

THE RELATIONS BETWEEN PREPOTENTIAL, RESTING  
POTENTIAL, AND LATENT PERIOD IN FROG  
MUSCLE FIBERS\*. †

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(Received for publication, September 19, 1955)

Excitation of nerve and muscle is accompanied by certain changes in the cell's transmembrane potential. If an adequate cathodal stimulus is applied, the cell depolarizes, fires, and generates an action potential. The cell does not fire unless the depolarization or prepotential reaches an adequate or critical value. The magnitude of this critical prepotential in muscle remains fairly constant in a given series of responses, whether recorded at the end-plate or in nerve-free regions of the fiber (Fatt and Katz, 1951; Nastuk, 1953). These authors have suggested that threshold might be a critical *membrane potential* rather than a critical *depolarization*. This statement was tested. The resting potential of the isolated frog sartorius muscle was altered by changing the  $K^+$  concentration of Ringer's fluid. The associated critical depolarization or prepotential at the foot of the spike was determined.

*Methods*

The isolated sartorius muscle of *Rana pipiens* was placed in a pool of flowing Ringer's fluid, composed of 0.110 M NaCl, 0.0013 M  $CaCl_2$ , 0.0024 M  $NaHCO_3$ , 0.001 M phosphate buffer at pH 7.3, plus various KCl concentrations. Two glass capillary microelectrodes, filled with 3 M KCl, were inserted as close together as possible into one fiber. The stimulating electrode led in square waves of various voltages and durations. The other electrode led through a cathode follower preamplifier to an oscilloscope. An electrode common to both of these circuits was placed in Ringer's fluid surrounding the muscle. All recording was d.c. The cathode follower was mounted within 1 inch of the microelectrode shank in order to reduce the input capacitance. Rise times of 50 to 150  $\mu$ sec. were obtained as routine. The recording error, caused by capacitative shunting, of the low frequency prepotential was probably negligible. Photographic records were made of prepotentials and action potentials at various latencies in response to subthreshold, threshold, and suprathreshold voltage shocks. The resting potential was measured by the displacement of the

\* A preliminary report appeared in *Fed. Proc.*, 1955, 14, 79.

† This work was aided in part by a grant from the Muscular Dystrophy Associations of America, Inc.

oscilloscope trace when the fiber was impaled. The prepotential at the time of excitation was measured on photographic enlargements of the oscilloscope trace. Excitation was assumed to occur when an abrupt change in potential, corresponding to the leading edge of the spike, was recorded. The magnitude of the prepotential at this time was a matter of interpretation and the error involved may have been as high as 5 to 10 per cent. It was not possible to stimulate with constant current shocks. The flow of stimulus current altered the resistance of the stimulating electrode and caused a 10 to 20 per cent decline in the current with 100 msec. shocks. The following abbreviations will be used: RP is the membrane resting potential; P\* is the magnitude of the prepotential at the firing point; CP is the transmembrane potential at the firing point and is equal to  $RP - P^*$ ; and  $t^*$  is the time interval between the "make" of the stimulus and the firing point.

#### RESULTS

*Excitation.*—The impaled fiber was tested with a series of single shocks which were oriented to pass current outward across the membrane like a conventional external cathode. Each shock was made stronger than the previous one and 15 to 30 seconds were allowed for recovery between shocks. As the shock strength approached threshold, the prepotential exhibited an increasing upward creep, indicating an increased depolarization rate. Just at threshold the rate of rise of the prepotential was accelerated and the prepotential turned smoothly into the front slope of an action potential. Fig. 1 represents such a series. These were the responses to increasing shock strengths of one fiber in  $K^+$ -free Ringer's fluid. As the stimulus was increased the fiber fired earlier and earlier. This was supplanted by repetitive firing of an increasing frequency. The adequate prepotentials ( $P^*$ ) in this fiber ranged from 42 to 52 mv. The resting potential (RP) was 98 mv. so that by definition the critical potentials (CP) ranged from 56 to 46 mv. This variation of  $P^*$  and CP will be discussed later. Fig. 2 also shows the behavior of the prepotential as threshold was approached. The adequate response had a CP estimated to be 44 mv. The largest inadequate response reached 42 mv. This gives an indication of the errors involved in this measurement.

More than 150 responses like these were obtained from fibers bathed in various modifications of Ringer's fluid. Only the KCl concentration was altered to set the RP at values between 54 and 124 mv. (The KCl concentration was varied between 0 and  $7.5 \times 10^{-3}M$ . No provision was made for the small osmotic pressure changes.) The RP,  $P^*$ ,  $t^*$ , and spike height were measured. In Fig. 3 the  $P^*$  of all these responses is plotted against the fiber's RP. There was a complicating factor, however. In Figs. 1 and 4 *a* it can be seen that a fiber which fired late had a larger  $P^*$ . In order to illustrate the time dependence of  $P^*$  in Fig. 3, the  $P^*$  data were separated into two groups depending on the length of the related  $t^*$ . It is rather obvious that  $P^*$  was not greatly dependent on  $t^*$  in fibers with a low RP, but did become more

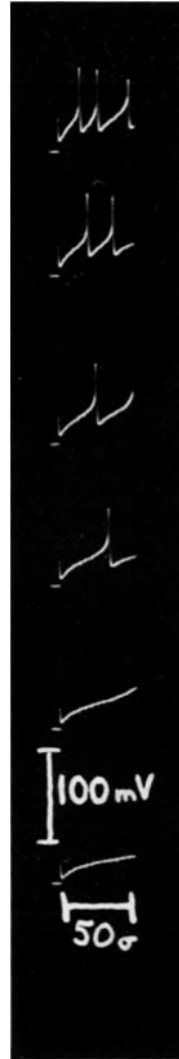


FIG. 1. These records were obtained by an intracellular electrode in one fiber in response to increasing shock strengths of 50 msec. duration. The muscle was bathed in  $K^+$ -free Ringer's fluid. The resting potential was 98 mv. The adequate prepotential and the latent period were measured at the point where the oscilloscope beam is rapidly accelerated upward. Note the marked drop in potential which followed the spike (see text for explanation). The shock strengths were increased between the bottom and the top records, but their magnitudes were not recorded.

dependent on  $t^*$  as the RP increased. This is further borne out by Fig. 5. This could mean that the  $P^*$ - $t^*$  relationship was somehow linked to the RP.

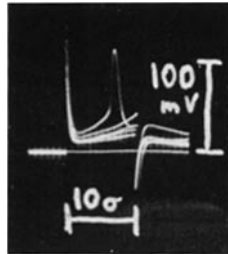


FIG. 2. This record from one fiber indicates the transition between electrotonus and excitation on a faster time base than that used in Fig. 1. The RP was 86 mv. The records from bottom to top were elicited by shocks which varied in 5 v. steps from 30 to 55 v. The largest inadequate electrotonic potential reached 42 mv., while the adequate prepotential reached 44 mv. at excitation. The acceleration of the oscilloscope beam at the excitation point can be clearly seen.

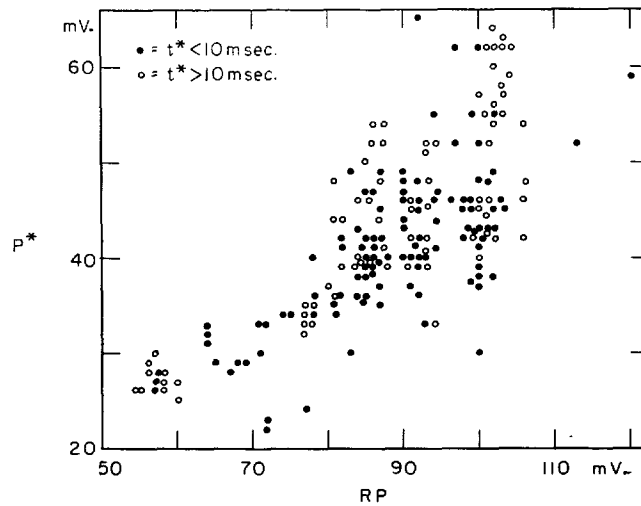


FIG. 3.  $P^*$  is the magnitude of the prepotential when the membrane is excited. RP is the resting membrane potential and was varied by altering the  $K^+$  concentration of the Ringer's fluid. The symbol  $t^*$  is latent period between the application of the stimulus and the excitation of the membrane.

The  $K^+$  concentrations could have been used as the abscissae of Fig. 3, but there were reasons for choosing the RP. In the summer it was convenient to store frogs in the refrigerator at 3–6°C. The muscles from these frogs had low RP's. Values were lower by 10 to 20 mv. from what was expected on the

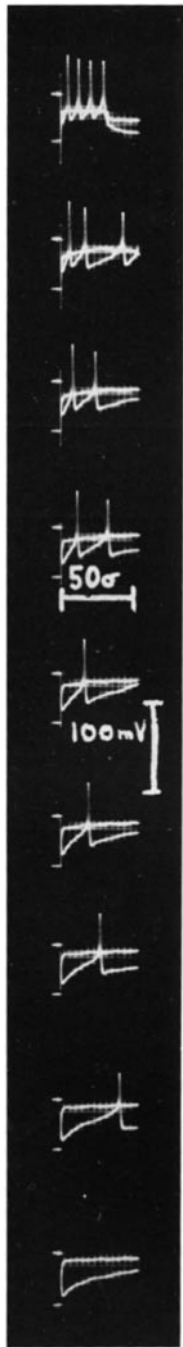


FIG. 4 a

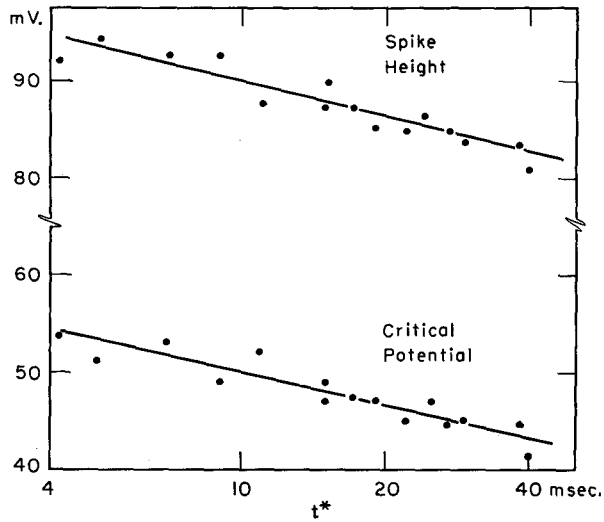


FIG. 4 b

FIG. 4 a. Consecutive responses of one fiber to increasing current shocks. The RP was 95 mv. The deflection of the upper trace of each set was proportional to the current shock. The shocks from bottom to top were 1.3, 1.4, 1.8, 1.8, 2.1, 2.7, 2.8, 3.2, 3.9,  $4.1 \times 10^{-7}$  A. It was not possible to prevent the decline of stimulus current during the pulse.

FIG. 4 b. The data obtained from the records depicted in Fig. 4 a. The spike height and the CP are represented as functions of the log of the latent period  $t^*$ .

basis of the  $K^+$  concentration of Ringer's fluid. The  $P^*$  of these fibers fell in the range associated with the RP and not the  $K^+$  concentration (see Table I). This table indicates that refrigerator storage caused some change in the muscle cell which led to a low RP and spike potential. This change was reversed when the animal was placed in an environmental temperature above the hibernating range, which is about  $10^\circ C.$  and below. The large RP in Experiment 14 was unexpected. Perhaps some shift in cell electrolyte concentrations had occurred. The small spike potential of Experiment 7 is indicative of the poor physiological status of the cold-stored muscle.

*P\* and the Latent Period  $t^*$ .*—The records showed that  $P^*$  was not always constant in responses of the same fiber. The variations were large and led to

TABLE I

Experiment No.	$K^+$ concentration	RP†	Spike†	$P^*$ †	CP†	No. of observations	Temperature $^\circ C.$	Treatment
16	2.5 mM	84	97	39	45	20	22	Frog fresh from $12^\circ C.$ storage.
7	0.5	81	80	36	46	9	28	From from $3^\circ C.$ refrigerator.
14	2.5	105	120	58	47	5	24	Refrigerated frog transferred to $12^\circ C.$ , 10 days before the experiment.
15	2.5	84	101	39	45	12	25	As in 14, but after 30 days at $12^\circ C.$

† These values are the averages from each experiment.

some concern. The source of these fluctuations was discovered to be the variation of  $P^*$  with latent period as illustrated in Figs. 4 *a* and 4 *b*. The stimulating pulse delivered to one fiber was varied in strength and spikes occurred at various intervals after "make." Careful inspection of the records showed that  $P^*$  (the upward inflection of the prepotential immediately preceding the spike) rose with late spikes. The spike height was also decreased as  $t^*$  increased. Figure 4 *b* represents, on a semilog plot, the spike height and CP from Fig. 4 *a* as functions of  $\log t^*$ . CP was plotted to draw attention to the similarity between the time dependences of CP and the spike height. These curves were analyzed statistically. The regression coefficient of the spike height on  $\log t^*$  is  $-11.4$ , while that for CP on  $\log t^*$  is  $-11.3$ . The correlation coefficients ( $r$ ) are 0.91 and 0.89, respectively. In some of the other fibers the CP and spike height did not decline in such a parallel fashion. The magnitude of the RP seemed to be a factor controlling the parallelism. It was

extremely difficult to obtain a large number of responses from one fiber in order to work out the relation between CP,  $t^*$ , and spike height. Some sort of accident usually occurred; the electrodes were dislodged or the RP decreased because of local damage. The scatter of data plotted from separate fibers was too great to allow any conclusion to be drawn beyond what has already been mentioned. Further work is in progress on this relationship. The data of Fig. 3 were replotted in Fig. 5 to further illustrate the time variation of the threshold.  $P^*$  was plotted instead of CP, since there is no essential difference between them. The spread in the data comprising this figure is

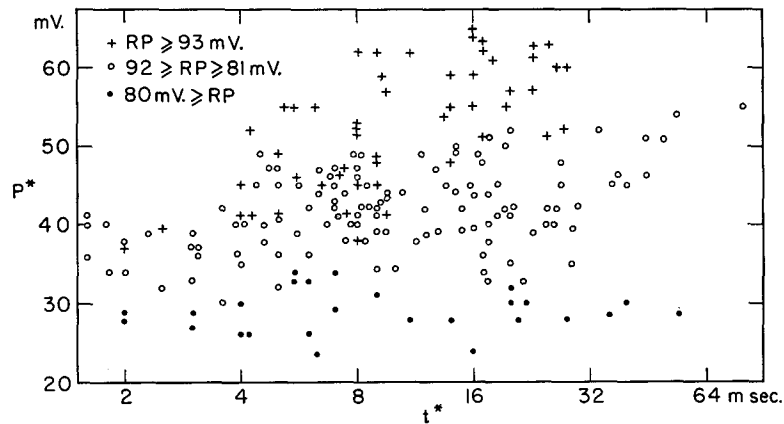


FIG. 5. The relation between the adequate prepotential ( $P^*$ ) and the latent period ( $t^*$ ). This figure contains all the experimental data. The slope of  $P^*$  as a function of  $t^*$  is dependent on the resting potential. To illustrate this dependence, the data were separated into three groups on the basis of the RP. It can be easily seen in spite of the overlapping that the three groups diverge as the latent period increases.

caused by at least two factors: (1) the usual variation in any experimentally determined value, (2) the variation of  $P^*$  with the RP.

The  $\text{Ca}^{++}$  concentration affected the  $P^*$ - $t^*$  slope. Only a few experiments have been done, but a typical result will be presented. The solid circles in Fig. 6 represent the data obtained after the normal Ringer's fluid was replaced by one containing a tenfold increased  $\text{Ca}^{++}$  concentration. (Note how the modified fluid raised the RP by 7 mv.) No action potentials were obtained later than 15 to 16 msec. after 'make,' even though this muscle previous to the fluid replacement responded as late as 58 msec. A characteristic change occurred in the prepotential shape. As the shock strength approached threshold, the responses began to show a small peak or overshoot. This maximum in the electrotonic potential first appeared at 25 msec., and moved closer to the stimulus "make" as the shock strength was increased. Not until the peak

occurred about 15 msec. was it large enough to fire the cell. Apparently spikes could not arise later because the prepotential is "pulled" away from the firing potential after this time (*cf.* Fig. 7). These early peaks were also recorded from fibers exposed to fluids with the normal concentration of  $\text{Ca}^{++}$  if the RP was near or below the value where conduction is blocked (55 to 60 mv., Jenrick and Gerard, 1953). Fig. 8 illustrates the peaks as seen in a fiber with a low RP in a normal  $\text{Ca}^{++}$  fluid. These records were obtained from a conducting muscle whose RP had been lowered to 58 mv. and which had been stored overnight in the refrigerator. As the shock intensity was increased, the monotonic discharging curves began to show a peak like the fibers in high  $\text{Ca}^{++}$

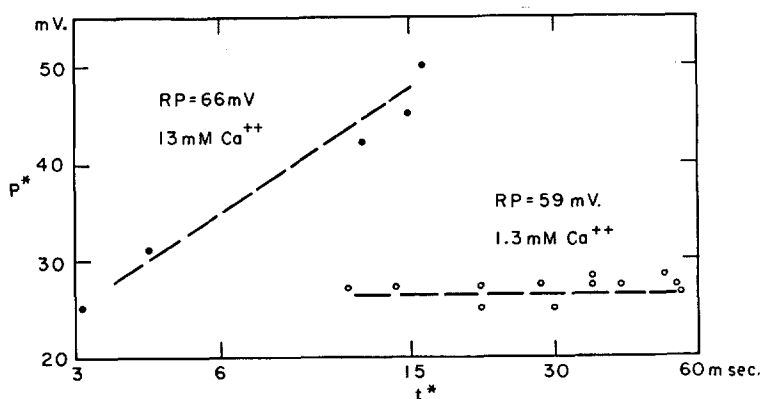


FIG. 6. The effect of the  $\text{Ca}^{++}$  ion concentration on the  $P^* \cdot \log t^*$  relation. The open circles represent the data obtained from one fiber in a muscle bathed in the normal Ringer's fluid. The fluid around this muscle was then changed for one containing a tenfold increase of  $\text{Ca}^{++}$ . 15 minutes later, another fiber was impaled. Its responses are indicated by the solid circles. Note that the RP was increased by 7 mv.

solutions. Record *a* of Fig. 8 shows a peak at 20 msec. The shock intensity was increased and the peak became larger and occurred earlier (record *b*). Threshold was not reached until very early at 7 msec. (record *c*). This was the latest that a spike appeared in the experiment. No multiple responses were seen, even though shocks of twice rheobasic strength were tried. It is our impression that these spikes were propagated, as evidenced by a vigorous twitch, even though the spike height was small, *ca.* 70 mv.

Some of the prepotentials which showed peaking were plotted on semilog paper so that  $\log$  (voltage) was plotted against time after make. The  $\log$  of the prepotential voltage at the height of the peak or overshoot varied linearly with the time of the peak. Larger peaks occurred earlier. The equation which fits the line connecting the prepotential peaks was of the form,  $\log$  (voltage) =  $A - B$  (time).

*Spike Shape.*—A large negative afterpotential is a prominent feature of the



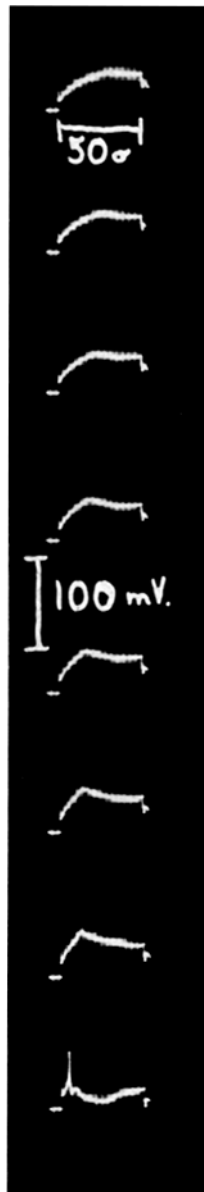


FIG. 7

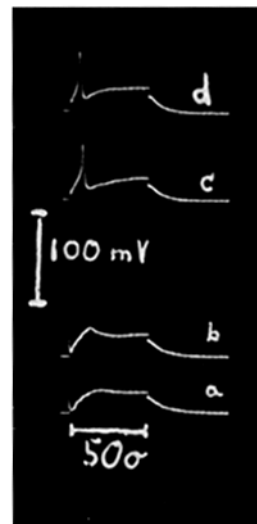


FIG. 8

FIG. 7. Prepotentials and an action potential from one fiber in a high  $\text{Ca}^{++}$ -Ringer's fluid ( $\text{Ca}^{++}$  increased tenfold to 13  $\text{mm/l}$ ). Responses to increasing shock strengths (from 7 to 10.5 v. in 0.5 v. steps) are plotted from above downward. Note the appearance of an overshoot in the prepotential in the second record from the top. The overshoot or peaking became more prominent and occurred earlier as the shock was increased. The RP of this fiber was 65 mv. This particular fiber responded only after short latent periods (5 msec. in the last record).

FIG. 8. These responses were obtained from one fiber of a muscle which had been stored 18 hours in the cold ( $4^{\circ}\text{C}$ .) in unmodified (0.0025 M KCl) Ringer's fluid. The RP was only 57 mv. The applied shocks were 7, 8, 9, and 10 v. for records *a* to *d*, respectively. The peaking or overshoot of the prepotentials in records *a* and *b* resembled those seen in fibers exposed to high  $\text{Ca}^{++}$ -Ringer's fluid. Similarly, action potentials appeared only after short latent periods (4 and 6 msec. in *d* and *c*, respectively).

muscle action potential. It will be noted that it does not seem to appear in any of our records. All the action potentials were recorded while the stimulating current was still flowing. The records were a composite of potentials generated (1) by the membrane activity, and (2) by the flow of stimulus current through the cell surface. In other words, the action potential was recorded simultaneously with the electrotonic potential induced by the stimulus. The electrotonic potential immediately after the spike was smaller than before because the membrane resistance decreased to 1 to 2 per cent of the resting value (Fatt and Katz, 1951). Therefore, the recorded potential approached the true value of the negative afterpotential (which is to be measured from the level of the original resting potential). As the membrane resistance was regenerated the recorded potential again increased. This sequence gives the appearance of a positive afterpotential following the spike, but it is obvious that a true positive afterpotential does not occur. The trace is never deflected beyond the original resting potential level.

*Incidental Observations.*—Once, when recording a number of prepotentials and spikes on one camera frame the stimuli were so spaced that the second spike of a multiple response exactly overlapped a single, late spike. (The film was too underexposed to make an adequate print.) This confirmed Fatt and Katz's statement that the effect of stimulus current on the spike seems to be almost nil, otherwise the two spikes which were caused by two different current strengths would not have been superimposed. Another important feature of this record was the overlapping of  $P^*$  from both spikes. Apparently, like the spikes,  $P^*$  was little affected by the stimulus strength.

One muscle that was placed in the chamber began twitching for some unknown reason. This spontaneous activity was localized in a few fibers. When the recording electrode was plunged into the end-plate free region of one cell, the following events were observed. The initial deflection of the oscilloscope trace indicated an RP of 80 mv. The trace, however, drifted slowly in a direction indicating depolarization. When it reached the 45 to 50 mv. level an action potential suddenly appeared and the fiber twitched. The trace flipped back to the 80 mv. resting potential level, then started slowly drifting again. This pattern of slow depolarization, action potential, and immediate repolarization was repeated every 4 seconds and continued for a minute or so. Even after the recording electrode was withdrawn the fiber kept twitching. The stimulating electrode was not inserted. Since a slow depolarization was recorded prior to the spike there are two possible explanations. Either the whole fiber was spontaneously depolarized and excited or a region near the electrode was acting as a pacemaker. Longitudinal decrement of electrotonus would prevent our recording the activity of a distant, localized pacemaker. We were not sure that this fiber was twitching before impalement. Perhaps the electrode damaged the cell membrane and caused a short circuit or current leak. The leak would allow adjacent membrane to depolarize and would act as a

stimulus. This would explain why the pacemaker appeared to be close to the electrode. Of major importance here was the  $P^*$  value of 30 to 35 mv. obtained with no external stimulation, only the cell's own electrical activity was involved. There was apparently no accommodation. The depolarization took 4 seconds to develop, yet the fiber fired. Since the RP was only 80 mv. in this fiber, an extrapolation of the data of Fig. 5 would bear this out.

A number of records were analyzed to determine the electrical resistance of the membrane as a cross-check on previous work. For this purpose the stimulus current was monitored and displayed on a dual beam oscilloscope along with the electrotonic potential. The membrane resistance was proportional to the RP as previously reported (Jenerick, 1953). There was a difference between the rates of charging and discharging a cathodal electrotonic potential. (The discharging curve is the decay after cathodal "break".) Discharging occurred more quickly than charging. The electrotonic potential was not fitted by exponential or error functions of one time constant, so that the membrane capacitance could not be calculated. Both make and break curves approached their final values more slowly than functions fitted to their early portions or, conversely, rose more quickly in their early portions than exponential or error functions of one time constant which fit their late portions. Part of this discrepancy may be attributed to the fact that the polarizing current was not constant because of the changes in the resistance of the stimulating electrode. However, this will not explain the events which followed "break" since no current was passing through the electrode.

#### DISCUSSION

Several features of this work have already been discussed in previous paragraphs. The slope of adequate prepotentials plotted against resting potentials is nearly  $45^\circ$  over the resting potential range from about 85 to 105 mv. Therefore, the difference of RP minus  $P^*$ , which is defined as the critical potential, becomes independent of the RP in this range. The average value of this CP lies somewhere between 45 and 50 mv. The variation of  $P^*$  with latent period caused the relative indeterminacy of CP. This CP range is comparable to the published values, either at the end-plate in response to the end-plate potential (50 to 58 mv. (Fatt and Katz, 1951) or 46 to 48 mv. (Nastuk, 1953)) or in nerve-free regions in response to cathodal shocks (47 to 49 mv. (Fatt and Katz) or 40 to 50 mv. (Hagiwara and Watanabe, 1955)). Fatt and Katz noted, as did Weidmann (1955 *b*) that raising the  $Ca^{++}$  concentration caused a lowering of the CP. All these CP values from fibers in normal Ringer's fluid are probably equivalent in view of the difficulties involved in their measurement.

The spike height and CP were decreased as the latent period increased. Was "sodium inactivation" (Hodgkin and Huxley, 1952) responsible for the CP alteration with time? The presently held view of the initiation of the action potential is this. Excitation occurs only after the membrane potential has

been pushed to the point where the inpouring sodium current becomes large enough to continue the depolarization. The inactivation process acts to decrease or oppose this influx; therefore, a larger membrane "opening" is required for late excitations. Conceivably this could be reflected by a related change in the critical potential. Alternatively, the spike height might have been lowered by the increased  $K^+$  conductance which accompanies cathodal depolarizations, but it is not clear what effect this would have on the CP magnitude. In all probability, both ionic processes were involved.

Because of the relative constancy of CP in fibers with RP's in the physiological range, the CP seemed to be an important factor in excitation. The CP did not have a fixed value if the RP was moved out of this range. It is not clear which is the more appropriate indicator of excitation, CP or  $P^*$ . CP is defined in terms of  $P^*$  and it might seem that both are equally valid. However, CP is a transmembrane potential. It must be related to the membrane properties, *e.g.* fluxes, structures, chemical reactions, etc., at the firing time and can be considered as a measure of some sort of the state of the membrane at the firing point. This in turn must be conditioned by the properties of the resting membrane. For these reasons, CP might be considered as the better measure of excitation.

The prepotential represents the time path of the membrane depolarization as it approaches the excitation point. Its shape and rate are governed by the growth of the excitatory processes. For this reason, several features of its development warrant further comment. When moderately strong subthreshold shocks were applied, a late, upward creep of the prepotential was seen. This acceleration of depolarization increased with current strength until excitation occurred (Figs. 1, 2, and 4 *a*). Katz (1948) recorded similar deflections with external electrodes and termed them local responses. These are very probably active responses in the sense that they represent some alteration of the membrane properties from the resting condition. If this is so, then the appearance of the accelerated depolarization signals the onset of membrane excitation. Examination of many of the records showed that the appearance of this creep was variable. Some records showed almost a straight line of depolarization, after the initial rise, leading right into the inflection at the foot of the spike. In other records the upward inflection which indicated an increasing rate of depolarization occurred about halfway in time between the shock make and the spike. This did not occur after any fixed time of depolarization. Records from fibers with large resting potentials were often concave downward right up to the foot of the spike (*cf.* Fig. 1). Therefore, it must be left unspecified when the local response appeared in our records.

The experiments were also analyzed to determine what conditions are necessary for the occurrence of multiple responses. Neither storing the animals in the cold or at room temperature nor using freshly dissected or stored muscles seemed to prevent this behavior. The only factor which appeared to play a

role was the magnitude of the RP. Fibers with a low RP never responded repetitively. Why this should be is by no means clear. One could say that the refractory period was greatly increased, but this begs the question. Wright, Coleman, and Adelman (1955) reported that the KCl concentration of the external fluid modifies lobster nerve behavior. When the KCl concentration was increased, the likelihood of multiple responses and the utilization time was reduced. This parallels our observations on muscle. Muscle fibers with a low RP did not fire repetitively in response to strong shocks and had short utilization times. One confusing point is this. Repetitive fibers had a large RP. The  $P^*$  of these fibers had a marked dependence on  $t^*$  (Fig. 5). Non-repetitive fibers with low RP's showed almost no variation of  $P^*$  with  $t^*$ . If the rate of rise of  $P^*$  with  $t^*$  is related to accommodation there is a paradox in muscle. Repetitive fibers accommodate well and non-repetitive fibers do not accommodate. Perhaps the solution lies in questioning the identification of accommodation with the time dependence of  $P^*$ . Yet, there are reasons for believing that they are related. The  $Ca^{++}$  ion has the same effect on both processes. In one sense, it seems that a fiber with no variation of  $P^*$  with latent period should not be able to accommodate. Excitation would occur as soon as the prepotential reached the adequate  $P^*$  value, whether the prepotential grew slowly or quickly. On the other hand, it may be that the stimulus efficiency or effect is so modified that accommodation, as it is commonly measured, is increased when the resting potential is lowered. However, fibers with a low RP have a low membrane resistance (Jenerick, 1953). If the membrane capacitance is unaltered, the membrane time constant is shortened as the RP is lowered. Electrotonus should be established more quickly in KCl-depolarized fibers. It seems that accommodation should be decreased as the RP is lowered, not increased as Wright *et al.* have shown.

There are other important processes that must be considered, however. Hodgkin and Huxley (1952) showed that the availability of the sodium carrier is lessened when the RP is lowered in the squid giant axon. This effect also occurs in Purkinje fibers (Weidmann, 1955 *a*), and suggests that this may be a general property of excitable systems. In addition, "sodium inactivation" proceeds more rapidly at lower membrane potentials (Hodgkin and Huxley). Therefore, if excitation is to occur in KCl-depolarized fibers, the stimulus must be increased at a rate rapid enough to overcome the changes that have taken place in the real excitatory process: the opening of the membrane to sodium. For these latter reasons it is felt that there is no real inconsistency between our data on the  $P^*$ - $t^*$  dependence and the data of Wright *et al.* on accommodation.

#### SUMMARY

1. Prepotentials and action potentials were recorded from amphibian striated muscle fibers. Intracellular electrodes were used for stimulating and re-

coding. The resting potential was varied from 55 to 120 mv. by alterations of the KCl concentration of the Ringer's fluid. The magnitude of the prepotential at the initiation of the spike potential was measured and compared to the resting potential and the latent period (time between stimulus "make" and excitation). The magnitude of this prepotential varied with the resting potential.

2. A large prepotential or cathodal depolarization was required to excite a fiber with a high resting potential. If a fiber with a high resting potential fired late (long latency), the adequate prepotential was larger than if the fiber fired early. Fibers with low resting potentials had smaller adequate prepotentials. Also, the adequate prepotential was independent of the latent period, in these depolarized fibers.

3. If the concentration of  $\text{Ca}^{++}$  was increased tenfold, the adequate prepotential of depolarized fibers became strongly dependent upon the latency.

4. Fibers with large or normal resting potentials were prone to respond repetitively during the passage of long duration shock, whereas depolarized and  $\text{Ca}^{++}$ -treated fibers were not.

5. The so-called critical membrane potential (which is defined as the transmembrane potential at the point of excitation) was not independent of the resting potential.

*Note Added in Proof.*—If the NaCl concentration of the Ringer's fluid is lowered to 30 mM/liter, the CP or P\* is not altered for fibers with normal RP. However, repetitive firing is abolished and all responses generally occur sooner than 20 msec. after "make." Cupferron (a chelating agent for Cu and Fe) at 10 mM/liter abolished all spike potentials within 10 minutes after application. At 3 mM/liter cupferron did not alter the RP, P\* or spike height in 60 minutes, but the maximum rate of rise and fall of the spike potential was decreased by 50 per cent.

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