

EXCITATION CHARACTERISTICS OF THE SQUID GIANT AXON:
A TEST OF EXCITATION THEORY IN A CASE OF
RAPID ACCOMMODATION

BY PAUL G. LEFEVRE

WITH THE TECHNICAL ASSISTANCE OF MARIAN E. LEFEVRE

(From the Department of Physiology and Biophysics, College of Medicine, University of Vermont, Burlington, and the Marine Biological Laboratory, Woods Hole)

(Received for publication, January 23, 1950)

Previous investigations into the relationship of the process of accommodation to the phenomenon of "break" excitation in frog nerve and muscle (Lefevre, 1946, 1948), met with some degree of uncertainty in interpretation because of the multi-unit character of these tissues. Bernhard, Granit, and Skoglund (1942) and Skoglund (1942) have discussed at some length these difficulties as they affected their accommodation studies. To simplify this aspect of the interpretation, therefore, investigations of a similar nature were undertaken on the squid giant axon, a readily available single neural unit.

The first measurements of the rate of accommodation in this cell, however, indicated an extreme rapidity in the process. Its time constant (Monnier's τ_2), calculated after the manner of Solandt (1935, 1936) from the pattern of the response to exponentially blunted shocks, apparently exceeded only slightly the time constant of the primary excitatory process (Monnier's τ_1). This made the evaluation of the two time factors difficult, since the formulations of threshold strength-duration relations commonly used in such analyses (Hill, 1935, 1936 *a*) presuppose that the ratio between the two time factors of excitation (τ_2/τ_1) is very high. In the calculation of these factors from experimental data in this instance, therefore, the usual shortcut methods could not be applied without special justification from further inspection of the predictions of the two-factor theories, omitting the simplifying assumption that the accommodative process is much slower than the excitatory process.

Monnier's representation of a model excitation system (1934), which for the form of threshold excitation data is indistinguishable from Hill's, does not include Hill's assumption about the relative slowness of the accommodative process; in fact, Monnier concludes that the two time factors are related in the ratio of 2, or at most perhaps 6. This decision, however, leaves Monnier without means of deriving in explicit form any counterpart of Hill's equations for threshold intensity-duration relations, for various stimulus forms. Rashev-

sky's system (1933) is still more general; if modified by one restriction (see below), it becomes completely identical with Monnier's model, and of course subject to the same limitations in the development of the desired relations. Since, therefore, the functions sought seem to defy a general mathematical statement, a number of specific cases were solved by substitution of particular ratios between the two time factors (τ_2/τ_1), and the degree of modification from Hill's analogous relations noted. In the range of experimental values encountered in the squid giant axon, the deviations from Hill's simplified treatment can be very considerable, as will be evident in the analysis of the data to follow.

Offner (1937) has shown that the physicomathematical excitation theories of Hill and Rashevsky, though developed from somewhat differing assumptions with respect to the accommodative process, predict identical strength-duration curves for whatever type of stimulus, if the counterpart of Hill's "normal" accommodation be imposed on Rashevsky's equations. This same restriction of Rashevsky's relations makes them absolutely identical with those of Monnier, so that all three treatments may be dealt with together, in the following remarks relating to general excitation theory.¹

Materials and Methods

The stellate nerves of *Loligo pealii* were dissected under running sea water, for a length of 5 to 10 cm. behind the ganglion, and ligated at each end. Occasionally, most of the smaller fibers were teased away from the giant axon, but more commonly the axon was used with the entire nerve trunk adherent; this did not interfere with recording of action potentials from the giant axon, and favored its survival in the moist chamber. The preparation was suspended horizontally on the stimulating and recording electrodes and additional supports in a closed Amphanol chamber filled with sea water up to 2 to 3 mm. below the level of the axon. In nearly all the experiments to be reported, the chamber was enclosed below and laterally by a jacket of rapidly circulating fresh tap water; this kept the chamber ordinarily at a temperature of about 20°C., and never in excess of 23°C.; under these circumstances, the axons regularly survived 3 to 4 hours and frequently 8 to 10 hours of experimentation.

¹ The only difference in the quantitative predictions of Hill's theory on the one hand, and Monnier's and Rashevsky's on the other, resulting from the different assumptions concerning the factor governing the secondary process (accommodation), appears in the dependence of the threshold on the ratio of the two time factors. The difference becomes significant only when this ratio (τ_2/τ_1) is small, as the present experiments indicate to be the case in the squid giant axon. Regardless of the form of stimulus applied, the local excitatory state at each particular instant, according to Hill's scheme, exceeds that predicted by Monnier's scheme by the factor $\tau_1(\tau_2 - \tau_1)^{-1}$. This distinction cannot be investigated experimentally without knowledge of the actual current flow through the excitable structure, and the means of varying the time factors without altering other properties of the system. Qualitatively, this dis-

Stimuli were applied through large calomel half-cells connected to the moist chamber through sea water-agar bridges to pools of sea water in which were vertically mounted soaked wooden wafers supporting the nerve. In all but the earliest experiments, these stimulating electrodes were separated by a distance of 23 to 27 mm.; the resistance across the two half-cells when the nerve was in place was typically about 12000 ohms. One or the other of the leads to the half-cells was grounded, depending on the direction of the applied stimulus. The recording electrodes were either silver or platinum wires, located at variable distances from the stimulating electrodes (between 11 and 45 mm. from the nearer stimulating electrode). These were condenser-coupled to the input grids of a three-stage push-pull amplifier included with the cathode-ray oscilloscope used to observe the electrical response of the axon. The stimulus artifact was minimized by adjustment of the relative resistances to ground from the two input leads; distortion of the form of the action potential was of no consequence to the present investigation. The oscilloscope was an experimental model Cardiograph Monitor, constructed by the Development Engineering Department of Rahm Instruments, Inc., and subsequently modified to permit driven sweep control with suppression of the beam between signals. This was coupled to the stimulator so as to sweep once at a suitable speed when triggered by either the "make," the "break," or the "test shock" stimulus, as the occasion demanded.

d. c. shocks in either direction, with supplementary condenser-discharge test shocks ($RC = 0.26$ msec.) at either anode or cathode, exponentially blunted shocks, and condenser-discharge stimuli were supplied from a battery-operated stimulator keyed by thyratrons controlled by a variety of condenser couplings. The performance of this stimulator was checked frequently by direct observation of the pulse form on the oscilloscope; this method was also used to calibrate the controls governing the timing and the amplitude of the test shocks. Within the limits of error of the method, there appeared to be perfect physical summation between the constant currents and the test shocks (whether they were similarly or oppositely directed), and complete independence between the magnitude of the test shock and its timing with respect to the "make" of the d. c. This "make" appeared to be perfectly free from disturbing transients usually seen with mechanical switching, and the wave front ascended as though condenser-shunted with RC on the order of 0.02 msec., with no perceptible overshooting. The "break" was similarly clean, but was about three times as delayed, probably because of the relative slowness of the deionization in the thyatron carrying the constant current.

The stimulator was usually set to deliver a test stimulus (of whatever form was in use) every 4 or 6 seconds; when stimuli of relatively longer durations were used, this

inction may be appreciated in that the Monnier theory predicts a much more pronounced rise in threshold as τ_2 approaches τ_1 than would be expected on the basis of the Hill theory.

Katz (1939) discusses another supposed divergence between these theories in connection with the form of the initial threshold change at the application of a constant current. But this seems to arise from a confusion of Hill's abstract threshold " U " with the actual measurable threshold (Hill's " $U - V$ "); the latter follows a double exponential curve of identical shape in both theories.

interval between shocks was commonly extended to 12 seconds. The d. c. shocks, including those exponentially blunted, lasted about half a second unless otherwise specified. The magnitude of the stimuli (other than "test shocks") was read on a voltohmmyst electronic voltmeter across the stimulating circuit. This circuit included series resistance of at least 200,000 ohms, usually a megohm, so that incidental variations in the resistance at the site of stimulation would have little effect on the current.

A. c. stimuli were obtained from a Hewlett-Packard audio oscillator, Model 200D, operating through a similar high resistance; the magnitude of the R. M. S. voltage applied was read also on the voltohmmyst electronic voltmeter. The manufacturers' specifications on both the oscillator and the voltmeter claim an essentially flat response throughout the frequency range used in these experiments; and their joint operation gave no reason to doubt their proper behavior in this respect. The specifications also limited harmonic distortion in the oscillator output to less than 1 per cent in the frequency range used.

Thresholds to constant currents, exponentially blunted currents, condenser discharges, and "test shocks" were approached from above, the amplitude being gradually decreased to the point at which two successive trials resulted in no responses, and the threshold taken as the last preceding (effective) shock. Thresholds to alternating currents were ordinarily taken in double form; the amplitude was very gradually increased, at a fixed frequency, and the point noted at which a maintained train of responses began; then the amplitude was gradually decreased and the "off" threshold also recorded. Additional readings were often taken with fixed amplitude and slowly varied frequency, in order to locate more satisfactorily the optimum frequency.

The excitation system in the squid giant axon (at least when the cell is suspended in a moist chamber after exposure to sea water) is particularly unstable when subjected to currents of relatively high magnitude or protracted duration. Marked sudden shifts in the threshold and in the size of the recorded action potentials are common. In the present investigations, the most disturbing stimuli required were the exponentially blunted direct current shocks considerably exceeding the rheobasic current. In the use of this type of stimulus, the rheobase was therefore determined again between each successive test, and the ratio of threshold to rheobase at each point was calculated in terms of the average between the immediately preceding and the immediately subsequent rheobase determinations. In this manner, satisfactory tests could be obtained with final current strengths up to about 4 to 5 times rheobasic. Beyond this point, abrupt and marked changes might interfere with quantitative measurements; in such cases, washing of the axon in sea water led to almost immediate recovery.

RESULTS

In the use of condenser discharges or exponentially blunted shocks, the threshold data on the squid axon could be reasonably fitted to the theoretical curves; Fig. 1 is a typical record of such measurements, with both sets of data taken on the same axon. The theoretical curves shown are derived with due allowance for the rapidity of accommodation observed in this preparation; the close fit with the observed points is evident. However, the wide

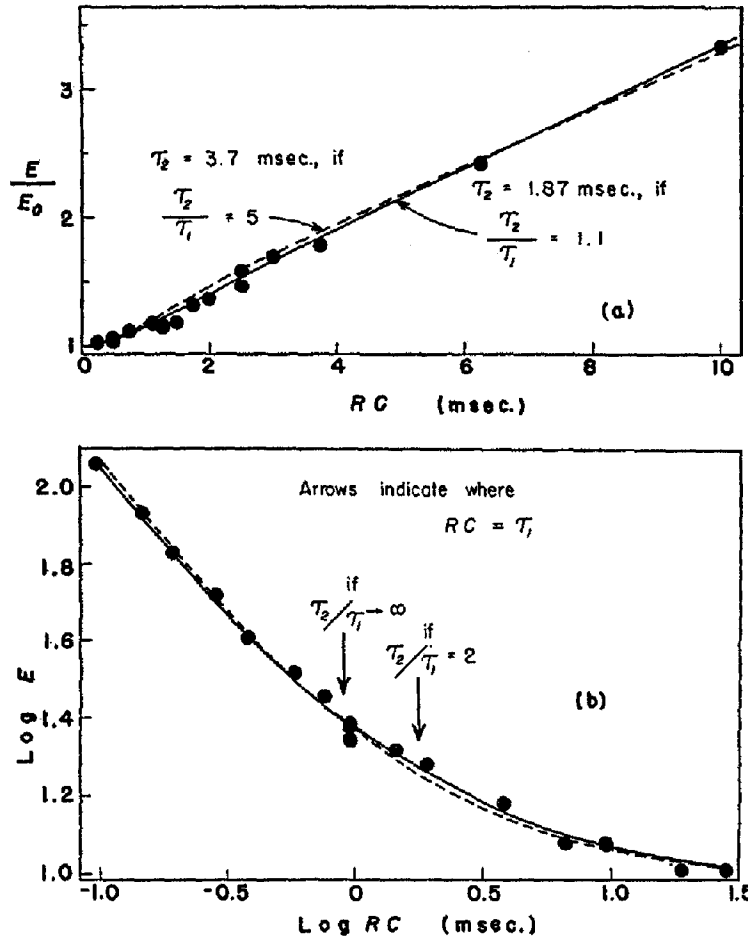


FIG. 1. Threshold patterns in squid giant axon.

(a) Exponentially blunted shocks. Threshold (in rheobase units) plotted against time constant of delay of rise of current strength.

(b) Condenser discharges. Log of threshold voltage plotted against log of time constant of discharge. Points are experimental observations; both sets of data are from the same axon. Curves are those predicted by theory, if time factors are such as indicated in figure.

range of theoretical values fitting the observed points in each case is indicated by the examples shown, and it is evident that further treatment of the data is required if the time factors are to be ascertained.

In the determination of τ_1 from condenser-discharge data, Hill (1936 b) recommends the use of the $\log E - \log RC$ plot (as in Fig. 1), which does not

necessitate measuring the rheobase. Fig. 2 shows the theoretical displacement of Hill's standard curve with several smaller values of τ_2/τ_1 . Note that with the low values of this ratio evident in the squid giant axon, the curve is considerably to the left of Hill's curve, but differs from it in shape only very slightly; so that any experimental data fitting one curve satisfactorily could

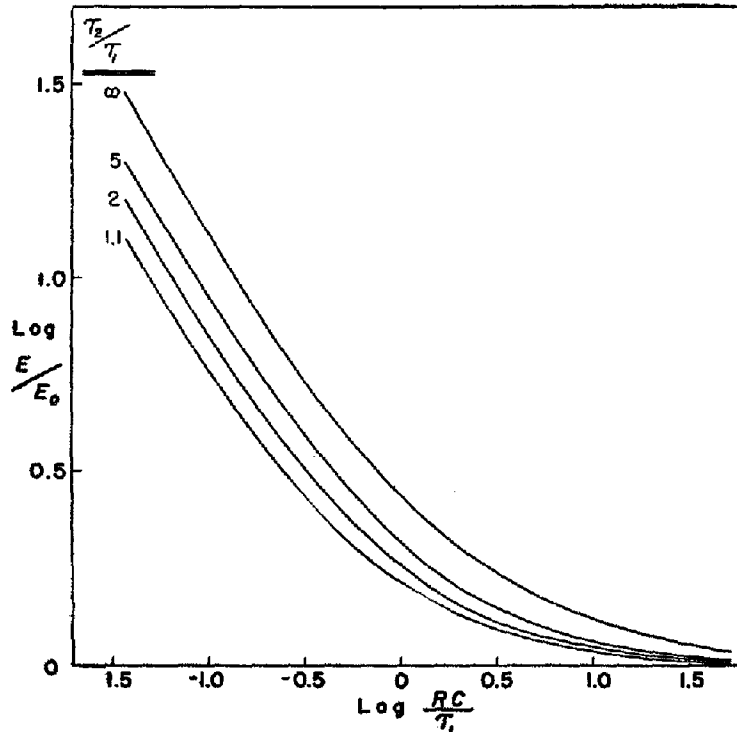


FIG. 2. Theoretical threshold pattern for condenser discharges, as affected by rapid accommodation. Log of threshold (in rheobase units) plotted against log of time constant of discharge (in tissue-constant units).

easily be fitted to another through suitable adjustment of the axes (*i.e.*, by suitable choice of values for E_0 and τ_1). This is reflected in the uncertainty of these factors in fitting the points in Fig. 1 *b*. If $\tau_2/\tau_1 = 2$, the usual procedure of applying the simple curve neglecting accommodation would lead to an underestimation of the time constant by about 50 per cent; the maximum divergence possible would show an apparent " τ_1 " equal to only $1/e$ of the true τ_1 . If, however, the rheobase is established experimentally, so that the position of the x-axis of the graph is fixed, a significant difference in the

configuration of the curves becomes apparent, and the threshold data indicate again a high rate of accommodation.²

Similar complications arise in the estimation of the time constant of accommodation, τ_2 , from data as in Fig. 1 *a*, employing exponentially blunted D. C. stimuli. It can readily be shown from the basic equations that, in a plot of the threshold E (in rheobase units) against RC (the time constant of the rise of current strength), the terminal slope must approach

$$\left(\frac{\tau_1^{\tau_1}}{\tau_2^{\tau_2}}\right)^{1/(\tau_2-\tau_1)}$$

For the extremely slow accommodation assumed by Hill, this becomes effectively τ_2^{-1} , and the slope becomes an inverse measure of the time constant. But with the rapid accommodation found in the squid axon, this simplification leads to a serious overestimate of τ_2 (by 100 per cent at $\tau_2/\tau_1 = 2$; even with a ratio of 20, the error is over 17 per cent).³ A further technical difficulty in working in this manner with the squid axon lies in the instability of the ex-

² As τ_2/τ_1 diminishes, the threshold for the slower discharges approaches the rheobasic level more gradually, since the increase in the rate of accommodation raises only slightly the threshold to the briefest shocks, and has most effect near the rheobase. The magnitude of this discrepancy is not such as to make the curves obviously different superficially, but is sufficient that a noticeably better fit with experimental data on the squid axon can be obtained with the theoretical curve for a very small value of τ_2/τ_1 than with Hill's curve. It should be stressed that, in the absence of the experimental determination of the rheobase, either curve fits equally well if the axes are suitably adjusted; this may give a false impression of conformity with the standard, and a totally improper evaluation of the time factor.

³ In setting τ_2/τ_1 effectively at infinity, Hill arrives at several other conclusions regarding classical excitation constants which do not apply to rapidly accommodating tissues. Lapique's "chronaxie" becomes equal to 0.693 τ_1 . For any finite value of the ratio, this overestimates the chronaxie, and the discrepancy becomes 100 per cent for a value of τ_2/τ_1 in the neighborhood of 2.5. Similarly the critical RC for condenser-discharge stimuli (the RC at which the threshold voltage is just twice the rheobasic voltage) diminishes with the ratio of the two time constants; from Hill's figure of 2 τ_1 (with no accommodation) the factor should fall to equal τ_1 at $\tau_2/\tau_1 = 4$, and continue to considerably less than τ_1 with smaller ratios. It may be noted that even with τ_2 as high as 100 τ_1 , Hill's simplification leads to an overestimate of 6 to 8 per cent in these time constants. The relation between the chronaxie and the critical RC (*i.e.*, Lapique's conversion factor in the use of condenser-discharge measurements to determine the chronaxie) is calculated by Hill at 0.347. This is not radically altered by diminishing the ratio τ_2/τ_1 ; it rises only to about 0.415 as a limit. Lapique's (1926) experimental average value of 0.37 corresponds to τ_2/τ_1 of 30 to 35, a reasonable figure for a variety of tissues.

citable system under exposure to direct currents in considerable excess of rheobase, and lasting for relatively long times, such as are required with this procedure. It is impracticable therefore to take threshold measurements with exponentially blunted currents with RC considerably longer than τ_2 , as very large currents would be required (the more so because of the very rapid ac-

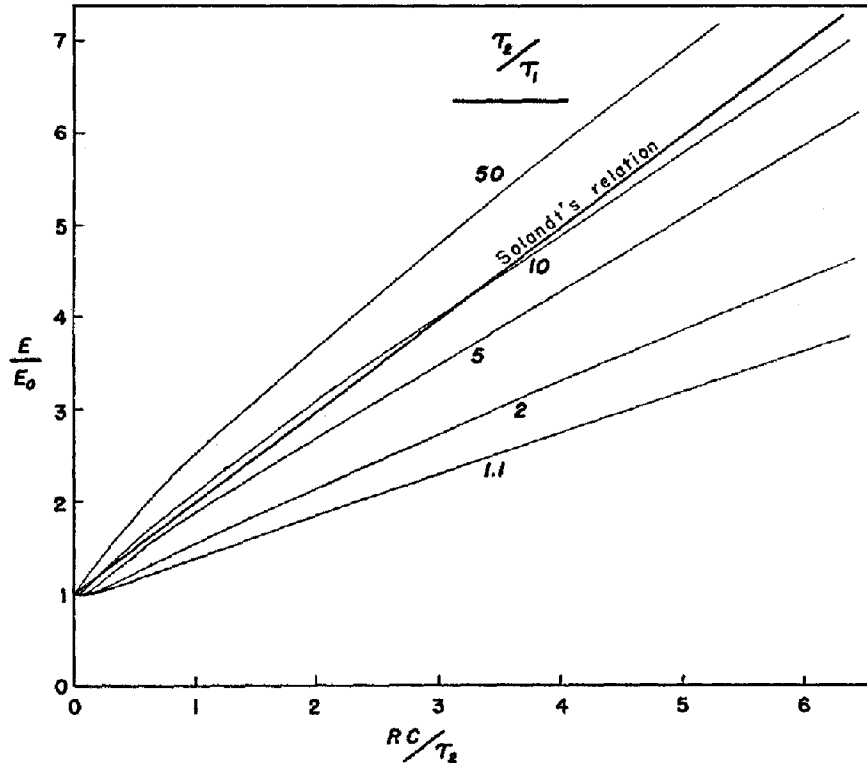


FIG. 3. Theoretical threshold patterns with exponentially blunted stimuli, as affected by ratio between the two time factors. Final magnitude of threshold stimulus (in rheobase units) plotted against time constant of delay of rise of current (in units of tissue accommodation time factor).

commodation). Satisfactory measurements can be taken with RC values sufficiently small that the terminal currents need not exceed 4 to 5 times the rheobase. But as Hill points out, unless RC is fairly large, the significance of the slope of the curve is lost; in the usable range, the slope and general configuration cannot even be stated explicitly, but as in the case of condenser discharges can be solved by substitution for any particular ratio of τ_2/τ_1 .

The appearance of the function is shown by the examples in Fig. 3.⁴ Initially, the slope is nearly zero (as has often been noted experimentally); in the neighborhood of $RC = \tau_1/2$ there is a rather abrupt rise which may (depending on the value of τ_2/τ_1) carry the curve above Solandt's simplified rectilinear relation. With higher RC , the slope progressively falls toward the limiting value stated above, and the curve passes permanently below the straight line (the graph of Fig. 3 does not extend far enough to show this in one case). In the range of useful observation (with E/E_0 up to about 5) the slopes, though not exactly constant, are reasonably definite following the abrupt rise. In order to arrive at some approximation of τ_2 from this sort of data, the slopes of these theoretical curves were calculated arbitrarily on the basis of the intercepts with $E/E_0 = 2$ and $E/E_0 = 4$. The reciprocal of such a slope from experimental data is taken as the "observed τ_2 " (following the usual procedure). The "observed τ_1 " is taken as that calculated from the application of Hill's curve to the condenser-discharge data. The observed " τ_2 "/" τ_1 " thus calculated is then an index of the degree of inaccuracy in these first approximations, and allows estimation of the true time constants. Fig. 4 shows the pattern of these conversions. Since the preferred methods of Hill's school lead to an overestimation of τ_2 and an underestimation of τ_1 , there is a lower limit to the possible observed ratio " τ_2 "/" τ_1 ", as the true ratio approaches unity; this limit is about 6.6, a surprisingly high figure. Moreover, if τ_2 is calculated on the basis of the *terminal* slope, as is customary, rather than from the slope as taken here, this limit is still larger, becoming e^2 , or 7.39. The occasional reports of such ratios in various tissues thus indicate a much more rapid accommodation than is generally appreciated.

In the application of these considerations to the present data, this limit of 6.6 becomes still more disconcerting, since all but one of the experiments show an apparent " τ_2 "/" τ_1 " less than 4.5. The single exceptional instance showed an apparent ratio of 11.1, corresponding to a true ratio of 7.1; the appropriate calculations here give $\tau_1 = 0.65$ msec.; $\tau_2 = 4.6$ msec. In fifteen other axons in which both of these types of threshold curves were obtained, the range of the apparent " τ_1 " was 0.38 to 1.0 msec. (average 0.696 msec.); the apparent " τ_2 " range was 0.85 to 4.0 msec. (average 1.71 msec.); and the average " τ_2 "/" τ_1 " was 2.43. Although, as has been indicated, this figure cannot be interpreted in terms of the simple theory so as to yield true figures for

⁴The curves of Figs. 2 and 3 were plotted from solutions by substitution in the general theoretical relations for threshold stimulation with the two types of stimulus in question. This involved determination by successive approximation of the utilization times (times of maximum excitatory disturbance), and substitution of this figure in calculation of the excitatory state.

the time constants, it is evident that extremely rapid accommodation is implied.

This failure of the two-factor theories militates against either the basic premises of the theories or the use of these stimuli with the squid axon, con-

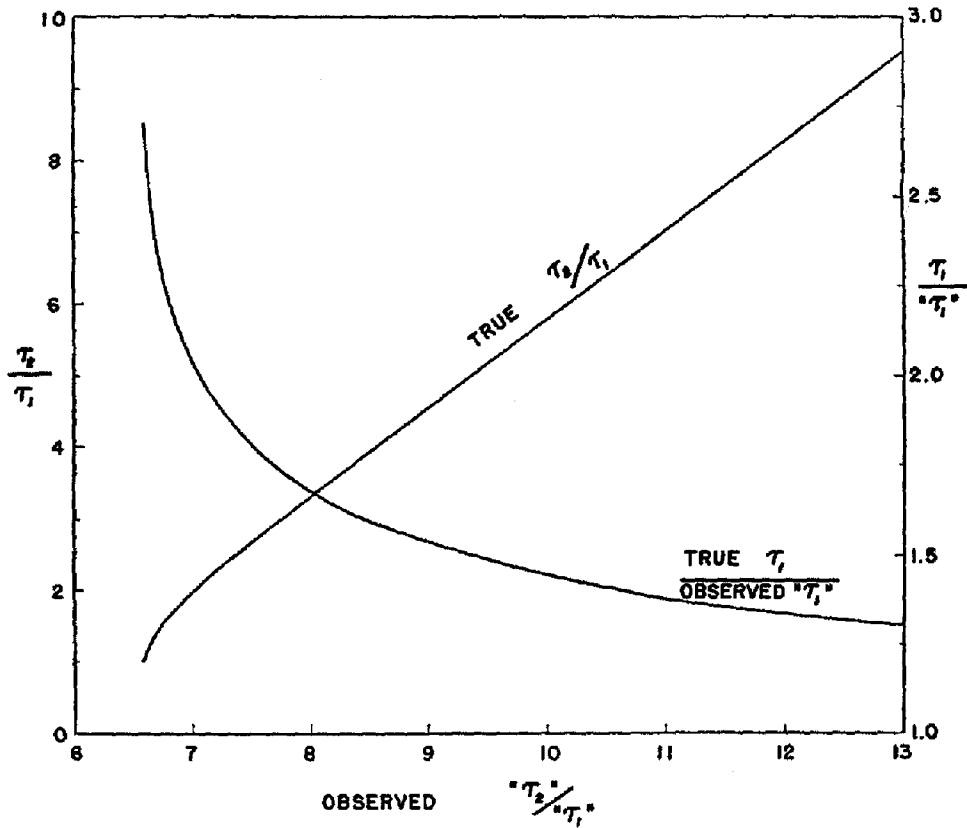


FIG. 4. Relation of true values of time factors to first approximations from conventional analysis of data, in cases of rapid accommodation. See text for further explanation.

sidering its susceptibility to disturbance by the relatively strong and prolonged currents used in the tests with exponentially blunted shocks. It might be suggested that the delayed entrance of accommodation, as reported by Erlanger and Blair (1931), could account for the incompatibility of the two types of threshold curves. However, even if this modification of the theory be allowed in the extreme, and the assumption made that the rapid accommodation has no influence at all on the determination of τ_1 from the condenser-discharge

data, the present observations could still not be explained. Ten of the fifteen sets of measurements would still show an apparent τ_2/τ_1 below the new theoretical limit of about 2.4. If the theory is to be retained at all in this application, it must be assumed that the more prolonged currents rapidly alter the time factors of the tissue, so that estimation of these factors by the methods favored by Hill and his school is not feasible.

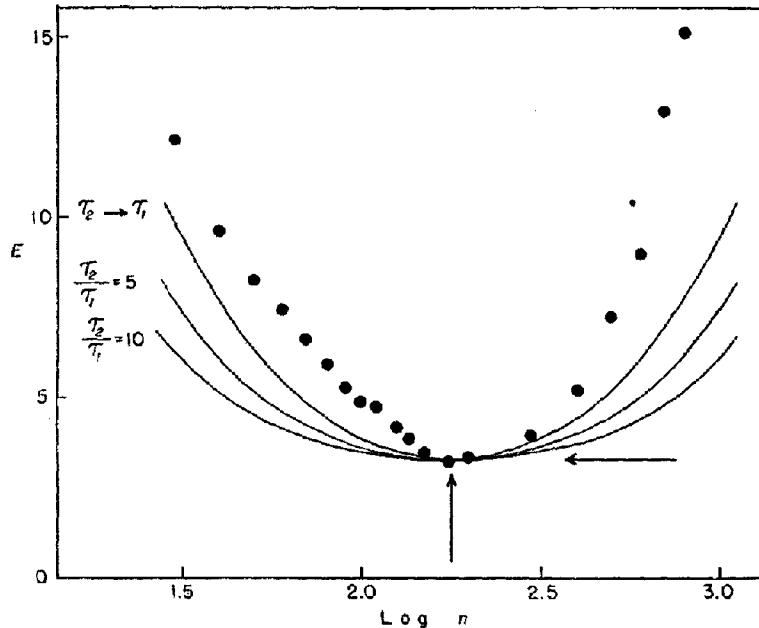


FIG. 5. A. C. steady stimulation of squid giant axon. Threshold voltage plotted against log of frequency (cycles per second). Points are experimental observations. Curves are those predicted from theory, with optimal frequency and minimal threshold fixed at levels indicated by arrows.

But measurement by means of the types of stimulus recommended by Monnier and Coppée (1939) proved similarly incompatible with unmodified two-factor theory. No estimation of the two time factors was usually possible in experiments with sinusoidal currents, or with brief "test shock" threshold determinations following the "make" of a direct current. The nature of this discrepancy is identical with that observed in decalcified frog nerves by Monnier and Coppée; in the pattern of the response to A. C., it becomes manifest in that the optimal frequency is more sharply defined than can be accounted for on the basis of the Rashevsky-Monnier-Hill theory. Fig. 5 shows a typical set of observations illustrating this behavior. The curves show the theoretical pattern of the threshold for the steady repetitive response to A.C. stimulation,

with three values of τ_2/τ_1 including the case where it just barely exceeds unity (the limit). In terms of the equation from which these curves are derived, the observed pattern (falling above this limit) implies that τ_1 and τ_2 are partly imaginary (being the roots of a quadratic equation in which $b^2 < 4ac$). This appears to be the normal state of affairs in the squid axon, and does not require the application of decalcifying agents.

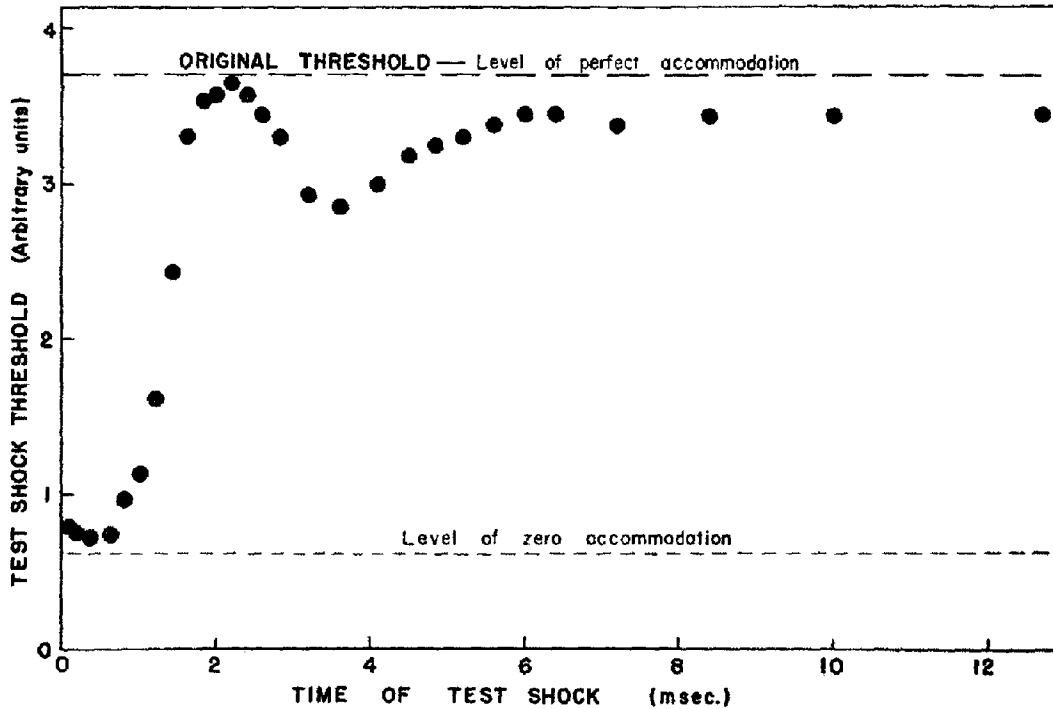


FIG. 6. Threshold changes at cathode at "make" of D. C. shock in squid giant axon. Test shock amplitude plotted against interval between "make" and test shock. Amplitude of D. C. is 83.5 per cent of rheobase.

The digressions from theoretical behavior seen in the pattern of the threshold changes at the "make" of a constant current are subject to the same sort of interpretation. The results of a typical experiment along these lines are presented in Fig. 6; damped oscillation of the threshold at the cathode of the steady current is very apparent. This sort of behavior has been studied extensively in decalcified frog nerves by Monnier and Coppée (1939), though, as Katz (1939) observes, their methods permitted estimation only of the direction of the excitability changes, not of their actual magnitude.

The degree by which the sharpness of the optimum frequency surpasses the limit set by the simple two-factor theories is not very pronounced in the

squid axon; it is never nearly so extreme as in some of the decalcified frog nerves observed by Monnier and Coppée. Their *aplatissement* factor ($\tau_a/\tau_o - \tau_b/\tau_o$, where τ_a and τ_b are the periods of the A. C. stimulus at which the threshold is twice that at the optimal period, τ_o) often fell to less than a tenth of the value of 3.464 critical for oscillatory behavior. In the present experiments, the lowest figures for *aplatissement* observed were on the order of 2.⁵ In most instances, the critical configuration was surpassed just sufficiently to make the relation unmistakable; and in about 15 per cent of the fibers observed, the *aplatissement* exceeded 3.464, so that the behavior of the cells could be treated simply on the two-factor basis. (These measurements showed an average τ_1 of 0.724 msec.; average τ_2 , 2.83 msec.; average τ_2/τ_1 , 4.19.)⁶ This distribution of fibers around the critical zone was also indicated by the fact that the oscillations in threshold observed at the cathode were almost critically damped; they were never obvious beyond the first two cycles (see example in Fig. 6) and in some fibers were not noticeable at all.

The minimal threshold A. C. voltage (at the peak of the sinusoidal wave) in the present experiments was commonly on the order of 0.7 to 0.75 times the rheobasic voltage. This is the range of the minimum possible figure on the basis of the two-factor theories, and is another indication of a very small ratio τ_2/τ_1 . The lowest figures of about 0.65 cannot be fitted to the theoretical predictions, and this may be a reflection of the discrepancies just considered. However, the rheobase determinations were made in the usual way, and not with rapidly repetitive direct current pulses such as Hill, Katz, and Solandt (1936) recommend as the only reasonable basis for comparison with A. C. stimuli.

There was always decidedly more accommodation at the cathode than at the

⁵ This figure would be somewhat lower if the curves relating threshold to log of frequency, with A. C. stimulation, were entirely symmetrical about the log of the optimal frequency. Actually, such curves, except in a very few cases, tilted somewhat toward the lower frequencies (see Fig. 5); for this reason the *aplatissement*, calculated as strictly defined, indicates a less acutely pointed curve than would appear from the logarithmic interval between τ_a and τ_b , since τ_a is displaced toward the shorter periods. The author is not prepared to evaluate the significance of this asymmetry; Coppée (1934, 1936) describes similar deviations under certain adverse circumstances, but these were always oppositely directed from that reported here, the curves leaning toward the *higher* frequencies.

⁶ In the calculation of these factors, some modification of Hill's procedure was again required because of the rapidity of accommodation, which made it inexpedient to refer intensities to Hill's "true rheobase," which is not a directly observable quantity. A much more readily and directly measured unit for expressing the slope is the observed threshold at optimal frequency. This quantity, unlike the abstract "true rheobase," varies with the relation τ_2/τ_1 , but the added complication permits the ready calculation of the two time constants from the data.

anode; this was observed also in frog nerves, where it has previously been reported by Parrack (1940). The data of Chweitzer (1935) show in fact the same relation, though this author treats the phenomenon in rather different terminology. But in the squid giant axon this difference is so pronounced that

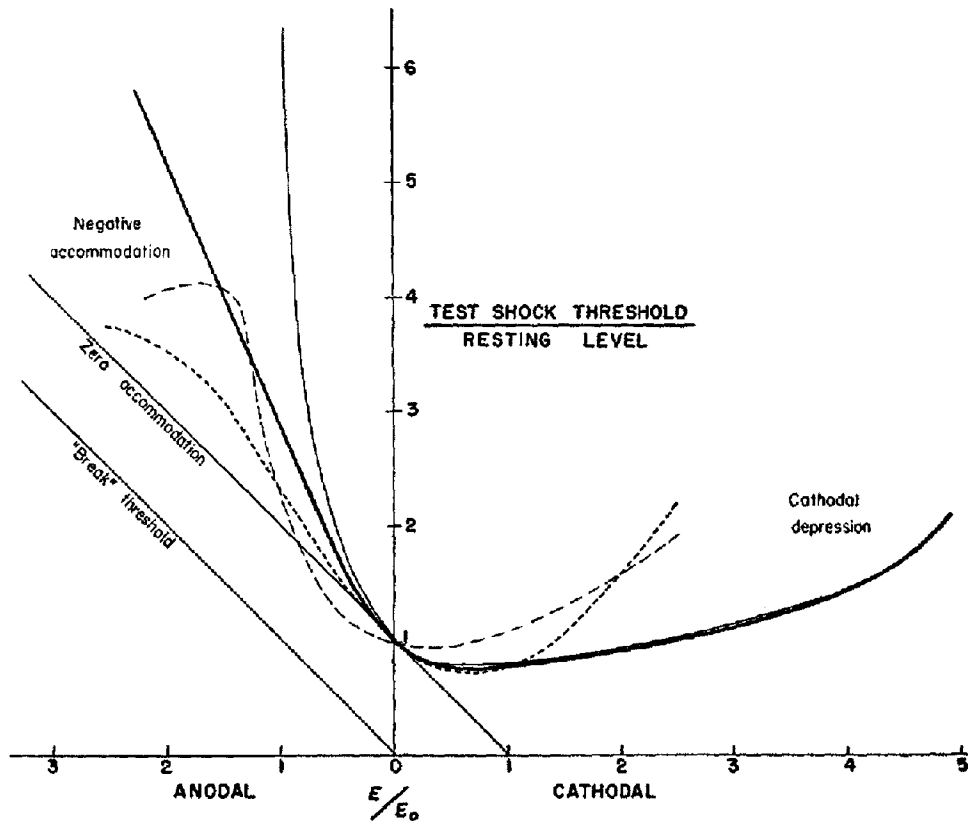


FIG. 7. Electrotonic threshold changes in squid giant axon. Test shock threshold plotted against steady current at same stimulating electrode. Each curve represents a different axon. Test shocks applied following D. C. "make" by: broken lines—102 msec.; heavy solid line—56 msec.; thin solid line—20 msec.

there is usually only *negative* accommodation at the anode, the threshold rising considerably above that anticipated from the proportional magnitude of the D. C. shock in terms of the rheobasic current. In other words, there is extreme anelectrotonus, making opening excitation very difficult to elicit, in spite of the fact that, in the usual sense, the tissue is highly accommodative.⁷

⁷ The threshold for opening excitation in these fibers was, in fact, regularly very high at the first determination; but the phenomenon of *décalage* (Cardot and Laugier, 1920) was very pronounced, and immediately subsequent determinations typically

This is illustrated in Fig. 7, showing several typical configurations in the plot of the final steady test shock threshold against the steady current (both in units defined by the resting thresholds), after the manner of Chweitzer. These curves resemble those Chweitzer presents for frog nerves, but show in general greater accommodation at the cathode, and less at the anode. The cathodal accommodation is in many instances almost "normal" (in the sense of Hill) with moderate strengths of current; and proceeds even to severe cathodal depression at relative current strengths at which the frog nerve threshold is still well below the resting level.

DISCUSSION

Prosser and Chambers (1938), using condenser-discharge stimuli, found τ_1 in the squid giant axon to be on the order of 0.4 to 0.6 msec.; this overlaps the lower values that would be obtained from the present data if treated in the classical manner, without regard for the rapid rate of accommodation. Prosser and Chambers used the mechanical response of the mantle as an index of neural activity, in a relatively intact animal; they mention that, under these conditions in their laboratory, Huntington found (using exponentially blunted currents) a value of τ_2 on the order of 200 to 400 msec., or about 100 times that reported here. It is therefore suggested that the rapid rate of accommodation seen in the present experiments may have developed only upon excision of the axon, since this would parallel the behavior of frog nerve as observed by Liesse (1938) in terms of his "CR2r" factor, and by Parrack (1940) using test shocks at the cathode of subrheobasic constant currents. However, the preparations used by Prosser and Chambers seldom survived more than about 20 minutes, so that it seems doubtful that the axons *in situ* were subjected to conditions more normal than when excised.

Monnier and Coppée's conclusion that the time constants (in their decalcified frog nerves) contained imaginary terms has been labelled by Katz (1939) as "somewhat astonishing" and the evidence dismissed as unconvincing on several counts. Katz feels that the finding of satisfactory time constants in such tissues, by the usual experimental methods, is evidence for their "reality." This seems hardly justified; with slight additional complications in the chemical or physical model systems of the two-factor theories, the excitatory state can, by appropriate relations of the constants, become underdamped and hence oscillatory. (Several simple models of this type have been discussed by Monnier and Coppée.) This would make possible the increase in the acuity of the sensitivity maximum at the optimal frequency beyond the limit dictated

showed the "break" threshold only 1.5 to 2 times the "make" threshold. The fact that the thresholds in these squid axons were rather easily disturbed by steady currents is in line with Chweitzer's (1935) interpretation of this *décalage* as resulting from residual anodal polarization acting as if to shift the y-axis to the left in curves such as in Fig. 7.

by the simpler system; but need not radically alter the form of the strength-duration curves and hence the measuring of *apparently* real time constants. Katz's objections to some of the details of experimental technique employed by Monnier and Coppée are not without basis, but they hardly invalidate the basic import of their results; the present experiments, free from these particular objections, are subject to the same general interpretation.

Direct evidence of underdamping in the excitable system in the squid giant axon is of course seen in the threshold oscillations at the cathode following "make" of a constant current, such as shown in Fig. 6. These oscillations in excitability are also undoubtedly reflected in the oscillations of local potential studied under similar conditions in the *Sepia* giant axon by Arvanitaki and coworkers (1939, 1941-43) and in the squid giant axon by Cole (1941) and Cole and Curtis (1941). In the present observations, no such oscillations of threshold were ever observed at the anode. This is in accord with Cole's description (1941) of the pattern of local potential changes across the membrane (or cortex) of this cell; Cole anticipated this pattern on the basis of the measured electrical characteristics of the membrane. (The lowering of the resistance component, which occurs at the cathode, and the elevation of the resistance at the anode, would, in his equivalent circuit, favor underdamping at the cathode and increased damping at the anode.) Similar oscillations studied in decalcified *Sepia* axons by Arvanitaki (1939, 1941-43) were apparently as easily elicited at either electrode; but Cardot and Arvanitaki (1941) found that the positive and negative undulations had very different critical thermal increments, and Arvanitaki (1943) described a difference between the events at the two electrodes in regard to the form of the relation between the amplitude of the primary undulation and the strength of the applied current. Thus it seems likely that the difference reported here in the behavior at the two electrodes is not merely a quantitative distinction.

The rapidity of the onset of recovery of the threshold back toward the resting level, after the initial increase in excitability at the cathode, is indicated in Fig. 6. The peak of the disturbance created by the application of the d. c. was, in fact, at a time (measured from the "make") considerably less than τ_1 as estimated from the various other measures. This is a briefer time than predicted by the theory,⁸ with even the smallest values of τ_2/τ_1 , and its significance is therefore questionable; it seems likely that its briefness is associated with the oscillatory character of the threshold that becomes evident immediately subsequently. At the peak of the excitability, there appears in all cases to be nearly perfect summation between the stimulating effects of the test

⁸ The theoretical time for the peak disturbance would be

$$\frac{\tau_1 \tau_2}{\tau_2 - \tau_1} \ln \frac{\tau_2}{\tau_1}.$$

shock and the steady current, since a fractional part of the rheobase lowers the test shock threshold by the same fractional degree. The example in Fig. 6 illustrates this, in the proximity of the first trough to the line indicating the level of "zero accommodation." This relation was also very reliable in the frog sciatic nerve, though Parrack (1940) in very similar experiments found no such agreement with the simple theory. In order to attain threshold in this tissue, he apparently had to increase the test shock amplitude to about 2.5 times the proportional fraction of the resting threshold that would be indicated by the relative magnitude of the applied constant current. Nothing in Parrack's recorded methodology suggests a basis for this point of difference from the present observations.

SUMMARY

1. The characteristics of the threshold excitation behavior of the squid giant axon were investigated with condenser discharges, exponentially blunted d. c. shocks, sinusoidal currents, and brief condenser-discharge test shocks superimposed at either electrode at controlled brief intervals following the closing of a constant current.

2. Data obtained by the use of condenser discharges and exponentially blunted currents followed theoretical patterns separately, but could not be mutually reconciled, under the system of the two-factor theories of excitation, since the accommodation was so rapid that the apparent ratio between the two time factors was incompatible with certain previously neglected theoretical limitations.

3. Data obtained with the other procedures indicated similarly rapid accommodation, but usually showed an imaginary component in the values of the time constants, associated with a capacity of the threshold to exhibit damped oscillatory behavior.

4. The anodal threshold behavior did not parallel that at the cathode, showing neither oscillation nor accommodation at ordinary current intensities.

5. The digressions from predictions of the two-factor theories of excitation are partly analyzed and compared with other reported deviations.

REFERENCES

- Arvanitaki, A., *Arch. internat. physiol.*, 1939, **49**, 209.
 Arvanitaki, A., *J. physiol. et path. gén.*, 1941-43, **38**, 147.
 Arvanitaki, A., and Cardot, H., *Compt. rend. Soc. biol.*, 1939, **131**, 1112.
 Bernhard, C. G., Granit, R., and Skoglund, C. R., *J. Neurophysiol.*, 1942, **5**, 55.
 Cardot, H., and Arvanitaki, A., *J. physiol. et path. gén.*, 1941-43, **38**, 9.
 Cardot, H., and Laugier, H., *Compt. rend. Soc. biol.*, 1920, **83**, 26.
 Chweitzer, A., *Compt. rend. Soc. biol.*, 1935, **120**, 626; *Arch. internat. physiol.*, 1935, **40**, 398.
 Cole, K. S., *J. Gen. Physiol.*, 1941, **25**, 29.

- Cole, K. S., and Curtis, H. J., *J. Gen. Physiol.*, 1941, **24**, 551.
- Coppée, G., *Arch. internat. physiol.*, 1934, **40**, 1.
- Coppée, G., *Cold Spring Harbor Symp. Quant. Biol.*, 1936, **4**, 150.
- Erlanger, J., and Blair, E. A., *Am. J. Physiol.*, 1931, **99**, 129.
- Hill, A. V., *Advances in Modern Biology*, Moscow, 1935, **4**, 131.
- Hill, A. V., *Proc. Roy. Soc. London, Series B*, 1936 *a*, **119**, 305.
- Hill, A. V., *Proc. Roy. Soc. London, Series B*, 1936 *b*, **119**, 440.
- Hill, A. V., Katz, B., and Solandt, D. Y., *Proc. Roy. Soc. London, Series B*, 1936, **121**, 74.
- Katz, B., *Electric Excitation of Nerve*, London, Oxford University Press, 1939.
- Lapicque, L., *L'Excitabilité en fonction du temps*, Paris, Presses Universitaires de France, 1926.
- LeFevre, P. G., *Biol. Bull.*, 1946, **91**, 223.
- LeFevre, P. G., *Biol. Bull.*, 1948, **93**, 256.
- Liesse, A., *Compt. rend. Soc. biol.*, 1938, **127**, 831, **128**, 1193.
- Monnier, A. M., *L'Excitation électrique des tissus*, Paris, Hermann & Cie., 1934.
- Monnier, A. M., and Coppée, G., *Arch. internat. physiol.*, 1939, **48**, 129.
- Offner, F., *J. Gen. Physiol.*, 1937, **21**, 89.
- Parrack, H. O., *Am. J. Physiol.*, 1940, **130**, 481.
- Prosser, C. L., and Chambers, A. H., *J. Gen. Physiol.*, 1938, **21**, 781.
- Rashevsky, N., *Protoplasma*, 1933, **20**, 42.
- Skoglund, C. R., *Acta physiol. Scand.*, 1942, **4**, suppl. 12.
- Solandt, D. Y., *Advances in Modern Biology*, Moscow, 1935, **4**, 44.
- Solandt, D. Y., *Proc. Roy. Soc. London, Series B*, 1936, **119**, 355.