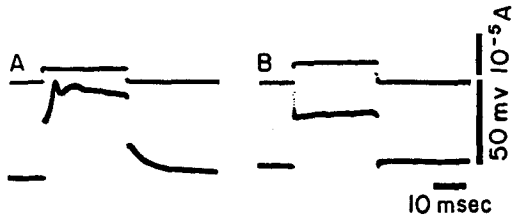

FIGURE 13. The effect of removal of Ca^{++}. *A*, The control response. *B*, the same cell 10 min. after addition of 90 m.eq./liter sodium citrate. There no longer was any response, the cell had depolarized, the resistance had decreased, and the time constant was no longer perceptible at the sweep speed employed.

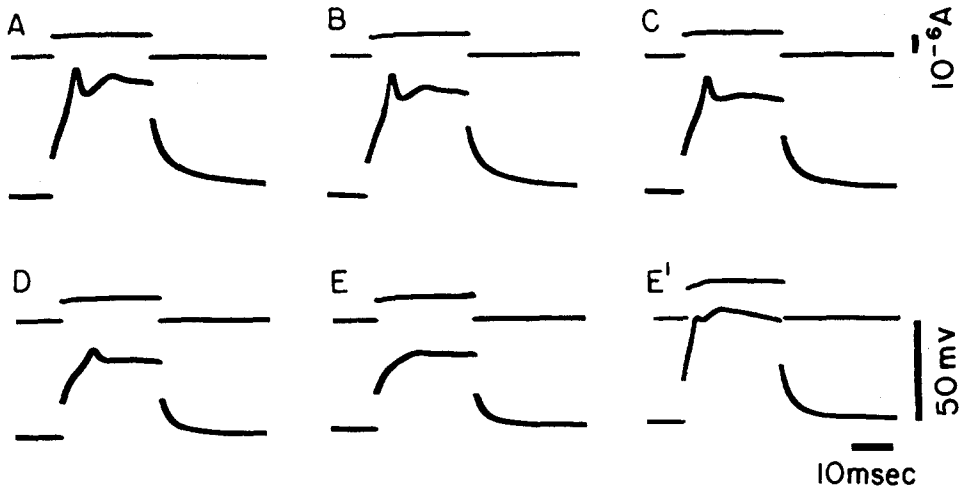

FIGURE 14. Temporal aspects of Ca^{++} removal. *A*, control response. The solution was replaced by a Ca^{++}-free Ringer's solution and records *B-E* were taken at 1 minute intervals. Note progressive depolarization, increase in conductance, and reduction in amplitude of the response. Seconds after *E* was obtained, the depolarizing current was increased (*E'*) and a small response was still evoked.

The increased membrane conductance which resulted from removal of Ca^{++} contrasts sharply with the decreased conductance obtained when K^+ was removed. The contrast suggests that presence of Ca^{++}, like that of Ba^{++}, tends to depress K^+ conductance. In the absence of Ca^{++} the muscle fiber was rendered inexcitable at least in part by increased conductance since

(*a*) stronger stimuli were required to produce adequate depolarization, (*b*) the responses that resulted were damped out by the low membrane resistance. Furthermore, inactivation for Na^+ may have been increased in low Ca^{++} as it is in the squid axon (16). Frog muscle fibers soaked in Ca^{++}-free Ringer's solution were depolarized, lost K^+, and gained Na^+ (48). However, responsiveness in the Ca^{++}-free medium was restored by hyperpolarizing the muscle fiber, showing that Ca^{++} is not essential to maintain responsiveness.

G. Mode of Action of TEA

Fatt and Katz (14) observed that application of TEA in relatively low concentrations to crustacean muscles converted their small responses into prolonged spikes. They were unable to correlate the concentration of TEA with the size

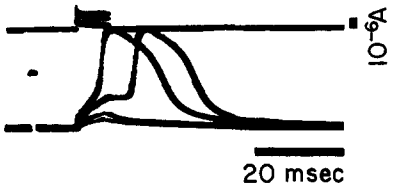

FIGURE 15. All-or-none spikes in lobster muscle fiber treated with TEA. One-half the Na^+ was replaced by TEA, and the cell subjected to increasing depolarizing currents. Four superimposed sweeps, two with subthreshold stimulations. Calibration pulse, 50 mv. Current monitoring in upper trace.

of the spike. They also noted, however, that Ca^{++} potentiated the TEA effect. The addition of TEA produced all-or-none spikes in crayfish (13) and in lobster muscle as well (Fig. 15).

The possibility that Ca^{++}, Ba^{++}, and TEA might have similar actions has been tested in the present work. Addition of TEA to the bathing solution was rapidly accompanied by increases in membrane resistance and by hyperpolarization. In some experiments, addition of as little as 10^{-5} *w/v* TEA caused an appreciable increase in resting resistance, and increases were invariably seen with 10^{-3} *w/v* TEA. The results of an experiment with a single fiber exposed to increasing TEA concentrations are illustrated in Fig. 16, showing increases in both resistance and resting potential.

TEA also prevented the depolarizing action of increased external K^+. Fig. 17 illustrates the results of a series of experiments in which the depolarizing effect of K^+ was opposed by 10^{-3} *w/v* TEA. To insure the maximum effect of K^+, which develops slowly in membrane that is poorly responsive to K^+ (66), the fibers were allowed to equilibrate over periods greater than 30 min. The

slope of depolarization was reduced from 52 mv. for a tenfold change in external K^+ to one of 35 mv.

In the experiment of Fig. 18 the resting potential increased slightly when the medium contained 10^{-2} *w/v* TEA as well as double the normal K^+. The membrane resistance remained at its original value. Thus, TEA like Ba^{++} (Table I) also counteracted the conductance change as well as the depolarization produced by high K^+. A higher concentration of TEA would have increased the membrane resistance, and in other experiments increases occurred at this concentration also. Thus, in lobster muscle fibers, TEA is seen to act similarly to Ba^{++}.

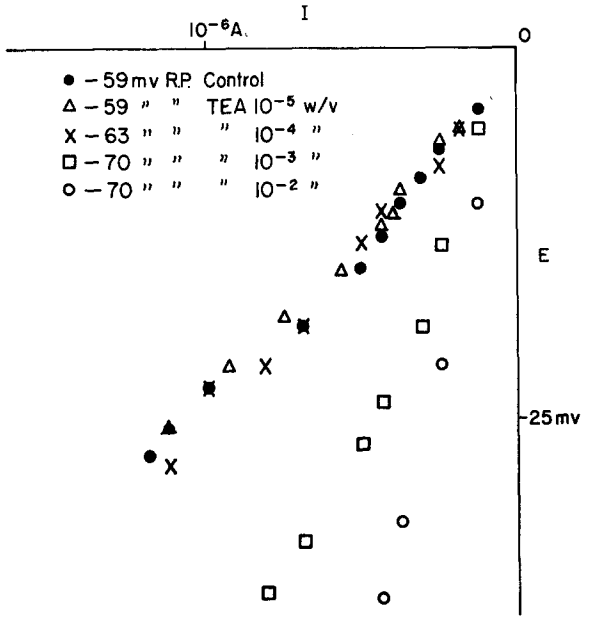

FIGURE 16. Effect of increasing concentrations of TEA on membrane potential and resting resistance. Measurements on a single fiber. The effective resistance increased almost fourfold in 10^{-2} *w/v* TEA. Units indicate hyperpolarization.

A test which indicates the equivalence of the actions of TEA and Ca^{++} was the ability of added TEA to prevent the marked changes associated with Ca^{++} removal. Fig. 19 summarizes the results from four fibers from each of two muscles soaked (for 30 min. or more) in Ca^{++}-free solutions to which various concentrations of TEA had been added. The average and extreme resistance values are shown by the dots and bars respectively. The numbers in parentheses indicate the average resting potentials in the eight fibers that are represented in each measurement. There was a progressive increase in both resistance and resting potential with increasing concentration of TEA. In

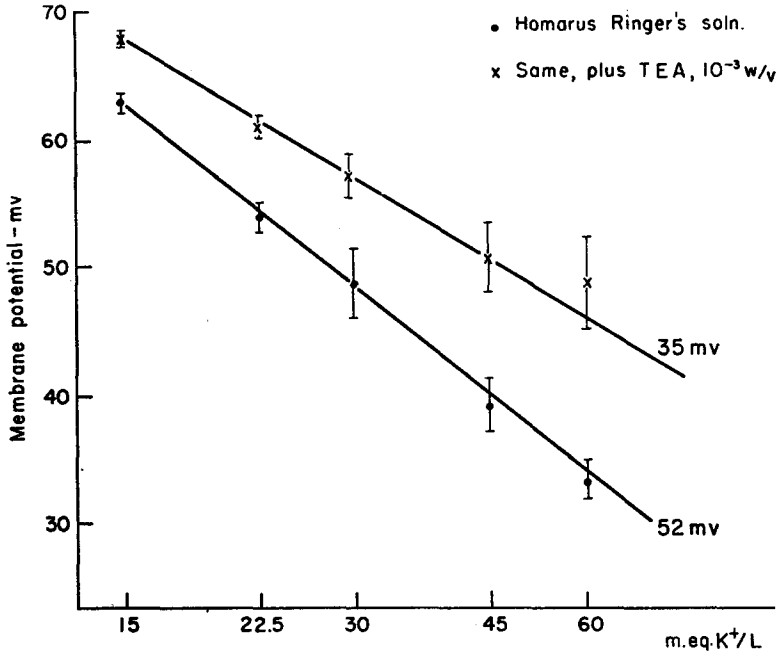

FIGURE 17. Protection by TEA against depolarization by K^+. Each point represents the mean of ten observations and the standard deviations are given. The dots represent the observations in the absence of TEA, while the crosses represent observations in the presence of 10^{-3} *w/v* TEA. Lines are drawn through the points with slopes of 52 and 35 mv. for a tenfold change in K^+ concentration respectively.

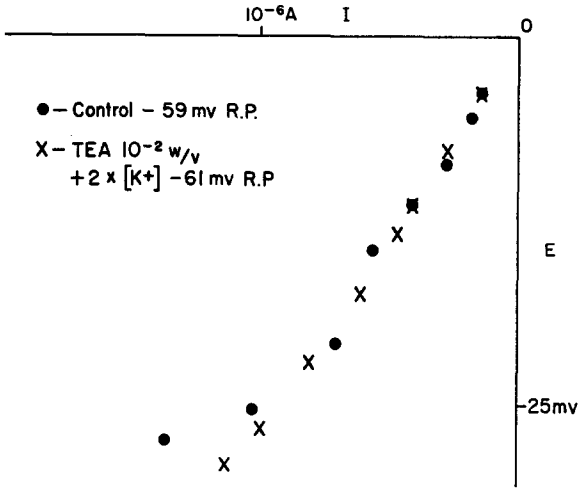

FIGURE 18. Loss of K^+ electrode property. An example is shown of resistance and resting potential measurements before and after doubling the usual K^+ concentration in the presence of TEA 10^{-2} *w/v*. Instead of the expected decrease in resting potential and resistance in the presence of high K^+, there is a slight hyperpolarization and no resistance change is seen. Measurements with hyperpolarizing currents.

solutions of 10^{-3} TEA *w/v* the muscle fibers became rsesponsive to depolarizing stimuli. Thus, TEA mimics the actions of Ca^{++} and Ba^{++} qualitatively in all respects tested.

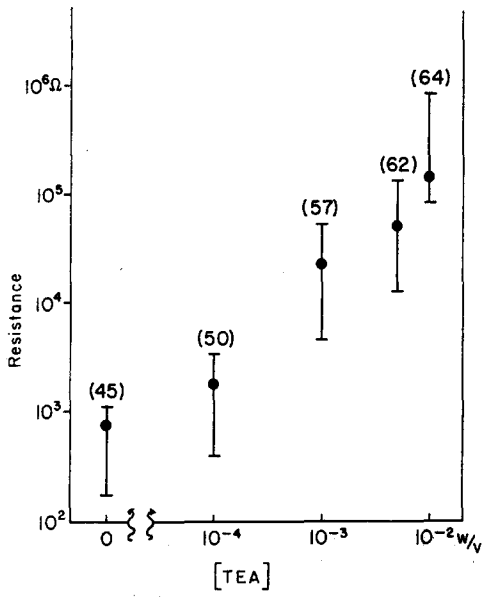

FIGURE 19. The ability of TEA to substitute for Ca^{++}. The filled circles represent the mean, and the bars the extreme values of effective resistance in four fibers from each of two preparations in which Ca^{++} had been omitted and the indicated concentration of TEA had been substituted. The mean resting potential is given in parentheses. The preparations were soaked in the appropriate solutions for 30 min. or more.

DISCUSSION

There are some differences in the behavior of muscle fibers of crayfish (13), *Romalea* (72), and lobster. However, there are also certain marked similarities. The graded responses to direct stimulation, which are produced by all the fibers in their respective, and very different, physiological media are converted to all-or-none spikes in the presence of alkali-earth cations. It seems reasonable to suppose that the conversion from graded to all-or-none activity might be due to the existence in the muscle fibers of similar conditions that were themselves responsible for the graded responsiveness, and that were affected in the same manner by the alkali-earth ions.

Fatt and Ginsborg (13) concluded that Ca^{++} is the carrier of inward current in the normal state of crayfish muscles fibers, the conclusion being partially based on the production of spikes in isotonic Ba^{++} or Sr^{++}. These ions can also act as the carriers of inward current in *Romalea* and lobster muscle

fibers. Nevertheless, the data presented above indicate that Na^+ is the major carrier whenever it is available. Thus, the addition of Na^+ potentiated the actions of Ba^{++} in producing all-or-none responses (Fig. 11). Furthermore, in the absence of Na^+ or when it was present only in very low concentration, Ba^{++} in a concentration of 250 m.eq./liter did not produce a spike (Fig. 10*E*) as against a concentration of 50 m.eq./liter which was effective in the presence of Na^+ (Fig. 11*D*). When an overshooting response was produced in higher concentrations of Ba^{++} (Fig. 10*F*) it was always briefer than the response evoked in solutions with only part of the Na^+ replaced by Ba^{++}. This potentiating effect of Na^+ was also manifested in *Romalea* muscle fibers (72), and was also described by Fatt and Ginsborg (13) on crayfish muscle. They reported that the spikes evoked in a mixture of Na^+, Ca^{++}, and Sr^{++} were reduced in amplitude and duration and had a lower rate of rise when more Ca^{++} was used to replace the Na^+ in the mixture. However, they did not comment further on this observation.

A brief résumé has already been given (72) of the conclusion that the activity of gradedly responsive muscles is converted to all-or-none responses partly as a consequence of the blocking of K^+ conductance by the alkali-earth ions, a manifestation of K inactivation (17, 24, 26).

The data of the present experiments do not deal directly with the postulated effect of alkali-earth and onium ions on the K activation mechanism (39), but do show that the resting K^+ conductance is markedly affected. Subsequent publications (26, 60), however, will present evidence that K activation is itself affected in the conversion of the graded responses of decapod muscle fibers to all-or-none spikes. What we interpret as interference with active K^+ conductance has recently been demonstrated in voltage clamp experiments on TEA-treated *Onchidium* neurons (33, Fig. 7). TEA diminished very markedly the delayed outward current of the neurons, while the initial inward current was diminished much less. The spike duration increased considerably, probably in part (26) as a result of the diminution of the repolarizing outward flux.

The present data indicate that another effect is also produced by Ba^{++}, a diminution of Na inactivation (39). This is suggested by the continued production of responses during sustained depolarization of Ba^{++}-treated muscle fibers (Fig. 5), which is in marked contrast to the rapid "accommodation" of normal fibers (Fig. 1). Calculations have been made of the ratios between the effective resistance at rest and during activity of the normal, gradedly responsive fibers and the spike-generating Ba^{++}-treated fibers. During the spike there was an increase at least of 30 to 55 per cent of net conductance above the conductance change during the graded response. These effects, which are consistent with diminution of Na inactivation by Ba^{++}, appeared to be independent of Ba^{++} concentration in the range of 10 to 50 per cent substitution of Na^+ by Ba^{++}. The continued high conductance during the prolonged spikes

of Ba^{++}-treated fibers (Fig. 7) is also consistent with diminished Na inactivation, for if the high conductance had been due to outflow of K^+ the muscle fiber should have repolarized rapidly. A very high conductance is obtained during the prolonged spikes in lobster muscle fibers that are treated with procaine and then with Ba^{++} (59, 61).

Thus, it would appear that not only does Ba^{++} reduce resting membrane conductance, but that it also reduces Na inactivation. These concurrent actions have an analogue in the squid axon where high Ca^{++} produces both changes (16). Reductions of sodium inactivation and of K^+ conductance by Ba^{++} are sufficient actions to account for conversion of the graded response to an all-or-none spike. It is therefore not necessary to invoke the hypothesis that dilute Ba^{++} is a more efficient carrier of inward current than the normally occurring Ca^{++}. However, the ability of the alkali-earth and onium ions to maintain activity of the muscle fibers in the absence of Na^+ indicates that they can also penetrate the membrane when the latter is excited, and can substitute to a limited extent their positive charges for those of Na^+.

Although the chemical properties of TEA and alkali-earth ions are dissimilar, the hydrated ionic radii, calculated by the Stokes-Einstein equation are remarkably close. The radii calculated for Ca^{++} and Sr^{++} are 3.0A° while the value for Ba^{++} is 2.80A° (50), and that calculated for TEA is 2.89A° (41). Although the Stokes-Einstein equation was not formulated for particles of this size, recent experiments show remarkable accuracy in the 2 to 5A° range (63). Thus, the common action of the alkali-earth and onium ions on arthropod muscle fibers can be accounted for, at least formally, by their similar ionic radii. These seem to be so dimensioned as to "plug" K^+ pores of the membrane, but not Na^+ pores (53).

The conversion of graded to all-or-none responsiveness of arthropod muscle fibers by alkali-earth and onium ions thus appears to be a resultant of several effects on different components of electrogenic processes in the electrically excitable membrane. The component of enhanced membrane conductance which is responsible for depolarizing changes in membrane potential is augmented relative to the component which produces repolarizing or hyperpolarizing changes. The depolarizing factor is probably a complex of Na activation and inactivation, as in squid giant axons (39), but in the membrane of arthropod muscle fibers the alkali-earth and some onium ions can also carry inward positive charge when the depolarizing conductance component is activated. The diminished repolarizing factor appears to be due to absolute or relative diminution of K conductance, and is one manifestation of K^+ inactivation (26).

These effects of alkali-earth and onium ions on arthropod muscle fibers also find their counterpart in similar actions on electrically excitable membranes of other tissues, and will need to be considered in attempts to formulate a

general theory of bioelectric activity. However, it is also important to note that the presence of different reaction components in a single electrogenic membrane implies the possibility that they may act independently of one another. Thus, electrically excitable membranes are now known in which the depolarizing component is absent (26, 28), as it is absent in certain kinds of electrically inexcitable membrane, including that of inhibitory synapses (25). The homologous components in different electrically excitable tissue may also react differently to various agents (27, 30, 60).

RELATED ACTIONS OF ALKALI-EARTH AND ONIUM IONS ON OTHER TISSUES

Effects on Membrane Potential Depolarization in Ca^{++}-deficient solutions has been described in frog nerve (11, 67); frog muscle (48, 49); and mammalian cardiac muscle (71). The effects of excess Ca^{++} have been more extensively studied. Hyperpolarization by excess Ca^{++} has been described in mammalian heart (45, 71), rat diaphragm (21), frog muscle (42–44), and insect chemoreceptors (52).

Effect on the K^+ Electrode Property Both types of changes in resting potential seem to be related to the effect of alkali-earth and onium ions on the K^+ sensitivity of the membrane. Protection of frog nerve by alkali-earth ions against K^+ depolarization, *i.e.* loss of the K^+ electrode property, was first described in 1905 by Höber (40) and has since been confirmed (35, 36). It has also been shown for crustacean nerve (32), frog dorsal root ganglion cells (54), frog rectus muscle (15), mammalian diaphragm (21), mammalian heart (45), and *Nitella* (55). Toad nerve fibers depolarized by 35 m.e.q./liter K^+ are repolarized when as little as 10^{-4} M/liter TEA is added to the high-K^+ solution (68).

A striking case of protection by Ca^{++} is that of the innervated membrane of eel electroplaques (62). The unreactive, uninnervated rostral face of the cell is depolarized with a slope of 35 mv./tenfold change in K^+ in solutions with external Ca^{++} ranging from 1 mM/liter to 12 mM/liter. The reactive innervated face responds similarly, however, only when the Ca^{++} is 1 mM/liter. At the normal value of Ca^{++} for eel Ringer's solution (6 mM Ca^{++}/liter), and above, the innervated face was not affected by the concentrations of K^+ used (0.5 to 50 mM/liter; normal value, 5 mM K^+/liter).

Resting Membrane Resistance The conductance components which determine the resting membrane resistance appear to have different pharmacological properties in different cells. Thus, prolonged spikes caused in lobster muscle fibers (59) or crayfish muscle fibers (26) by procaine are not associated with increased membrane resistance, whereas the alkali-earth ions and TEA increase the latter as well as causing prolonged spikes. The prolongation of

the spikes of *Onchidium* neurons by TEA (33), and to a lesser degree of those of toad muscle fibers (34), is not associated with increased resting resistance. Squid axons exhibit another variant. TEA is only effective when injected, and it prolongs the spikes without changing markedly the resting resistance or that during the plateau of the spike (70). Thus, it seems likely that TEA blocks K activation in this membrane, but only when applied to its inner surface. Asymmetrical behavior of the exterior and interior of the squid axons membrane has also been observed with respect to other ions (23, 29). TEA increases the resistance of frog axons. The fibers become insensitive to increased K^+. Rectification is also diminished (51*a*, Fig. 8).

Delayed Rectification The repolarizing or hyperpolarizing conductance change that is termed delayed rectification (*cf.* reference 38), can occur in the "electrically inexcitable" frog slow muscle fibers (5), and *Raia* electroplaques (28). It is abolished or diminished in both tissues by applications of Ba^{++} (2, 9). TEA markedly diminished delayed rectification in *Onchidium* neurons (33) and toad muscle fibers (34). Of particularly interest as a demonstration of the independence of different conductance components is the finding that urethane suppresses much of the early inward current flow in *Onchidium* neurons, while not affecting the late outward flux which is associated with delayed rectification (33).

Prolonged Spikes First described by Lorente de Nó (51) in frog B and C fibers on substituting quaternary ammonium compounds for Na^+, prolonged spikes have been produced in many other cells by the action of alkali-earth or onium ions. Thus, Ba^{++} prolongs spikes of frog dorsal root ganglion cells (54, 69), of puffer supramedullary cells (3), of mammalian and frog B and C fibers (22, 65), of mammalian smooth muscle (7), and of cardiac muscle (46). Increased, prolonged contractions of amphibian muscle caused by Ba^{++} were described by Brunton and Cash (4) nearly 80 years ago and later Führer (18) observed similar effects in leech muscles.

Prolongation of spikes by onium ions, has been observed in crustacean nerve fibers (6), toad skeletal muscle (34), *Onchidium* ganglion cells (33), and frog dorsal root ganglion cells (47). Squid axons are not affected by externally applied Ba^{++} (64) or onium ions (70), but as noted above, microinjection of the latter causes marked prolongation of the spike (70).

Effects on Na Inactivation The very prolonged spikes that are produced in frog dorsal root ganglion cells treated with Ba^{++} (69) have an extremely low resistance during the plateau (54, 65). This suggests that the membrane of these cells responds to Ba^{++} by a large diminution of Na inactivation. Addition of Ca^+ decreases Na inactivation in cardiac muscle (71) and squid axons (16). Unfortunately, few experimenters have determined conductances

of resting as well as active membrane under various conditions, and comparative data are therefore meagre.

CONCLUSION

The nature of graded responsiveness and the mode of action of substances which convert graded to all-or-none activity proposed in the present work may be summarized briefly. Na^+ is the major carrier of inward current during the response, but its depolarizing action is counteracted by a K^+ conductance that is large relative to the potential Na^+ conductance. The Ca^{++} concentration controls both K^+ conductance and Na inactivation, increase of Ca^{++} serving to decrease both. However, Ca^{++} is much less effective in the muscle fibers than are Ba^{++} and Sr^{++} or onium ions. The enhanced depolarization made possible by lower Na inactivation and the lowered repolarization due to K inactivation both operate together to generate an all-or-none spike in the fibers treated with alkali-earth or onium ions.

The ions can also act as carriers of inward positive charge, and they can therefore replace Na^+ in the external medium. The response, nevertheless, continues to have a peak about like that when Na^+ is present (Fig. 10). This may be due in part to the counterbalancing effects of the remaining K^+ conductance, which would increase as the membrane became more inside-positive. A similar effect has been demonstrated in *Chara*, a plant cell in which the spike appears to be caused by enhanced Cl^- conductance (19).

Thus it appears that the basic processes postulated by Hodgkin and his coworkers for squid axons (37, 39) can also account for graded responses in arthropod muscle fibers. The major difference appears to be in the resistance "state" of the membrane, which is probably determined by the effectiveness of Ca^{++} in affecting a fourth factor, K inactivation (26). In lobster muscle fibers as much as 25 mM Ca^{++}/liter does not lead to the high resistance state of the membrane, but smaller amounts of Ba^{++}, Sr^{++}, or TEA do so. The frog axon (66), the reactive membrane of the eel electroplaque (62), and probably many other electrically excitable spike-generating cells are more responsive to Ca^{++}.

There are several notable features about the effects observed on different cells. One ion or another may fail to act upon a particular type of cell. Thus, choline chloride causes all-or-none prolonged responses and increased resistance in crab muscle fibers (14). However, choline is inert for lobser muscle fibers. Amphibian A fibers are blocked by TEA (51) as they are by Ba^{++} (22). Frog B and C fibers may also be blocked in 100 per cent replacement of Na^+ by Ba^{++}, but give prolonged responses in lower concentrations of Ba^{++} (65). Frog muscle fibers, which have high Cl^- conductance (39), exhibit prolonged

responses in the absence of penetrating anions (12). Thus, it seems likely that specific configurations of membrane structure may determine whether one or another of these ions may have some effect on different components of various electrically excitable membranes.

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