# ANODE BREAK EXCITATION IN SPACE-CLAMPED SQUID AXONS

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ABSTRACT Strength-duration curves for space-clamped squid axons, using square wave anode breaks as stimuli, established the existence of four distinct regions. For the average experimental axon the intersection of the first two regions, $\tau_1$, occurs at about 7 msec. This agrees with computations based on the Hodgkin-Huxley (HH) equations and corresponds to the accommodation time constant found previously for a linearly rising ramp, as given by the HH equations and as found experimentally. The second break in the curve, $\tau_2$, at about 200 msec, and the third break, $\tau_3$, at 1 sec, are far beyond the range of the HH equations and may be the counterpart in the excitability of the long time constants, which have been apparent from a number of other types of experiments. The regions of the curve before 1 msec and beyond 2 or 3 sec are quite variable and may represent breakdown. Rheobase increases in both experimental and computed axons when temperature is raised. In both experimental and computed axons $\tau_1$ decreases slightly when the temperature is raised from 10 to 15°C. At 20 and 25°C, $\tau_1$ of the experimental axon increases markedly.

## INTRODUCTION

According to the two-factor theory of excitation formulated by Rashevsky (1933), Monnier (1934), and Hill (1936), and developed by Cole (1933, 1941), during and immediately after the passage of a constant hyperpolarizing current, the local potential of the nerve membrane falls and this is soon followed by a fall of threshold. When the current is terminated, the potential returns quickly to the resting level, while the threshold remains depressed for some time. During this period the excitability at the anode is enhanced and with strong enough current "break excitation" occurs. This entire process is the reverse of cathodal excitation at the "make" of a constant current. The fully accommodated nerve membrane behaves before the break of the constant current as if no current were flowing. Thus the break at the anode is equivalent to the application of a cathodal pulse to a resting nerve membrane. Early work on strength-duration curves for break excitation occurring at the anode was done by Keith Lucas (1907), Cluzet (1908), and Cardot and

Laugier (1912). Solandt (1936) found quite good agreement between results from break excitation and determinations utilizing exponentially rising currents.

It has been known for a very long time that the time scale for the local excitatory process is much shorter than for accommodation. Discussions of the time constant of excitation $k$ and of accommodation $\lambda$ can be found in the papers of Hill and Solandt already cited and for modern work on squid axons in a paper of Guttman (1968). Both exponentially and linearly rising currents were used for these studies. The considerable contributions of Cole to the problem of excitation are summarized in Cole (1968, see especially Fig. 2.5).

The phenomenological concepts of excitation and accommodation have proven extremely useful when a more detailed analysis is not available or is not needed. They have, however, been completely superseded by the far higher level of empiricism given by Hodgkin and Huxley (1952). Wherever applicable, these equations have been computed for comparison with the experimental findings. All of these calculated values as presented in the figures or discussed in the text were computed by Richard FitzHugh and John Shaw (see Appendix). Since such computations are tedious and rather expensive, however, they have usually been limited to the immediate objective, and separate print-outs of sodium, potassium, and leakage currents have not been obtained.

The present study on squid giant axons involves only break excitation after square wave hyperpolarizing pulses, i.e., anode break excitation. As was found by the earlier workers (cf. Katz, 1939), strength-duration curves for anodal break shocks involve much higher intensity of voltage than do such curves for depolarizing pulses. As will be discussed, special precautions and techniques were required to prevent injury from such large pulses and to insure reproducibility of results; however, increased leakage was quite possibly a source of the variability of results that was encountered.

The present work may be of possible interest to those studying sensory mechanisms, especially vision. For in several molluscan systems primary inhibition is observed, i.e., these systems respond only to decreasing illumination (cf. Wiederhold and MacNichol, 1970; McReynolds and Gorman, 1970).

## MATERIAL AND METHODS

The dissection of the giant axon of the squid, the axon chamber, the solutions, the temperature control, and the instrumentation used were identical with that described in previous work (cf. Guttman and Barnhill, 1970).

Space clamp was maintained by the double sucrose gap technique. The measured experimental area of membrane was usually about 0.01 $cm^2$. Constant current stimuli were applied through a 470 k$\Omega$ isolating resistance to platinum electrodes.

### *Difficulties of Measurement*

Investigation of this subject posed a number of unique difficulties. For one thing, in the early experiments it was necessary to make probes over five decades of durations before the three

time constants could be identified; however, once they were fairly well pinpointed, the problem became somewhat less difficult, because it was then known roughly in which areas it would probably be necessary to concentrate readings to determine time constants in future experiments.

Also, the injuriousness of stimuli at (*a*) long durations and (*b*) short durations and high voltages posed a serious problem. In general, we had to be content with establishing only one time constant for a particular axon before irreparable injury was inflicted and further readings were rendered unfeasible. It was only after considerable experience and improvement of technique that finally it was possible to establish all three time constants on one axon (Fig. 1).

When readings are attempted for durations much beyond $\tau_3$, the amount of scatter of data points increases markedly, resulting in considerable deviation from the line that represents rheobase$_3$. This scatter is undoubtedly associated with breakdown of the membrane. If such long duration stimuli are used repeatedly, irreversible injury occurs and the experiment must be abandoned.

Stimulation at the other extreme of the curve, where durations are very short but voltages extremely high, is also quite drastic. One can stimulate with greater impunity in the middle region of the curve, i.e., in the vicinity of $\tau_2$ and rheobase$_2$.

It is interesting that after one has with considerable difficulty recorded an anode break excitation curve, taking many precautions (such as repeatedly treating the fiber with three or four subthreshold cathodal stimuli between readings, to restore the membrane to its original

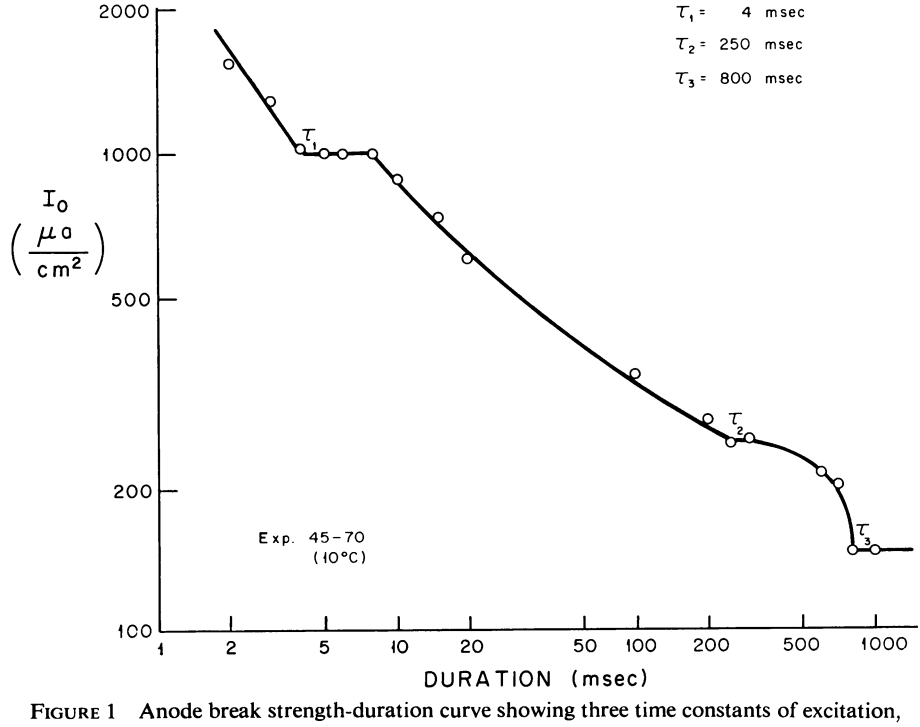


FIGURE 1 Anode break strength-duration curve showing three time constants of excitation, $\tau_1$, $\tau_2$, and $\tau_3$, occurring at 4, 250, and 800 msec, respectively. Threshold current $I_0$, in microamperes per square centimeter vs. duration in milliseconds, both on log scales. All data points obtained on same axon at 10°C.

condition), and then finally given up the possibility of continuing anode break readings on that axon, one is surprised to find that a good and reproducible cathodal threshold curve can subsequently be obtained. Apparently, the membrane does not respond as regularly to "unphysiological" anode break stimuli as it does to "physiological" stimuli such as cathodal stimulation.

## RESULTS

The strength-duration curves of the axons studied varied in the following ways. Axons in good condition with relatively high resting potentials exhibited strength-duration curves associated with lower threshold currents and relatively high time constants compared with axons in poorer condition.

### *Absence of Threshold Oscillation at Anode*

We were able to corroborate LeFevre's finding (1950) that there is no evidence of threshold oscillation at the anode (Fig. 2). As LeFevre points out, this is in accord with Cole's description (1941) of the pattern of local potential changes across the membrane. Cole anticipated this pattern on the basis of measured electrical characteristics of the membrane: the lowering of the resistance component, which occurs

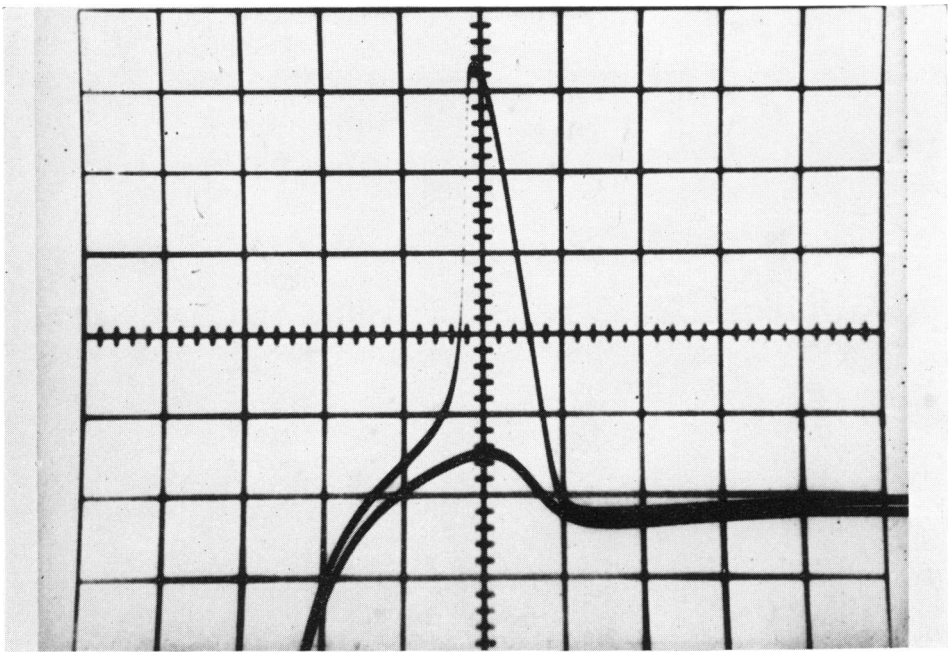

FIGURE 2 Typical anode break responses (subthreshold and threshold, superimposed) of space-clamped squid axon. Calibration, 20 mv and 1 msec; duration of stimulus, 25 msec; temperature, 10°C.

at the cathode and the raising of the resistance at the anode, would, in Cole's equivalent circuit, favor underdamping at the cathode and increased damping at the anode. Mauro et al. (1970) discuss this feature in relation to the HH parameters.

*Time Constants and Constant Quantity Regions*

In the experiment shown in Fig. 1, where all three time constants were established on one axon, the values obtained for the breaks in the strength-duration curve, $\tau = 3.8$, $\tau = 250$, and $\tau = 800$ msec, are quite similar to the average for all axons studied, 7 msec, 200 msec, and 1 sec, respectively (Fig. 3).

In the experiment shown in Fig. 1, where such a large range of durations was covered on one axon, we had to content ourselves with fewer data points for each break than when just one break was being established for an axon; however, data obtained on other axons confirm that the 45° lines preceding $\tau_1$ and $\tau_3$ are real and that constant quantity regions can be expected there. The constant quantity aspect at durations less than $\tau_1$ is displayed in Fig. 4, which is a composite of six runs on five axons. In this composite, individual runs have been displaced vertically and

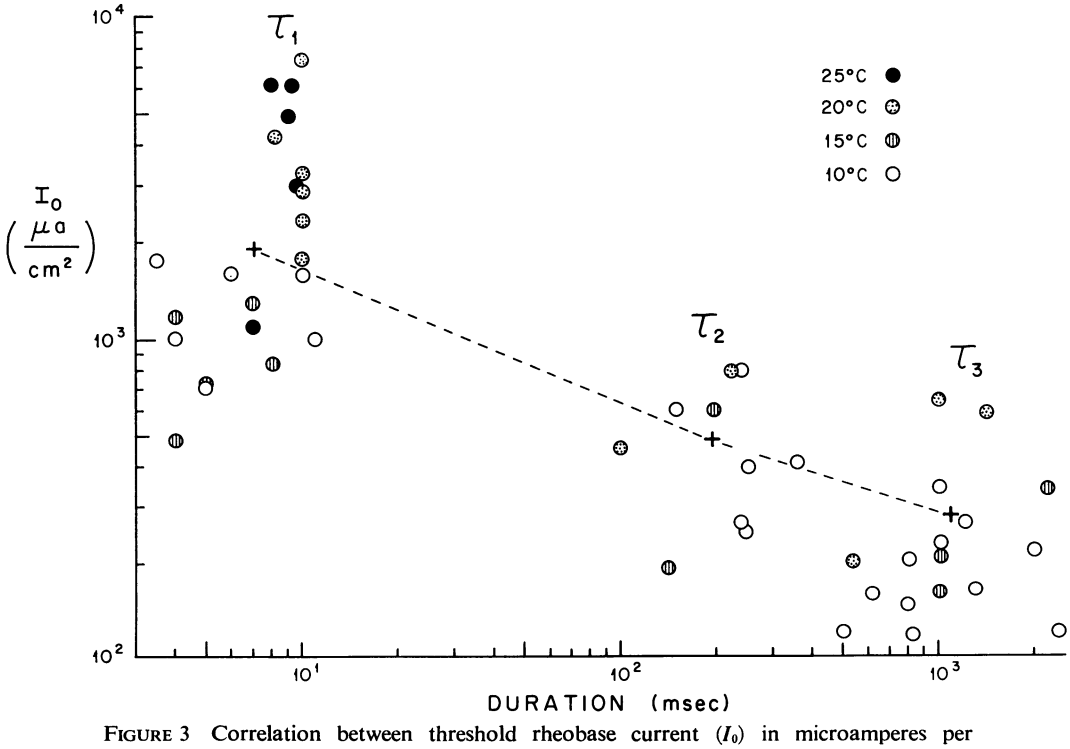


FIGURE 3 Correlation between threshold rheobase current ($I_0$) in microamperes per square centimeter and value of time constants ($\tau_1$, $\tau_2$, $\tau_3$) in milliseconds, both on log scales. Data from 47 runs on 37 axons at 10, 15, 20, and 25°C as indicated. Broken line connects points which represent the means of averaged $\tau$'s at 10, 15, and 20°C, respectively. (Since 25°C data were not taken for $\tau_2$ and $\tau_3$, these data are omitted from the averages.)

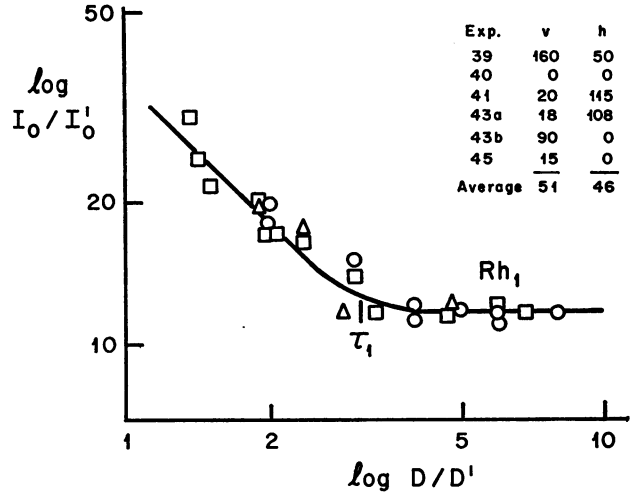


FIGURE 4 Composite graph showing data from five runs on four axons normalized to experiment 40. Log threshold current vs. log duration for short durations. Average amount (absolute value) of vertical displacement is 51% and of horizontal displacement is 46%. Note constant quantity region (45° line) preceding $\tau_1$.

horizontally by the amount indicated on the graph for best fit to a particular run (experiments 40–70). The data suggest that the time factors ($\tau_1$, $\tau_2$, and $\tau_3$) do not remain constant during an experiment and on the assumption that they change by a factor, the curves have been translated to the right or to the left in order to show that they are of the same general form. Similarly, there were changes in the amplitude of threshold stimuli and a vertical displacement has been used in the same way. Since we are interested primarily in the shape of the curve and not in absolute values, these considerable displacements are felt to be justified. In our excitation studies of the past decade, we have invariably found very large variation in rheobase, reflecting presumably differences in physiological condition of the various axons studied. During the course of our experiments, the axons presumably become more leaky, action potential amplitude decreases, and resting potentials occasionally decline. The values used are those obtained when the axons were in the freshest condition.

The values of the breaks in the curves of all axons as a function of threshold current are shown in Fig. 3, which represents data taken in 47 runs on 37 axons at various temperatures. Although there is variation from fiber to fiber, the breaks fall into three clusters, and the means of the averaged $\tau$'s at 10, 15, and 20°C for each cluster are indicated by crosses. Disregarding the influence of temperature for the moment, the average for the first break, $\tau_1$, occurs at about 7 msec, $\tau_2$ at about 200 msec, and $\tau_3$ at about 1 sec.

These times agree fairly well with the time constants of the slow processes described in papers by Adelman et al. (1965), Adelman and Palti (1969), and Chandler and Meves (1970). Narahashi (1964) has described an effect of $[K_0]$ on the transient

sodium current which has a time constant of about 1 sec. Slow sodium inactivation has been suggested by Cole (1958) as an explanation for these slow processes.

There is thus a considerable body of literature on voltage-clamped, perfused, and other squid axons, utilizing quite different techniques, with which this present study can be compared, and our results do violence to none of these observations.

The first break in our curve, $\tau_1$, agrees with the experimental values found for accommodation time in experiments using linearly rising ramps as stimuli and with computations based on the HH equations (Guttman, 1968). But while $\tau_1$ may represent the time constant of the accommodation process, the evidence given above is not yet sufficient to identify the processes represented by the time constants $\tau_2$ and $\tau_3$.

Fig. 3 indicates that there is significant correlation in the anode break excitation curves between time constant values and $I_0$, the intensity of threshold stimulation; i.e., the value of the associated rheobasic threshold decreases as the value of the time constant increases.

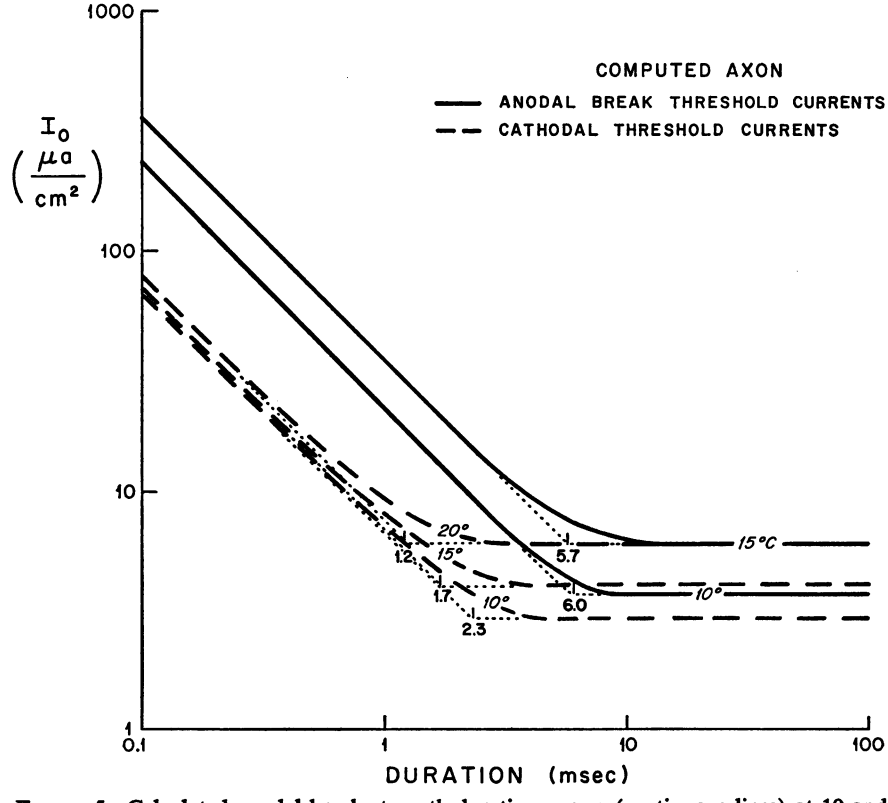


FIGURE 5 Calculated anodal break strength-duration curves (continuous lines) at 10 and 15°C and cathodal strength-duration curves (broken lines) at 10, 15, and 20°C for short durations. Stimulus duration in milliseconds and threshold current in microamperes per square centimeter, both on log scales. The time constant of excitation, $\tau_1$, for these short times is 1.7 msec for cathodal and 5.6 msec for anodal threshold currents at 10°C.

*Comparison of Experiments with Computations*

Computations based on the HH equations (1952) generally confirm the value found for $\tau_1$ in the experiments here reported: computations give 5.6 msec and experiments 5.9 msec for $\tau_1$ at 10°C (Fig. 5). This figure also agrees, as has been mentioned, with previous experiments on accommodation time calculations (cf. Guttman, 1968), but the empirical HH equations do not apply to data taken with stimuli of longer durations. Therefore, the possible correlation of experiment and calculation could not be tested for $\tau_2$ and $\tau_3$ ; the portion of our experimental anode break excitation curve at durations longer than $\tau_1$ and its associated $\text{rheobase}_1$ is not predicted at all by the equations.

Computations based on the HH equations give values for $\text{rheobase}_1$ which are about an order of magnitude lower than found experimentally. One suggestion to explain this discrepancy was given by H. Lecar and R. Nossal (1971). They used FitzHugh's *Vm*-reduced HH equations (cf. FitzHugh, 1969) to show the sensitivity of rheobase to the leakage current and suggest that better agreement may be obtained if more realistic leakage values are used in the HH equations.

*Comparison of Excitation Time Values Obtained from Cathodal and Anode Break Stimuli*

Computed and experimental results for the time constant of excitation using cathodal pulses and for the first time constant of excitation ($\tau_1$) using anode break stimuli are compared. In both the computed axon (6.0 msec compared with 2.3 msec) and the experimental axon (5.8 msec compared with 0.8 msec) the anode break time constant, $\tau_1$ , is longer than the excitation time obtained using cathodal currents. The agreement is very good for the anode break time constant, $\tau_1$ , in experimental and computed axons (5.8 and 6.0 respectively).

*Effect of Temperature*

It was difficult to study the effect of temperature upon the anode break strength-duration curve of real axons since the experimental procedure was so drastic that repeated runs at different temperatures could usually not be carried out on the same axon. Rheobase values varied greatly from fiber to fiber, depending upon how much the fibers had previously been stimulated. But in one experiment, it was possible to determine $\text{rheobase}_3$ at three different temperatures on the same axon, and the rheobase was found to increase strongly with temperature, especially at high temperature (Fig. 6). This is consistent with the result obtained previously from strength-duration excitation curves involving cathodal stimuli where rheobase increased with temperature, the temperature effect being more marked at the higher temperatures (cf. Fig. 4, Guttman, 1966). This finding is also consistent with the result obtained for the calculated axon, where rheobase increases as temperature is raised in both anode break and cathodal strength-duration curves (Fig. 5).

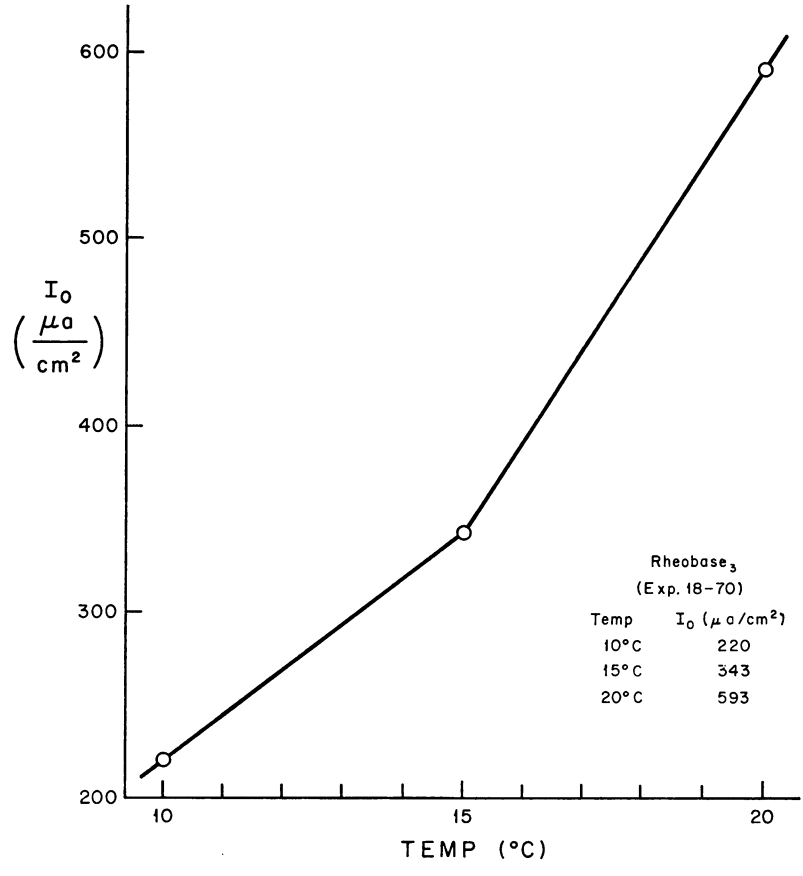


FIGURE 6 Values for rheobase$_3$ (rheobase after the third break, $\tau_3$, in anode break excitation curve) in microamperes per square centimeter vs. temperature in degrees centigrade. The three points are taken from three runs on same axon.

TABLE I

EFFECT OF TEMPERATURE ON $\tau$'S OF ANODE BREAK STRENGTH-DURATION CURVES OF COMPUTED AND EXPERIMENTAL AXONS

| Temperature | Computed $\tau_1$ | Experimental | | |
|---|---|---|---|---|
| | | $\tau_1$ | $\tau_2$ | $\tau_3$ |
| °C | | | | |
| 10 | 6.0 | 5.9 | 250 | 1132 |
| 15 | 5.7 | 5.6 | 171 | 1225 |
| 20 | | 9.7 | 163 | 977 |
| 25 | | 8.6 | | |

In both computed and experimental axons, raising the temperature from 10 to 15°C decreased the value of $\tau_1$ slightly in the anode break strength-duration studies (Table I); however, when the temperature was raised to 20 or 15°C the value of $\tau_1$ increased markedly in the experimental axon. Preliminary experiments at 20°C resulted in shorter $\tau_2$'s and $\tau_3$'s as compared with values for $\tau_2$ and $\tau_3$ obtained at lower temperatures (Fig. 3 and Table I).

## CONCLUSIONS

The following possible explanations are offered for the portion of the anode break excitation curve at (*a*) short durations and (*b*) durations longer than those that give $\tau_1$ and rheobase$_1$.

### *Short Duration Portion of Anode Break Excitation Curve*

During the stimulating pulse, $V$ goes negative (hyperpolarized direction) and as a result $m$ and $n$ approach small values and $h$ becomes large ($m$, $n$, and $h$ are terms in the HH equations). Because $m$ and $n$ are small, so are $g_{Na}$ and $g_K$, leaving the leakage current as the main current. $V$ settles down to a value just far enough from the leakage potential to drive the stimulating current through the membrane principally as leakage. Then when the stimulating current is switched back to zero at the end of the stimulus, $V$ starts back toward $V_L$ exponentially with the $C/g_L$ time constant. The first conductance variable to respond to this change of $V$ is $m$, which has the smallest time constant. $m$ increases quickly to the point where $g_{Na}$ is larger than at rest, because $h$ is larger than at rest and has not had time to decrease much. This makes the sodium current so large that excitation takes place. This explanation is essentially an elaboration of that given by Hodgkin and Huxley (1952) and was suggested to us by R. FitzHugh and H. Lecar (personal communication).

### *Long Duration Stimuli*

The above may account for the upper left part of the exponential curve (Fig. 1) down to the first leveling off. What may happen beyond there? There is recent experimental evidence for very slow Na and K inactivations not included in the HH equations; Narahashi (1964), Adelman et al. (1965), Adelman and Palti (1969), Chandler and Meves (1970), and Cole long ago (1958) suggested slow sodium inactivation as an explanation for these slow processes.

It is suggested by R. FitzHugh and H. Lecar (personal communication) that such slow "inactivations" decrease the conductance for depolarizations; for hyperpolarizations they might be expected to increase them. A slow Na inactivation would act in the same way as the HH inactivation $h$, decreasing threshold still more, as we found experimentally. A slow K inactivation would thus tend to make it more difficult to stimulate and would increase them. A slow Na inactivation would act in the same way as the HH inactivation $h$, decreasing threshold still more, as we

found experimentally. A slow K inactivation would thus tend to make it more difficult to stimulate and would increase the threshold as the stimulus durations increased. This is the opposite of what we observed experimentally. Or there may be some totally different effect than the above appearing during very long stimuli, i.e., the accumulation of some ion at some interface setting up an E.M.F. to favor excitation.

We are indebted to Richard FitzHugh and John Shaw for the computations in this paper.
We are also very grateful to Kenneth S. Cole for suggestions and discussion.

This work has been aided by National Science Foundation grant GB-28527 awarded to Dr. Guttman.

*Received for publication 7 September 1971 and in revised form 2 December 1971.*

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## APPENDIX

### COMPUTATION OF THE ANODE BREAK THRESHOLD FOR THE HODGKIN-HUXLEY MODEL OF THE SQUID AXON

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The threshold computations were done using a PDP-10 time-sharing computer with a 340 cathode-ray display. The Hodgkin-Huxley equations were solved using Runge-Kutta integra-

tion with step size automatically adjusted to control truncation error. The automatic search procedure for threshold was monitored by watching curves of $V$ vs. $t$ on the display for each stimulus value, a useful way to eliminate errors. The criterion for distinguishing subthreshold responses was not the usual one of comparing peak $V$ with an arbitrary criterion level. The choice of criterion level can affect the threshold value at higher temperatures, where responses become more graded. Instead, the search was for the inflection point on the stimulus-response curve (peak $V$ vs. stimulus strength), to within an accuracy limited by roundoff error.

R. FitzHugh and J. Shaw wish to thank the staff of the PDP-10 computer group, Division of Computer Research and Technology, National Institutes of Health, Bethesda, Maryland, for making this computer available.