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STRENGTH-DURATION CURVES FOR REPETITIVE STIMULATION OF MEDULLATED NERVE.

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THE recent work of Bugnard [1934] on the "fatiguing" effect of high-frequency and the "conditioning" effect of low-frequency stimulation in frog's medullated nerve made it desirable to determine the effect, if any, on the time relations of excitation, of repetitive stimulation at various frequencies. Bugnard's results themselves were first confirmed, employing as a measure of the response, not the heat production but the action current (the "negative variation") recorded with a sensitive high-resistance galvanometer of the moving-magnet type. For the study of the time relations of the excitatory process the method employed was that suggested by Hill [1934, p. 211], which has shown itself capable of great accuracy and rapidity. A constant response of the nerve itself was used as an indicator of a constant stimulus, instead of a minimal twitch in a muscle attached to it. This allows any kind of nerve to be used, and avoids the complication of fatigue in muscle or neuro-muscular junction.

The nerve was mounted in a moist chamber and connected by non-polarizable electrodes to the sensitive galvanometer so as to give a summated monophasic response. Its injury current was balanced and it was stimulated, by means of a rotating commutator, with a regular series of condenser discharges. The response (galvanometer deflection) can be varied either by changing the capacity of the condenser (more strictly its "discharge time" CR, where C is capacity and R is total discharge resistance), or by altering the potential to which the condenser is charged. When the former is altered the latter can be adjusted so that the response remains the same, in which case we can equate the excitatory effect of one capacity and one potential to that of another capacity and another potential.

APPARATUS.

The nerve chamber was of paraffin wax and had two pairs of electrodes, one pair for stimulation, the other pair connected to the galvanometer. The galvanometer electrodes were 14 mm. apart, and were separated from the stimulating electrodes by 26 mm., the distance between these latter being only 7 mm. All the electrodes were non-polarizable calomel half-cells connected to the nerve by means of moistened filter papers held end-on by slits in small blocks of vulcanite. The surface of contact between the edge of the filter paper and the nerve was small, being of the order of 0·3–0·5 mm.

The resistance of the calomel half-cells was reduced to a minimum by decreasing the length of the fluid electrical path from the calomel to the filter paper and increasing the diameter of the tubing used. The total resistance between the terminals of the stimulating electrodes, including 7 mm. of nerve, was reduced to 5500–6000 ohms in this way. In order that the calomel half-cells might remain non-polarizable, even under extreme conditions, the diameter of the tube containing the mercury and calomel was increased to about 18 mm., and no trouble from this source was experienced.

The galvanometer was a Downing moving-magnet instrument adjusted to a period of about 2 sec. at a sensitivity of about 2×10^{-11} amp. per mm. at 3 metres distance. It was read on a scale. Stimulation was by the usual sequence of condenser discharges employed in neurothermic experiments [Hill, 1934, etc.], only discharges being used. With the cathode nearer the galvanometer electrodes this insured that all the shocks were effective and that no anode block could occur. The condensers were of the mica insulated type and had been previously calibrated so that their values were known to within 5 micro-microfarads. To avoid leaks from the stimulating circuit the commutator was mounted on a separate bench and connected by carefully insulated lines to the condenser, and an earthed silver electrode was laid on the middle of the nerve. Since small capacities were used for the shorter times of discharge it was important that the capacity of these lines should be kept at a value well below that of the smallest condenser used. It was necessary also that the inductance of the discharge circuit should be negligible. It was further essential at these very short times of discharge to have a high linear speed of the surface of the commutator under the central brush, since otherwise a discharge time of 1 micro-second might well be almost complete before the brush had had time to make good contact with the oncoming segment of the commutator. In that case the resistance between the brush and the segment might be very high and an unknown quantity, thus seriously disturbing the time relations of the discharge. For this experiment, therefore, a special two-segment commutator was used and run up to 3000 r.p.m. to give 50 one-way shocks per sec.

To vary the voltage to which the condenser was charged, a rotary potential divider was placed across five 2-volt accumulators in series; for higher values a 60-volt high-tension accumulator was used, having taps every 2 volts. By the further addition in series of a 120-volt dry battery, 190 volts could be obtained. Any desired potential therefore could be applied and the value adjusted as accurately as required by means of the rotary potential divider. The potential was read with a multirange voltmeter.

A small resistance box was used in the stimulating circuit, so that various non-inductive resistances could be placed in series and in parallel with the nerve. A fixed resistance of 10,000 ohms was used in series with the nerve and a shunt resistance across the whole: the shunt could be altered by means of three switches to be 5000, 2500 or 100 ohms respectively. By varying these shunt resistances the effective resistance in the discharge circuit could be changed over a fiftyfold range. This, together with the variation of the capacity of the condenser, provided "discharge times" CR of from 1 to 10,000 micro-seconds. The "discharge time" is the time required for the condenser to discharge until $1/e$ of its original charge remains, e being the base of the Napierian logarithms.

METHOD.

The sciatic nerve of a Hungarian frog (*Rana esculenta*) was dissected and left to soak in Ringer's solution for 2 hours in order that it should become adjusted to this medium. It was then mounted in the nerve chamber so that the stimulating electrodes were at the proximal end with the cathode nearer to the galvanometer electrodes. The nerve was then crushed with forceps between the galvanometer electrodes, and three to five drops of isotonic KCl were placed on the crushed end. This procedure was found to give the largest injury potential, the average value being about 12 millivolts. When thus prepared the cell was covered with a strip of glass fitted with a moistened filter paper to prevent the nerve from drying, and moist oxygen was admitted. Before each experiment the chamber was carefully dried out to prevent any condensed moisture from forming a short circuit.

With the stimulating circuit connected and the commutator running at a constant speed the nerve was given a series of maximal stimuli at regular intervals (every 30 sec.) and the average size of the deflection measured. These stimuli consisted of a 5 sec. tetanus, and after three or four applications gave an almost constant response. It was found that 1·5 volts, 2·0 microfarads and a 5000 ohms shunt gave a satisfactory stimulus for this purpose.

The maximum deflection having been found the next procedure was so to stimulate the nerve at all "discharge times" as to produce a response one-half of this maximum. With this semi-maximal response kept constant throughout the experiment the result was capable of great accuracy. In order to maintain the state of excitability of the nerve throughout, it was stimulated with 5 sec. tetani every 30 sec., in the same way as for the maximal stimuli, since any variation in the interval would have left the nerve in a slightly different condition and liable to give a different response. The nerve does not fully return to its resting state, as regards its electrical response determined in this way, for about 1½ min. after a tetanus has been applied; its condition, however, after 30 sec. is a close approximation to this, while tetani given every 15 sec. lead to much more uncertain results.

Stimulating regularly as described, the condenser and resistance were set to give the longest discharge time, and the potential divider was adjusted until the response was semi-maximal. The longest discharge time used was 7970 micro-seconds; this was sufficient to give the rheobase in nearly all cases and was as large as could be allowed considering the frequency of stimulation employed. Longer discharges would have been cut off to some degree by the commutator even at such low frequencies as 10–20 per sec. When the semi-maximal response to the longest discharge time had been found, the condenser and the resistance were altered to give about half the above discharge time and the potential divider was again adjusted to give a semi-maximal response.

Thus the discharge time was altered in steps, each approximately half the previous value, and for each step the potential was found for which the nerve gave a semi-maximal response.

The shortest discharge time used was 0·862 micro-second, shorter discharge times than this requiring potentials of more than 150 volts, which for danger of leakage were avoided. After the reading at the shortest discharge time, a reverse series was at once taken in order to eliminate, by averaging, any progressive change in the condition of the nerve; although results showed that in nearly all cases the values obtained in the reverse series were so nearly the same as in the forward one that this extra precaution was unnecessary. At the higher voltages tests were made at intervals for any leak from the stimulating circuit by switching all of the condensers out and then "stimulating" the nerve. Occasionally small deflections would result, in which case a thorough drying of the cell removed the error.

The effective resistance is made up of (*a*) the resistance of the nerve, (*b*) the resistance in series with it, and (*c*) whatever resistance is placed in the shunt. The simplest and most reliable method of measuring the resistance of the nerve itself was found to be by a bridge method where the stimulus was used instead of an alternating E.M.F., and the null point was found with a pair of telephones. If the nerve was still connected to the galvanometer the size of the E.M.F. used could be chosen to give a response similar to that found during the tetani used in the experiment proper. Measurements on several different nerves agreed closely, and the resistance between the electrodes was taken to be 6000 ohms: this was correct to within ± 500 ohms. Assuming 6000 ohms in the nerve itself, the total effective resistance could be calculated.

RESULTS.

The strength-duration (potential-discharge-time) relation obtained by the above procedure is best plotted logarithmically, giving curves as in Fig. 1 [see Rushton, 1931, p. 283]. For the longest discharge times the curve is nearly horizontal and gives a close approximation to the rheobase; doubling the voltage, *i.e.* adding $\log 2 = 0{\cdot}301$ to its logarithm, gives the "chronaxie" or "excitation time." The point where the energy necessary for a given response is a minimum is that where the curve has a slope of $-\frac{1}{2}$ [Rushton, 1931]. This point can easily be marked off with a parallel ruler. The point where the quantity of electricity necessary to give a constant response is a minimum is that at which the slope is -1. As a matter of fact this is not a point but a wide region, since for very short discharge times the curve approaches a slope of -1 with considerable accuracy. This means that for short discharge times the quantity of electricity necessary to give a constant response is constant. In all the experiments performed on the sciatic nerve of *Rana esculenta* at room temperature (about 20° C.) this constant slope of -1 was reached for all discharge times shorter than 20 micro-seconds.

Fig. 1 shows two strength-duration (voltage-discharge-time) curves taken with different frequencies: (*A*) with 9 per sec. and (*B*) with 400 per sec. In the latter case the curve ceases to have any meaning for long discharge times, since discharge will necessarily be cut off by the commutator before it is nearly complete. To determine just how much of a condenser discharge can be cut off in this way without diminishing the effectiveness of the stimulus a commutator was designed by Prof. A. V. Hill, and constructed by Mr A. C. Downing, in which it was possible to alter the time of contact of the brush with the segment without altering the frequency. This was done by making the segments triangular in shape, so that moving the centre brush with a rack and pinion from one side to the other varied the duration of contact for discharge from zero on one side up to a maximum on the other. There were four pairs of segments, so that the maximum time corresponded nearly to a quarter of the whole circumference. The contact time for charging was bound to be altered in the converse manner as the brush was moved across, but the resistance on the charging side was made so small that the charge time was of the order of 5 micro-seconds, and the condenser was presumably completely charged even with the shortest duration of contact.

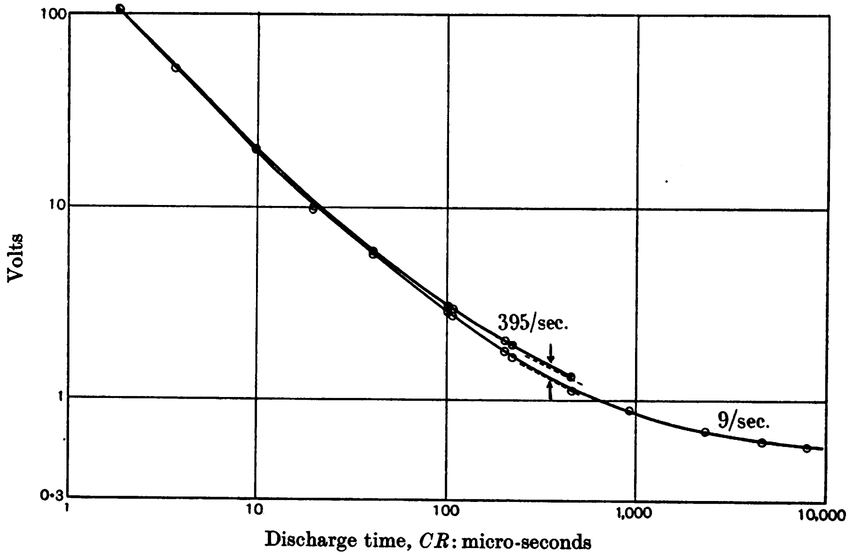

Fig. 1. Strength-duration (potential-capacity × resistance) curves of medullated nerve for repetitive stimulation: two different frequencies: logarithmic scales of potential and time.

With a given "discharge time" *CR* the voltage necessary for a constant response was determined as the centre brush was moved across the commutator from side to side. In this way it was possible to find the shortest time of contact at which the effectiveness of the stimulus was not diminished. The result can be expressed in two other ways, either (*a*) as the fraction of the full discharge which is necessary to give the full effect, the remainder of the discharge being ineffective, or (*b*) as the greatest frequency with which strength-duration curves can be made when including a given discharge time *CR*. The results are given below:

Discharge time *CR* μ sec.	Minimum contact time for undiminished response μ sec.	Least percentage of complete discharge for undiminished response	Maximum frequency allowable per sec.
9115	2810	26·6	100
1805	1690	60·8	200
456	560	72·3	600

Thus, for a frequency of 400 per sec. the longest "discharge time" that it is safe to use, without danger of reducing its effectiveness by cutting it off too soon, is about 600 micro-seconds, beyond which point the curve has not been continued. It is clear that if values for the rheobase and the chronaxie are to be obtained, the maximum frequency that can be used is less than 100 per sec. The above results show that it is not a constant fraction of the discharge that is necessary to give a full response, but that the fraction is much less with slow discharges than with rapid. This was to be expected in a general way, since with the slower discharges there is more time for "accommodation" of the tissue to set in, so the rest of the discharge becomes ineffective. It is in general agreement with the results of Lapicque [1926].

It is clear from the preceding results that for investigating the time relations of nerve at relatively high frequencies of stimulation the rheobase and the chronaxie are of no value and some other point on the strength-duration curve must be chosen. Such a characteristic point is that at which the energy necessary for a given response is a minimum. This is clearly defined on the curve as the point where the slope is $-\frac{1}{2}$, and with fresh nerves it usually occurs at a discharge time *CR* of about 400 micro-seconds. Since this lies within the available curve for 400 per sec. it is suitable for comparison in the strength-duration curve at all frequencies. This point has been marked on the curves taken at 9 per sec. and 400 per sec. The difference is negligible in spite of the great difference of frequency, as it was in a similar experiment where for the same two frequencies the points of minimum energy were respectively at 461 and 478 micro-seconds. Thus not only is there little difference between the shapes of the curves at 9 per sec. and at 400 per sec., but they both become asymptotic to a line drawn at 45° to the base, and in both the points for minimum energy occur at about the same discharge time. Over the range, therefore, in which the method used in these experiments is available, it has not been possible to show any important difference between the strength-duration curves for frequencies as widely different as 9 and 400 shocks per second.

To examine the effect of continuous prolonged stimulation on the strength-duration relation, the procedure was reversed so that the response taken as indicator of constant stimulus was not the deflection on applying the stimulus but the return of the galvanometer when the stimulus was removed. The nerve was stimulated continuously for 40 min. at 50 per sec. with a maximal stimulus, and then the stimulus was removed for 5 sec. and the size of the back swing of the galvanometer noted. The same precautions as before were taken to keep the preparation in a constant state of activity, but in this case the stimulus was removed for 5 sec. regularly every minute, instead of being applied regularly for 5 sec. every 30 sec. When the maximum back swing was found the discharge time CR was set to its maximum and the potential adjusted to give a steady maximal back swing on removing the stimulus. This procedure was followed over the whole range of CR, the result being a strength-duration curve for a nerve continuously stimulated, the periods of rest being only 5 sec. in each minute.

Each series of readings under continuous stimulation was preceded for comparison by a series taken during rest by the method described earlier, while after the series under continuous stimulation the nerve was given 1 or 2 hours' rest and another series taken during rest. Comparison of the three curves showed that the rheobase under stimulation was higher than during rest, although the curve taken after recovery from continuous stimulation never coincided with the one taken before, being always somewhat higher.

These curves all approach the slope of -1 for short discharge times, and all have the same general shape as the curves taken during rest. If plotted on the same sheet as in Fig. 2 the curves taken during continuous stimulation converge somewhat at shorter times on those taken during rest; although the potentials are much larger for values near the rheobase they are not proportionately larger for short discharge times. Corresponding to this, under continuous stimulation the point of minimum energy is shifted towards shorter discharge times. In Fig. 2 this characteristic point is moved from 365 micro-seconds for the resting nerve (curve A) to 122 micro-seconds for the nerve under continuous stimulation (curve B), returning to 232 micro-seconds after 2 hours' rest (curve *C*). It was found that the stronger the stimulus the shorter was the time to which the point of minimum energy could be driven, and that the point would move to the right even during the short intervals of an experiment. Curve *B* could not be taken during true continuous stimulation, there being 5 sec. rest in every minute to allow the response to be measured. Thus, if it were possible to make the curve while the nerve was being maximally stimulated without any rest at all, the point of minimum energy would be found at still shorter times.

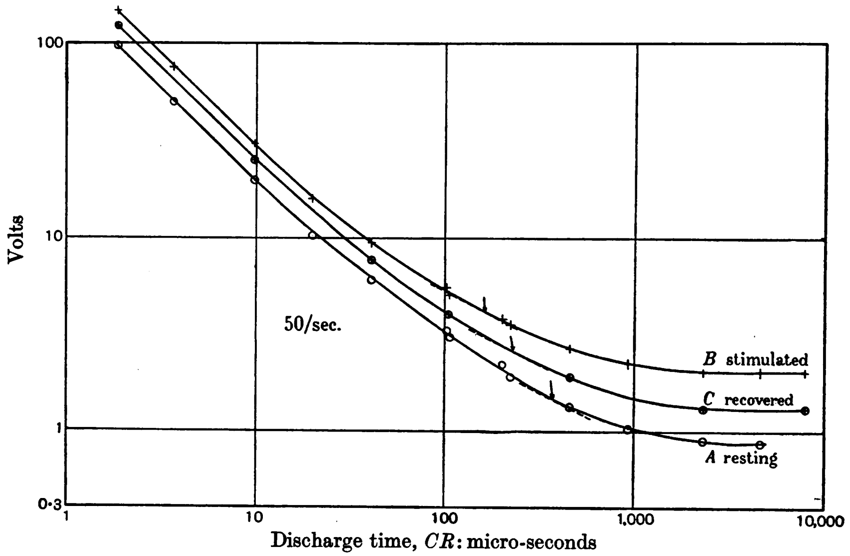

Fig. 2. Strength-duration (potential-capacity × resistance) curves of nerve for repetitive stimulation at 50 shocks per sec. *A*, stimulation 5 sec. every half min.; *B*, after prolonged stimulus, stimulation 55 sec. every 1 min.; *C*, after long rest, stimulation 5 sec. every half min.

The effect of continuous stimulation was investigated with regard to frequency, and the same result was observed at 200 per sec. as at 50 per sec., the magnitude of the effect remaining about the same. Its extent seems to depend solely on the degree to which the nerve has been stimulated.

The results just recorded confirm in some respects the observation made by Boyd and Gerard [1930], using a muscle-nerve preparation from a green frog and blocking the nerve by cold below the stimulus during the time that the continuous tetanus was applied. It was then necessary to remove the cold block, before any measure of the rheobase or chronaxie could be made in the usual way by observing a just noticeable twitch. They found an increase of 27 p.c. in the rheobase but only a slight shift (if any) in the chronaxie towards shorter times. In the present experiments the increase in the rheobase was as great as two to three times the resting value, but far more significant was the shift in the discharge time for minimum energy, which was shown to change from 365 micro-seconds for the resting nerve to 122 micro-seconds for the nerve under conditions approximating to continual stimulation. It was found that a very large change both in rheobase and in the point for minimum energy took place very rapidly when the nerve was allowed longer rests than in the present experiments. If the nerve was allowed to rest for 10 sec. every minute instead of 5 sec., the effects on the rheobase and on the time for minimum energy were reduced considerably. The present results, therefore, agree with the findings of Boyd and Gerard, considering that they had to wait about 25 sec. between the moments at which the continual tetanus was removed and at which the first measure of the rheobase could be made. Considering also that an appreciable further interval must have elapsed before the measurement of the chronaxie could be completed, the nerve would be nearly returned to a resting condition before the cold block was removed and the necessary values obtained for rheobase and chronaxie.

When measurements were made of the potential-capacity relation during constant repetitive stimulation a double series was always taken, starting with long discharge times and going to short discharge times and returning. The double series had been preceded by continuous stimulation without gaps, and when the gaps began, starting with long discharge times in the double series, it was observed that the first few values of the potential necessary for constant response decreased in spite of decreasing discharge time. This was due to the rest provided by the 5 sec. gap. When the same discharge times were examined in the reverse series they gave almost a constant value for potential, since by now the nerve had become adjusted to the new steady condition corresponding to 55 sec. stimulus, 5 sec. rest.

In order that the tetanus should remain maximal the stimulus had initially to be made considerably supermaximal: a stimulus which was just maximal at the beginning was soon considerably less than maximal.

Field and Brücke [1926] found a lengthening of the refractory period during continual stimulation, and showed that this effect continued for ten minutes after a ten minutes' stimulus. Boyd and Gerard [1930] found that the increase in rheobase referred to above persisted for about 4 min. after 5 min. continual stimulus.

SUMMARY.

A method is described for determining the strength-duration (potential-capacity × resistance) relation for the electric excitation of nerve, employing the action current of the nerve itself as an indicator. A semimaximal electrical response is used instead of a threshold muscle twitch. With repetitive stimulation the limitations of the method in respect of frequency of stimulation are discussed.

Very consistent results are obtained. For very short discharge times (less than 20 micro-seconds) with a frog's sciatic nerve the quantity of electricity required for a constant degree of excitation becomes accurately constant, and independent of the time of discharge.

The frequency of stimulation as such has little or no effect on the time relations of nerve excitation. By a slight alteration in the method the strength-duration relation can be determined during continual repetitive stimulation. Steady activity causes a considerable increase in the rheobase and an appreciable quickening of the excitatory process.

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