

# Nerve Excitation by Alternating Current

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## I—INTRODUCTION

When an alternating current is passed through a nerve a relation exists between the intensity  $I$  necessary for steady threshold excitation, once to each cycle at the electrode considered, and the frequency  $n$ . For a pure sine-wave current applied to a nerve lying on two non-polarizable electrodes, and taking account of both time-constants ( $k$  of the local excitatory disturbance,  $\lambda$  of accommodation), this relation (Hill, 1936a, p. 343) should be:—

$$\frac{I}{I_0} = \sqrt{(1 + 4\pi^2 k^2 n^2)(1 + 1/4\pi^2 \lambda^2 n^2)}, \quad (1)$$

where  $I_0$  is the "true rheobase" for steady excitation by a regular series of constant-current pulses, each of sufficient duration to attain the minimum threshold.

The theory, in its present form, takes no account of the phenomena of absolute and relative refractoriness after an effective stimulus, so that equation (1) need not be expected to apply rigorously at frequencies so high that successive waves fall within the refractory periods, absolute and relative, of their predecessors. Nor can it allow for the fact that, owing to effects of electrical capacity at surfaces or membranes in the nerve, the current distribution in the nerve must change with change of frequency. It was important, however, to examine the relation between  $I$  and  $n$  over the wider range, and experiments were made at frequencies from nearly zero to 10,000 cycles per second. Those at the higher frequencies are referred to in § XI below; in the rest of the paper we deal only with the lower range (up to 300–1000 cycles per second, according to the temperature).

The theory takes no account of the “supernormal phase” of excitability (Adrian, 1920, 1921) which occurs under certain conditions, and may affect the threshold during repetitive stimulation of appropriate (rather low) frequency.

In fig. 1*a*, *b*, *c* the process of steady threshold excitation by sine-wave alternating current is illustrated graphically, for a typical case of frog's nerve at 15° C., and for three frequencies 10, 100, and 1000 cycles per second. The curves were drawn from equations (35) and (37) of a previous paper (Hill, 1936*a*). The full line represents the local potential (the local excitatory disturbance  $V$ ), fluctuating with the applied current, but lagging behind it by a phase angle  $\tan^{-1} 2\pi nk$  which is greater the greater the frequency. ( $A$  in each diagram denotes phase zero of the alternating current.) The broken line represents the threshold  $U$ , fluctuating with the applied current and with  $V$ , lagging behind the former by phase angle  $(\tan^{-1} 2\pi nk + \tan^{-1} 2\pi n\lambda)$ , behind the latter by phase angle  $\tan^{-1} 2\pi n\lambda$ .

Threshold excitation occurs when the  $V$  curve *touches* the  $U$  curve; if the former *crosses* the latter the stimulus is unnecessarily strong. The moment of such excitation is at  $E$ , the point where  $U$  is at phase zero; that is to say, at threshold excitation by alternating current the nerve is exactly unaccommodated (see Hill, 1936*a*, p. 342). For low frequencies the phase angle  $AE$  is small, for high frequencies it approximates to  $\pi$ ; for the optimum frequency it is  $\pi/2$ . Expressed otherwise, a threshold current of optimum frequency stimulates at the crest of each wave; of lower than optimum frequency, on the rising phase; of higher than optimum frequency, on the falling phase. These are deductions which could be tested experimentally. With an alternating current of any strength, both the local excitatory disturbance  $V$  and the threshold  $U$

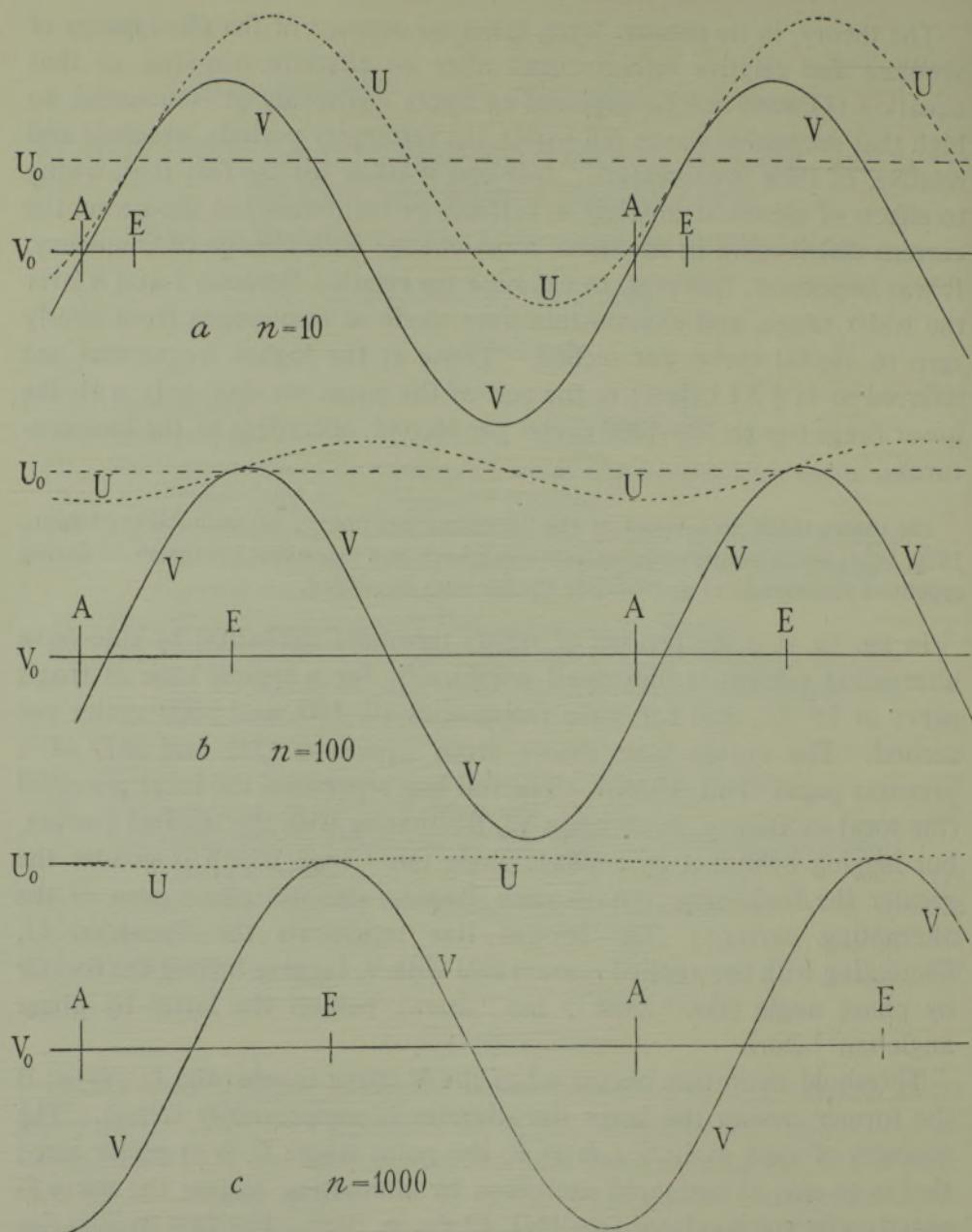


FIG. 1, *a*, *b*, and *c*—To show the time course of  $V$  and  $U$  in steady threshold excitation by alternating current, for  $n = 10$ , 100 and 1000 cycles per second respectively.  $A$  = commencement of A.C. wave;  $E$  = moment of excitation. Note (i) that  $V$  and  $U$  lag behind the current by a phase angle depending on the frequency, and (ii) that excitation occurs (at  $E$ ) at the moment when  $U = U_0$ , i.e., when there is no "accommodation". The case taken (*cf.* Hill, 1936*a*, p. 342) is for a frog's nerve at  $15^\circ\text{C}$ . with  $k = 0.5$  msec.,  $\lambda = 10$  msec. (fairly rapid accommodation). The currents required to excite in the three cases are, as multiples of the true rheobase:  $n = 10$ ,  $I/I_0 = 1.88$ ;  $n = 100$ ,  $I/I_0 = 1.06$ ;  $n = 1000$ ,  $I/I_0 = 3.32$ . The optimum frequency is  $n = 71$ , for which  $I/I_0 = 1.05$ .

fluctuate more at a lower, less at a higher frequency. At a very high frequency, and with a current of threshold strength,  $V$  fluctuates with amplitude  $(U_0 - V_0)$ , while  $U$  scarcely fluctuates at all.

The optimum frequency  $n_{op}$  is given by

$$n_{op}^2 = 1/4\pi^2 k \lambda, \quad (2)$$

the strength  $I_{op}$  of the optimum stimulus by

$$I_{op}/I_0 = 1 + k/\lambda. \quad (3)$$

Equation (1) may be written,

$$I/I_0 = \sqrt{\left(1 + \frac{k}{\lambda} \frac{n^2}{n_{op}^2}\right)\left(1 + \frac{k}{\lambda} \frac{n_{op}^2}{n^2}\right)}, \quad (4)$$

or

$$I/I_{op} = \frac{\sqrt{\left(1 + \frac{k}{\lambda} \frac{n^2}{n_{op}^2}\right)\left(1 + \frac{k}{\lambda} \frac{n_{op}^2}{n^2}\right)}}{1 + k/\lambda}. \quad (4')$$

If we write  $n/n_{op} = 10^x$ , so that  $x = \log_{10} n/n_{op}$ , equation (4) becomes,

$$I/I_{op} = \frac{\sqrt{\left(1 + \frac{k}{\lambda} 10^x\right)\left(1 + \frac{k}{\lambda} 10^{-x}\right)}}{1 + k/\lambda}. \quad (4'')$$

The expression on the right of equation (4'') is unaltered by changing the sign of  $x$ . If, therefore,  $I$  be plotted against  $\log_{10} n$  a symmetrical curve results, with its vertex at  $I = I_{op}$  and its vertical axis of symmetry at  $n = n_{op}$  (fig. 2). The symmetrical character of the  $I - \log n$  curve was established experimentally by Coppée (1934c): it is confirmed by the experiments described in § IX below.

The relation between  $I/I_{op}$  and  $\log n/n_{op}$  is invariable in all respects save one, viz., in the value of  $k/\lambda$ . If  $k/\lambda$  were constant, *i.e.*, if the behaviour of an excitable tissue were characterized by a single time-constant, then the relation in question would be completely invariable. The experiments given in § VIII show that it certainly is not; the reason is that  $k/\lambda$ , as pointed out by Solandt (1936a) on other evidence, can vary over a wide range. Coppée's experiments, indeed, already made this clear, for he refers to the fact (1934c, p. 16) that the *aplatissement* of his curves (the width of the  $I - n$  relation at twice the minimum height) varied rather widely. In fig. 2 are three calculated curves of  $I/I_{op}$  versus  $\log_{10} n/n_{op}$ , for  $k/\lambda = 1/10, 1/30$ , and  $1/100$  respectively. Plotted on the same scales of  $I/I_{op}$  and  $\log_{10} n/n_{op}$ , the curves are more pointed the

greater the value of  $k/\lambda$ , more rounded the less that value. A 55% change in the width of the  $I - \log n$  curve at twice the minimum height means, in this range, a 10-fold alteration of  $k/\lambda$ . In fig. 3a are observed points on a calculated curve for which  $k/\lambda = 0.0624 = 1/16$  approximately.

The values of  $k$  and  $\lambda$  can be determined separately from the relation, experimentally observed, between  $I$  and  $n$ , provided that the said relation extends sufficiently far on either side of the optimum frequency. We

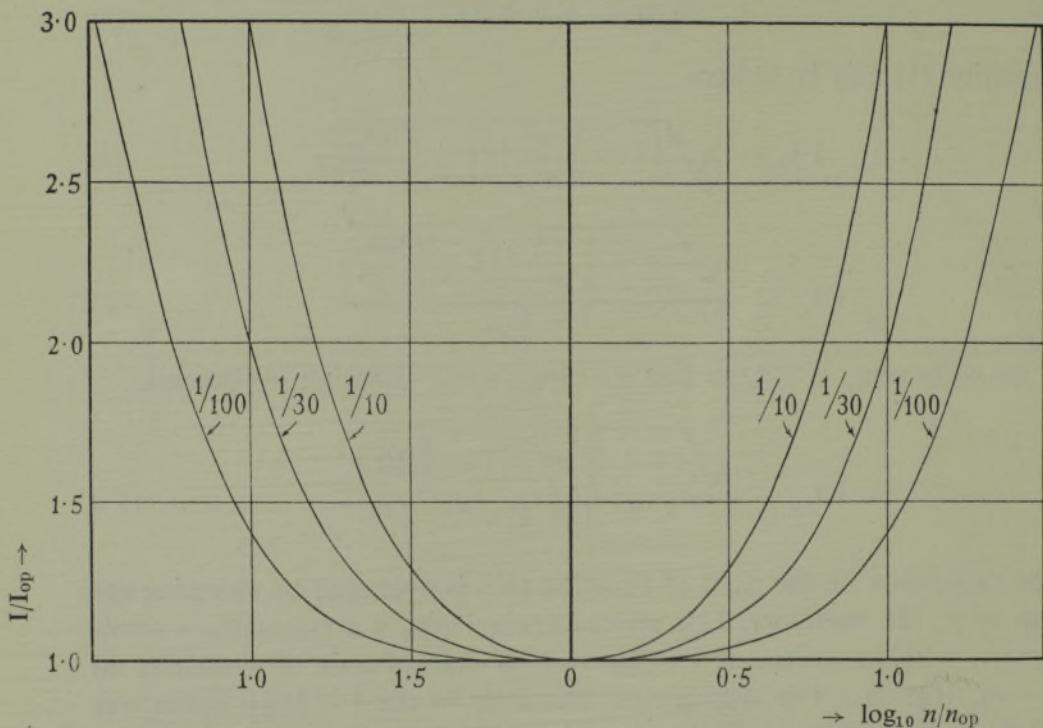


FIG. 2—Relation between threshold alternating current and the logarithm of the frequency, for different values of  $k/\lambda$  shown on the curves; drawn from equation (4'). Note that the vertices are made to coincide by dividing the current by the optimum current, and the frequency by the optimum frequency.

may, for example, plot  $I/I_{op}$  as observed against  $\log n$  (fig. 3a) and then move about a family of curves, similar to that of fig. 2 but drawn on tracing cloth, over the observed points, until we get the best fit of the points with one of the family. The particular member of the family gives  $k/\lambda$ , while the optimum frequency gives  $k\lambda$  (equation (2)): from these,  $k$  and  $\lambda$  can be calculated separately. This method works quite well, but a simpler one is as follows. This depends on the fact that  $k/\lambda$  is usually small, so that if either  $n^2/n_{op}^2$  or  $n_{op}^2/n^2$  is rather small, one or other of the factors on the right-hand side of equation (4) is nearly equal to unity. Thus if  $k/\lambda = 1/20$  and  $n^2/n_{op}^2 = \frac{1}{2}$  the effect on  $I^2/I_0^2$  of putting

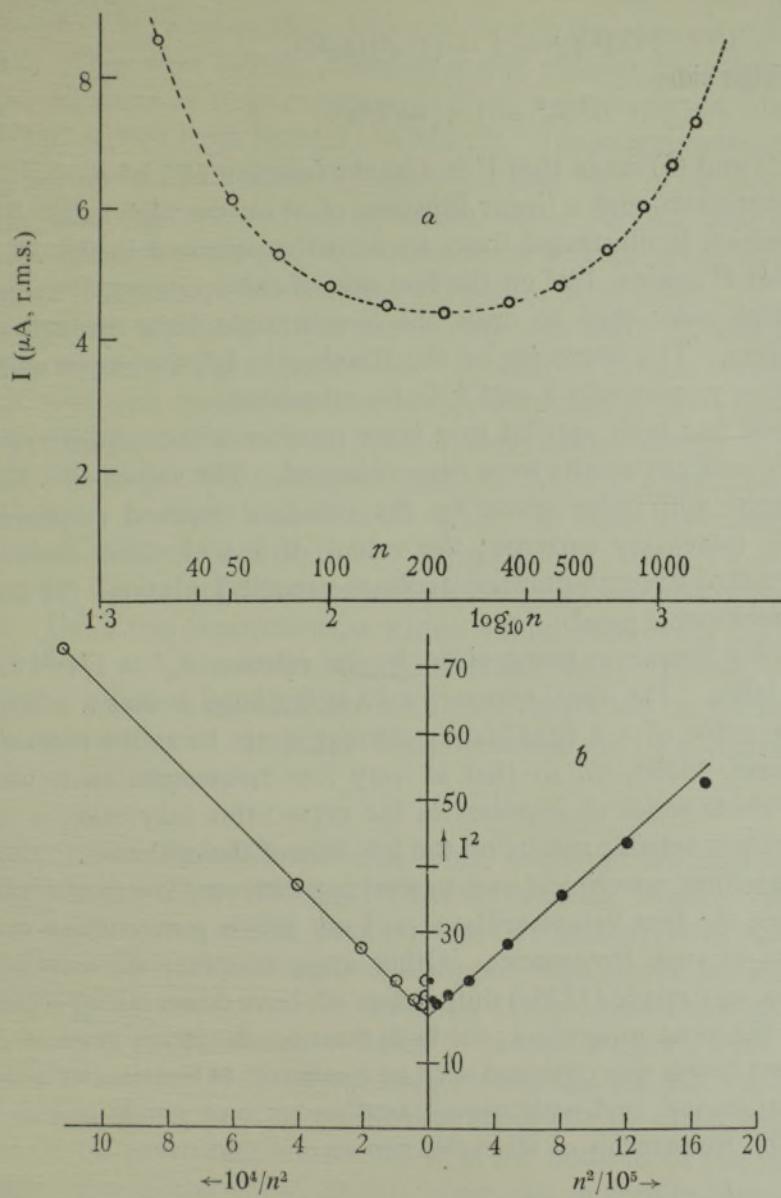


FIG. 3—Experiment of 9 January, 1936; Table XIV, § IX. Nerve treated with  $6 \times \text{Ca}$ , at  $32^\circ \text{C}$ . Muscle response as index. (a)  $I$  plotted against  $\log_{10} n$ , and a theoretical curve (from equation (1)) with the constants given in Table XIV. (b)  $I^2$  plotted against ( $\rightarrow$  right)  $n^2$ , and ( $\leftarrow$  left)  $1/n^2$ , showing linear relations on the high and the low sides respectively of the optimum frequency.

the first factor equal to unity is to make it only  $2\frac{1}{2}\%$  too small. Hence on the low side of the optimum frequency, with sufficient accuracy, equation (1) becomes,

$$\frac{I^2}{I_0^2} = 1 + (1/n^2)/4\pi^2\lambda^2 \quad (5)$$

and on the high side,

$$\frac{I^2}{I_0^2} = 1 + 4\pi^2k^2n^2. \quad (6)$$

Equations (5) and (6) mean that  $I^2$  is a linear function of  $1/n^2$  on the low side of the optimum, and a linear function of  $n^2$  on the high side. The procedure, which is illustrated from an actual experiment in fig. 3b, is simply to plot  $I^2$  against  $1/n^2$  on the low side of the optimum,  $I^2$  against  $n^2$  on the high side; then to draw the best straight lines through the observed points. The intercepts on the  $I^2$  axis give  $I_0^2$ , the slopes of the two lines allow respectively  $\lambda$  and  $k$  to be calculated.

This method has been applied to a large number of experiments, and unexpectedly constant results have been obtained. The values of  $\lambda$  have been compared with those given by the standard method employing exponentially increasing currents; the values of  $k$  with those deduced from the standard strength-duration (voltage-capacity) relation. In both cases the agreement is good.

The optimum frequency being given by the relation  $n_{op}^2 = 1/4\pi^2k\lambda$ , is low if  $k\lambda$  is large. The usual reason for  $k\lambda$  being large is that  $\lambda$  is large. With a large value of  $\lambda$  a long-lasting current is apt to evoke repetitive response (Katz, 1936*b, c*), so that at very low frequencies each wave produces a whole series of impulses in the nerve; this may make it impossible to obtain reliable results on the low side of the optimum. There is another objection, a technical one, to working with very low frequencies, viz., that even the best valve-oscillator will not give a pure enough sine-wave current at such frequencies. Other apparatus may be employed in this region, as Coppée (1934*c*) did, and as we have done, but an oscillator is by far the most convenient; for both reasons, therefore, most of the evidence given below was obtained with an oscillator, at frequencies above 25 cycles per second, and with nerves used under such conditions as to have optimum frequencies of 60 cycles per second and over.

## II—EXPERIMENTAL

*Preparation*—The sciatic nerves of large Hungarian frogs (*Rana esculenta*) were employed throughout. Those of English frogs were not long enough for convenient use and for the avoidance of leaks when the action current was taken as index of response. The work has extended over 16 months, so that animals at various seasons have been used. The

latest and most decisive experiments were made during the winter and spring (January–April) of 1936 on a batch of frogs which arrived in the laboratory in October, 1935, and were kept in the open air in a pond on the roof. They were usually placed in a sink indoors for a few days before use. In some of these experiments frogs were employed which, recently caught, arrived from Hungary in March. The experiments of December, 1934, to June, 1935, were on frogs which had been in the laboratory since October, 1934, some in an aquarium, some (for the later experiments) in a cold store at about  $5^{\circ}$  C.; the latter were kept in the warm for a few days before use. No consistent differences were noted.

In many experiments the nerve only was used, its electric response being taken as index of excitation. In others, a muscle-nerve preparation was employed, muscle response to nerve excitation being observed. The preparation, in either case, was soaked before use in Ringer's fluid, "normal Ringer" consisting of 6.75 gm. NaCl, 0.2 gm. CaCl<sub>2</sub>, 0.15 gm. KCl, made to 1 litre with distilled water. In many experiments, as noted below, CaCl<sub>2</sub> was added to the Ringer's fluid: 10  $\times$  Ca (*e.g.*) denoted that the Ringer's fluid contained 10 times the normal concentration of Ca. For a few experiments, a Ringer's fluid poor in Ca (*e.g.*, 0.4  $\times$  Ca) was made up; for others, the animal had 40 to 100 mg. of CaCl<sub>2</sub>, in isotonic solution, injected into its dorsal lymph sac on the previous day. The nerves from Ca-injected frogs were soaked in a high-Ca Ringer's solution before use.

*Electrodes*—Calomel half-cells were employed throughout, both for stimulating and for recording the action current. A galvanometer placed in the stimulating circuit gave no sign of rectification of the alternating current. The calomel half-cells dipped into pools of Ringer's fluid. Contact was made with the nerve by sharp-edged strips of soft wood, soaked for a long time previously in normal Ringer's fluid. When the nerve was soaked in abnormal Ringer's solution the soft wood strips were soaked in it too, and the pools were filled with it. The strips were held fast by being pushed into plasticine at the bottoms of the pools. Their edges were usually about 20 mm. apart, but in certain experiments (§ V) they were brought as close as 1.5 mm. (*see* Hill, 1936*b*, pp. 443, 448; Solandt, 1936*a*, p. 357).

*Chamber*—This was fashioned from a block of paraffin wax. For a muscle-nerve preparation there were two (or three) pools, dug out in the wax, for the calomel half-cells. The muscle was pinned to a cork sunk in the wax. A glass cover, preferably with a strip of moist filter paper on

it, was used to prevent evaporation. The muscle was connected to a spring lever, for ease in observing the response. For a nerve preparation, there were four (or five) pools for the calomel half-cells, two being used for leading off the action current.

*Galvanometer*—A very sensitive moving-coil galvanometer, of period about 4 seconds, was used for recording the monophasic action current. This was critically damped with an external resistance of about 2000 ohms. Maximal stimulation at moderate frequency gave a steady deflexion of 50 to 200 mm. As index of constant response, either a small galvanometer deflexion (1 to 3 mm.) or (at first) a constant small fraction of the maximal response for the frequency considered was taken. The latter was later discarded, since at high frequencies the stimulus for maximal response is so great that it tends to alter the threshold, and at very high frequencies the true maximal response cannot be determined (*see* Katz, 1936a).

*Oscillator*—For the earliest experiments an oscillator constructed by the Cambridge Instrument Company was used. This was calibrated from 50 to 3000 cycles per second with the aid of tuning forks. It was tested with a cathode ray oscillograph (by the kindness of Professor D. T. Harris) and the conditions necessary in order to obtain an approximately pure wave-form were worked out. At low frequencies, however, the wave was never sufficiently pure, and the range was not wide enough. With the aid, therefore, of a grant from the Royal Society, a Beat-Tone Oscillator of N.P.L. design (Type 3A) constructed by Messrs. Muirhead & Co., of Beckenham, Kent, was obtained. This was calibrated by the National Physical Laboratory for frequency, from 10 to 10,000 cycles per second: it can be set precisely from time to time by a “trimming condenser” and two reeds contained in the equipment. The root mean square value of all the upper harmonics in the wave given by a Muirhead amplifier connected to the oscillator, according to the N.P.L. certificate, is given in Table I. For the present investigation the oscillator was used without the amplifier, so that the harmonic content was presumably less. The oscillator has six ranges, controlled by a potentiometer in the input circuit of the detector valve, and to keep the wave as pure as possible the highest range (No. 6), which gives a rather large grid swing to the last valve, was not used, except sometimes at high frequencies when the whole available E.M.F. was required.

The oscillator was carefully tested at low frequencies (10 to 200 cycles per second) with a Downing moving-coil oscillograph viewed with a set

of revolving mirrors. Below 20 cycles per second the wave form was perceptibly distorted, but at and above 25 cycles per second a very good wave was obtained. It is essential for the argument that a pure sine-wave should be used, otherwise (particularly at low frequencies) entirely false thresholds may be obtained. The wave-form, for example, of Coppée's earlier oscillator (1934a, fig. 5, p. 245) might well lead to anomalous results.

TABLE I—HARMONIC CONTENT OF WAVE, AS A PERCENTAGE OF THE FUNDAMENTAL

	Cycles per second	%
Range 4 .....	100	3·0
	500	2·5
	2000	<1
Range 5 .....	100	4·5
	500	4·1
	2000	2·2
Range 6 .....	100	6·1
	500	5·7
	2000	3·1

For very low frequencies an apparatus was constructed by which the total light through an aperture was made to vary sinusoidally with the time. The frequency could be varied from zero upwards to about 250 cycles per second. The light was concentrated by a condensing lens into a vacuum photo-cell, which was connected to an amplifier. The form of the wave so produced was tested while running by photographing it with an oscillograph, or at "zero" frequency by setting the disk (which was calibrated round its edge), in a series of positions, reading the current with a galvanometer, and plotting. The wave form was good and was independent of frequency. The apparatus worked well but was much slower to set and less convenient to use than the oscillator, since variation of frequency meant stopping a motor and altering belts on pulleys.

The photoelectric arrangement need not be further described here, since the results obtained with it are the less important, and analogous systems have been described before (*see e.g.*, Nicolai, 1930, and the mechanical arrangements of von Kries, 1884a, and of Coppée, 1934c).

*The Measurement of the Current*—In the later experiments a Westinghouse "instrument type" rectifier connected to a microammeter, together with a potentiometer arrangement of Muirhead reactionless resistance

boxes, was employed (fig. 4). The rectifier was calibrated at various frequencies, up to the highest used, and with various intensities of current, against a pair of Cambridge "vacuo-junctions" (in series) connected to a very sensitive moving-coil galvanometer. The vacuo-junctions themselves were calibrated with direct current measured by the same microammeter. It was found that the "1 mA." rectifier could be used safely to well below 100  $\mu$ A., and at all frequencies from 25 to 10,000 cycles per second.

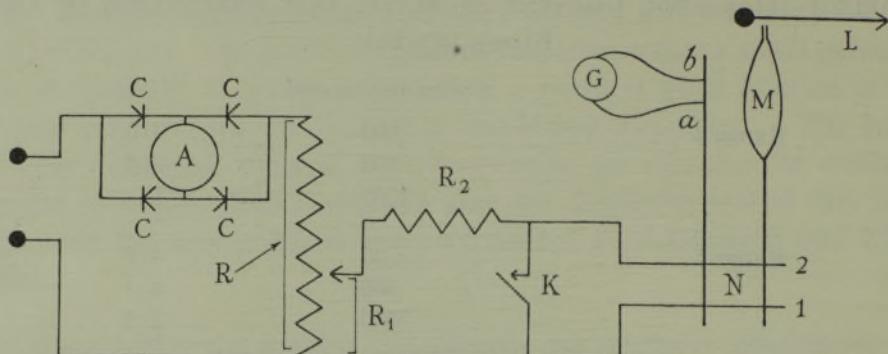


FIG. 4—Diagram of connexions for adjustment, and measurement of strength, of alternating current stimulus.  $R$ , reactionless resistance box, 110,000 ohms.  $R_1$ , adjustable part of  $R$ .  $R_2$ , calibrated "grid leak" resistance, about 1 megohm.  $C$ , Westinghouse 1 mA. metal rectifier, instrument type.  $A$ , microammeter connected to rectifier.  $G$ , sensitive galvanometer for recording monophasic action-current.  $K$ , short-circuit key.  $L$ , lever to muscle  $M$ .  $N$ , nerve on stimulating non-polarizable electrodes (1) and (2), (1) being in a well-injured region so that excitation occurred only at (2); connected either to muscle or galvanometer.  $a, b$ , non-polarizable electrodes, the nerve being well injured at  $b$  for monophasic response. Note.— $R_2$  was so large that opening  $K$  did not affect the current read on the microammeter.

The calibration number to turn "average" rectified current, measured on the microammeter, into root-mean-square is theoretically  $\pi/2\sqrt{2} = 1.11$ , (i) if the wave-form is perfect, (ii) if there is no frequency error due to capacity in the rectifier, and (iii) if there is no appreciable leak in the rectifier with the strength of current used. Actually several calibrations carried out at intervals during the course of the work gave extreme values of 1.11 and 1.14, with a mean of 1.13 which has been used throughout. The consistency of the calibrations is a tribute to the rectifier, and (if one were needed) to the purity of the wave-form. See Table II.

In the comparison of alternating current with other forms of stimulation it is the "crest" value  $I$  which is needed, for the actual current at time  $t$  has been written  $I \sin 2\pi nt$ . With a pure sine-wave current the

crest value is  $\sqrt{2}$  times the r.m.s. value, so that the factor to turn "average" rectified current into "crest" current was  $1.13 \times \sqrt{2} = 1.60$ .

The current  $i$  from the oscillator, read by the rectifier and microammeter, was usually about 100  $\mu\text{A}$ . Let  $R_1$  be the resistance, from the ends of which current is led off to the nerve. Let  $R_n$  be the resistance of the nerve with electrodes;  $R_2$  the resistance of the "grid-leak" in series with it. Then the current  $I$  through the nerve is given by

$$I = R_1 i / (R_1 + R_2 + R_n).$$

TABLE II—CALIBRATION OF WESTINGHOUSE RECTIFIER ("1 mA., INSTRUMENT TYPE") WITH TWO CAMBRIDGE VACUO-JUNCTIONS CONNECTED TO VERY SENSITIVE MOVING-COIL GALVANOMETER. THE FACTOR IS THAT REQUIRED TO TURN RECTIFIED CURRENT INTO ROOT-MEAN-SQUARE.

Cycles per second . . . . .	50	150	500	1500	4000	10000
Current, $\mu\text{A.}$ , (r.m.s.) . . . . .	89	93	95	92	78	44
Factor . . . . .	1.13	1.135	1.13	1.135	1.135	1.135

The resistance of the nerve and electrodes was measured at the end of an experiment. It was usually about 60,000 ohms, a comparatively small fraction of the resistance in series with it. The resistance (strictly, the impedance) of the nerve varies somewhat with frequency (*see below*), but this variation is altogether swamped by the high resistance  $R_2$  in series with it.

Since  $R_1$  could be as high as 110,000 ohms and  $R_2$  was about a megohm, and since  $i$  could be as great as 200  $\mu\text{A.}$  (r.m.s.), the maximum current available through the nerve was about 20  $\mu\text{A.}$  (r.m.s.). This is about 20 times the average threshold for a normal nerve at optimum frequency, 7 times that for a nerve treated with Ca: it allowed us usually to go to as high a frequency as was needed; when necessary the resistance  $R_2$  was reduced to 500,000 ohms.

The resistance  $R$  (Muirhead's "Universal" resistance boxes with non-reactive windings) should show no measurable effects of capacity or inductance up to the highest frequency we have used. According to the makers, indeed, the percentage error in impedance is completely negligible up to 50,000 cycles per second. The leads to the rectifier and to the nerve were kept well apart to avoid effects of capacity. The effects of inductance are negligible in circuits of such high resistance.

In the earlier experiments a Cambridge vacuo-junction connected to a sensitive galvanometer was used instead of the rectifier, the rest of the

connexions being analogous to those in fig. 4. This was accurate but not nearly so quick or convenient.

It would have been simpler, had it been possible, to avoid the potential division of fig. 4, to vary the current simply by varying a large series resistance, and to put the rectifier directly in the nerve circuit. This could not be done, since rectification is not complete with currents as low as the 1  $\mu$ A. required for the minimum threshold of isolated nerve.

*Leaks and Earth Connexions*—The precautions to be taken under this heading are among the most important of all for alternating current stimulation, but there is little mention of them in the literature of the subject. Where our results differ from those of others we are inclined to attribute the divergence, in some cases at least, to neglect of necessary precautions. A capacity of various parts of one's apparatus to earth can provide another path for an alternating current, particularly of high frequency, and when one is dealing with an indicator as sensitive as an isolated frog's nerve a fraction of a microampere may altogether spoil one's results.

The same effect might occur with very rapid condenser discharges; expressed mathematically, the Fourier analysis of a series of condenser discharges would involve very high harmonics, and these, if there were earth capacities to various parts of one's apparatus, might—by leaking across via the earth—cause considerable disturbances. The constant-quantity relation for very short times has probably been long obscured for such reasons; even the simplicity of the strength-duration (voltage-capacity) relation in frog's medullated nerve has probably escaped detection by the same cause.

It is not possible to avoid leaks of this kind by interposing even the best condensers in the two lines from one's oscillator; these can eliminate only constant current disturbances. The ordinary laboratory bench, although apparently dry, is often a serious cause of trouble; it may act as a large earthed plate of a condenser, of which the other plate is some part of one's apparatus. Trouble from this source can be avoided by heating the laboratory bench and tables (*e.g.*, by radiators placed beneath them) and then a suitable earth connexion to some part of the stimulating circuit, found by trial, usually eliminates any further disturbance. Large earthed objects near any part of the stimulating circuit are to be avoided, and any sign of leaks to the nerve with high frequency current should be explored and the experiment proper be postponed until the leaks have been "earthed". Batteries, not D.C. power mains, should be used for the oscillator, for the mains invariably cause trouble by leaks. These and other elementary precautions were taken in all the later experiments, and the consistency of the results is partly due to them.

*Nerve Impedance and Frequency*—In discussing the stimulating circuit shown in fig. 4 we referred to the well-known fact (see, e.g., Lullies (1930a)) that the impedance of nerve varies with frequency, and therefore that the series resistance ( $R_2$ ) must be high compared with that ( $R_n$ ) of the nerve. A few determinations were made of the variation of impedance with frequency, under the conditions of the present experiments. A substitution method was used. Current from the oscillator, measured by the rectifier connected to a quick mirror-galvanometer, was passed either through the nerve or through a reactionless resistance box, and the resistance was varied until the same reading was given by either. From the resistance so determined was subtracted that of the electrodes alone, when connected together either by a thick piece of filter paper wet with Ringer's fluid, or by direct contact at their tips; the electrode resistance was independent of frequency. In one typical experiment at 30° C. the following results were obtained, for 15 mm. and 1·5 mm. respectively of nerve.

$n$ .....	30	500	2000	10000
$R_n$ (15 mm.) .....	30250	28500	26700	24800
$R_n$ (1·5 mm.) .....	9000	8900	8200	7200

In another at 29° C., for 22 mm. and 2 mm. respectively:—

$n$ .....	30	100	500	1000	2000	5000	10000
$R_n$ (22 mm.) ....	67300	67000	66000	64700	63000	60800	59500
$R_n$ (2 mm.) ....	15000	14700	14300	14000	13300	12500	11800

In another at 27° C., for about 6 mm. length:—

$n$	20	50	100	200	500	1000	2000	5000	10000
$R_n$	42200	42000	41800	41900	41700	40900	39700	38200	37100

In eleven such determinations the impedance fell, on the average, about 18% between 30 and 10,000 cycles per second. With a nerve of 50,000 ohms and a total resistance of rather over a megohm the variation of nerve impedance with frequency, between the extreme limits of frequency used, is less than 1% of the total impedance, and so can be neglected. If, however, the resistance  $R_2$  were small, the variation with frequency would not be negligible, and serious error might result.

### III—THE COMPARISON OF THE OPTIMUM CURRENT WITH THE RHEOBASE, AND OF THE "TRUE" WITH THE "OBSERVED" RHEOBASE

According to the theory (Hill, 1936a) the threshold current  $I_{op}$  at the optimum frequency  $n_{op}$  is related to the "true rheobase"  $I_0$  by the

equation  $I_{op} = I_0 (1 + k/\lambda)$ . If, therefore,  $I_{op}$  and  $k/\lambda$  be measured,  $I_0$  can be calculated. Moreover, as shown above,  $I_0$  can be obtained by plotting  $I^2$  against either (i)  $1/n^2$  on the low side of the optimum, or (ii)  $n^2$  on the high side of the optimum, when  $I_0^2$  is the intercept of either of the straight lines so obtained on the vertical axis. It is interesting to compare the values of  $I_0$  determined by these methods with  $I_1$  the "observed rheobase".

In measuring the rheobase  $I_1$  it is not sufficient to find the threshold for a single response to a single constant current, since the threshold may be appreciably, sometimes considerably, lower for repetitive stimulation than for a single stimulus. The reason for this is not known, but the fact itself is certain as the following experiments show. A frog's muscle-nerve preparation was placed in the usual chamber at 18·5 to 19·5° C., with the nerve on non-polarizable electrodes. The rheobase for a constant current was determined in the ordinary way, the index being a single minimal twitch. A series of constant current pulses was then sent into the nerve, at a frequency of 40–22 per second, each pulse being of duration 2½–4½ msec., which was definitely greater than the *Hauptnutzzeit*, so that the minimum threshold was attained. With a rather large value of  $\lambda$ , the interval, viz., 22–41 msec., between the pulses might not be quite long enough for the accommodation of the nerve to be fully reversed; in some of the experiments, therefore, in order to avoid any accumulating accommodation to repetitive one-way pulses, or any possible polarization in the nerve itself, each pulse was followed, after an interval of 0·5–1·0 msec., by a reverse pulse of precisely the same strength and duration in the opposite direction. The cathode of the reverse pulse was in an injured region of the nerve so that its threshold was much higher; thus no excitation could result from the reverse pulse, which was used only to wipe out any possible after-effect of its predecessor and to leave the nerve more or less in its resting state. The cycle was therefore as follows:—

Pulse 2½–4½ msec.; interval 0·5–1·0 msec.

Reverse pulse 2½–4½ msec.; interval 20–35 msec.

Excitation at break did not occur at the end of the reverse pulse, as shown by the fact that a series of reverse pulses only had no effect except with a much stronger current.

The index taken in the case of the repetitive pulses was a minimal steady tetanus. The threshold strength  $I_1$  was measured and compared with that ( $I_1'$ ) for a single constant current.  $I_1$  was always less than  $I_1'$ , sometimes considerably less, as the results in Table III show. There is clearly

some accumulating, or "facilitating", effect of a series of subthreshold pulses, which finally become adequate at a lower threshold than a constant current. This effect has no explanation in the present theory of excitation, but it is evident that it must be taken into account when comparing the threshold for alternating current with that for constant current. Alternating current, as used, provides one stimulus to each cycle, and in any comparison with another form of current the latter should, if possible, be employed in a way which also permits repetitive stimulation.

The experiments in Table IV were performed in January, 1936, on the relation between the optimum threshold  $I_{op}$  by alternating current and the rheobase  $I_1$  by repetitive pulses.  $k$  and  $\lambda$  were separately determined by equations (5) and (6), and so the "true rheobase"  $I_0$  was calculated

TABLE III—COMPARISON OF RHEOBASE FOR CONSTANT CURRENT ( $I'_1$ ) WITH RHEOBASE FOR REPETITIVE PULSES ( $I_1$ )

	*	*	*	*		
Preparation number .....	1a	1b	2a	2b	3a	3b
$I_1/I'_1$ .....	0.95	0.94	0.92	0.92	0.96	0.95
			*			
Preparation number .....	4a	4b	5a	5b	6a	6b
$I_1/I'_1$ .....	0.92	0.93	0.76	0.87	0.92	0.84

1 megohm in series with nerve.

\* Two-way pulses to avoid accommodation or polarization—see text.

from  $I_0 = I_{op}/(1 + k/\lambda)$ . In all that follows each of the repetitive pulses was followed by a reverse pulse, to avoid polarization or accommodation, as described above.

The mean values, for the 16 experiments of Table IV, are:—

of $I_{op}/I_1$ .....	1.05
of $I_0/I_1$ .....	0.985

The experiments of Table V were performed in January and February, 1936, on the ratio  $I_0/I_1$ , where  $I_0$  is the "true rheobase" obtained by alternating current as described above (equation (5)) by extrapolation on the low side of the optimum frequency, and  $I_1$  is the rheobase observed by pulses.

The mean value of  $I_0/I_1$  for the 15 experiments of Table V is 1.02.

The experiments of Table VI were performed in January and February, 1936, on the ratio  $I_0/I_1$ ,  $I_0$  in this case being obtained (equation (6)) by extrapolation on the high side of the optimum frequency. The mean value of  $I_0/I_1$  for the 19 experiments of Table VI is 0.995.

TABLE IV—COMPARISON OF OPTIMUM STIMULUS WITH RHEOBASE

Temperature, °C.	31*	29*	16	29	28	28	27	27
$I_{op}$ , $\mu A$ , crest	4.13	2.98	1.87	2.47	1.78	1.86	2.18	1.81
$n_{op}$ , cycles per sec.	200	190	54	155	140	150	140	200
$I_1$ , pulses, $\mu A$	3.76	2.75	1.62	2.10	1.63	1.65	2.42	1.815
Pulse frequency, per sec.	80	80	78	80	57	57	60	61
$I_0/I_1$	1.10	1.08	1.15	1.18	1.09	1.13	0.90	1.00
$k/\lambda$	0.107	0.10	0.037	0.069	0.07	0.12	0.078	0.078
$I_{ob}$ , $\mu A$ , crest	3.73	2.71	1.80	2.31	1.67	1.66	2.02	1.68
$I_0/I_1$	0.99	0.99	1.11	1.10	1.02	1.01	0.84	0.925
Temperature, °C.	29	31*	24	21	23*	22	23*	Mean
$I_{op}$ , $\mu A$ , crest	2.35	6.2	1.34	1.55	7.14	1.36	5.05	—
$n_{op}$ , cycles per sec.	186	245	128	40	95	77	102	—
$I_1$ , pulses, $\mu A$	2.31	5.9	1.33	1.57	6.55	1.40	4.80	—
Pulse frequency, per sec.	60	60	44	42	43	43	43	—
$I_0/I_1$	1.02	1.05	1.01	0.99	1.09	0.97	1.05	1.05
$k/\lambda$	0.085	0.051	0.074	—	0.03	0.069	0.032	—
$I_{ob}$ , $\mu A$ , crest	2.17	5.90	1.25	1.53	6.93	1.27	4.90	—
$I_0/I_1$	0.94	1.00	0.94	0.97	1.06	0.91	1.02	0.985

\* Signifies a nerve treated with high-Ca concentration.

TABLE V—COMPARISON OF "TRUE" WITH "OBSERVED" RHEOBASE FROM LOW SIDE OF OPTIMUM

Temperature, °C.	34	24	24	23	23	24	24	25	24	25	24	25	26	27
$I_0/I_1$ .....	1.04	0.99	0.99	1.06	1.02	1.06	1.05	1.02	1.03	1.00	1.02	1.00	1.03	0.96

In most of these experiments the pulses were 43 per second and of duration 2.6 msec.

TABLE VI—COMPARISON OF "TRUE" WITH "OBSERVED" RHEOBASE FROM HIGH SIDE OF OPTIMUM

Temperature, °C. ....	30	34	34	32	32	32	32	23	23	24	24	24	23
$I_0/I_1$ .....	0.86	1.04	1.01	1.04	1.04	1.02	1.02	0.99	0.99	0.95	0.95	0.99	1.07
Temperature, °C. ....	22	23	24	24	24	24	24	25	25	25	25	26	26
$I_0/I_1$ .....	0.92	1.02	1.00	1.02	1.03	1.03	1.00	1.01	1.01	1.00	1.00	1.00	1.00

The net result of these 50 determinations by three different methods is that the mean value of  $I_0/I_1$  is exactly unity, 35 of them (70%) being between 0·95 and 1·05. The mean value of  $I_{op}/I_1$ , in 16 experiments, was 1·05. This is in complete disagreement with Coppée's conclusion (1934c, p. 14) that "the threshold crest potential at the optimum frequency is always less than the rheobase", and his statement that in most of his experiments the former was 65% to 85% of the latter. The divergence must be due in part to the fact that Coppée neglected to use repetitive pulses of constant current to determine the rheobase, so that his observed rheobase was too high (see Table III above) and his ratio too low.

In § IV it is shown that the "true rheobase"  $I_0$  deduced from alternating current measurements is approximately equal to the rheobase extrapolated from the E-RC relation with repetitive condenser discharges, being probably slightly less. The results were rather more variable than those just referred to, but they add general confirmation to them.

The fact that the mean value of  $I_0/I_1$  is unity was not expected. According to the theory (Hill, 1936a, pp. 321, 322, 325)  $I_0/I_1$  is less than unity in the ratio  $1 : (\lambda/k)^{\frac{1}{\lambda/k-1}}$ ; for a usual value of  $\lambda/k$  of (say) 25,  $I_0/I_1$  should be 0·88, for  $\lambda/k = 100$   $I_0/I_1$  should be 0·95, for  $\lambda/k = 10$   $I_0/I_1$  should be 0·78. This quantitative divergence from the theory is probably related to another observed fact, viz., that the "*Hauptnutzzeit*" is nearly always rather greater than predicted (Hill, 1936a, p. 322). For  $\lambda/k = 40$ , the calculated utilization time of a threshold constant current is  $3 \cdot 79k$ , which for  $k = 0 \cdot 35$  msec. is 1·33 msec. A more usual experimental value is about 2 msec. Dr. H. Rosenberg informs us that in experiments which he made in April, 1929, on frogs' nerves at about 18° C., the make-response times ranged from 1·31 to 1·98 msec., for currents which gave just noticeable action-potentials. The low value occurred only once, and it is probable that the current was slightly above the threshold and that the longer durations are more correct. Thus the theory gives rather too high an "observed" rheobase  $I_1$  and rather too short a "*Hauptnutzzeit*".

It was emphasized (Hill, 1936a, pp. 319, 346) that the theory takes no account of electrotonic changes of excitability (Pflügers law) and that in consequence exact quantitative agreement with experiment may not always be found. The two examples just mentioned are probably of this kind. The process of accommodation tends to make the threshold higher, the electrotonic increase of excitability at the cathode tends to make it lower, and apparently for currents of rheobasic strength *on the average* the two effects just balance one another at the utilization time. Fig. 5 describes diagrammatically the supposed interaction of accommodation and the electrotonic rise of excitability. The full line

shows U and V (threshold and local potential) according to the original theory (cf. Hill, 1936a, fig. 3). The broken lines illustrate how a fall of threshold due to electrotonus, and setting in rapidly, followed by the usual rise due to accommodation, may (i) lower the observed rheobase until it is approximately equal to the true rheobase, and (ii) increase the utilization time of a current of rheobasic strength.

There would be no value at present in a mathematical discussion of the effect of electrotonus in this connexion, since not enough is known

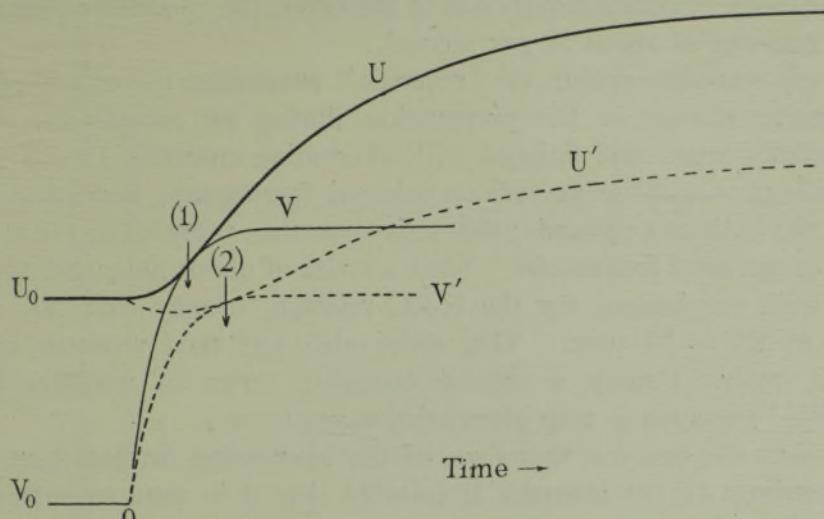


FIG. 5—Diagram to illustrate the effect of electrotonic increase of excitability on “utilization time” of threshold constant current. Changes of local potential (V) and of threshold (U) as functions of time on making the current at O. Full lines show “normal” accommodation, in which the final value of ( $U - V$ ) is the same as the initial value ( $U_0 - V_0$ ); arrow (1) at moment of excitation. Broken lines show the usual effect of electrotonus, in which the final value of ( $U' - V'$ ) is less than the initial value ( $U_0 - V_0$ ); arrow (2) at moment of excitation. The initial downward trend of  $U'$  is supposed to be due to electrotonus setting in early and lowering the threshold quicker at first than accommodation raises it. The effect of this is to increase the “*Hauptnutzzeit*”.

about it. It is clear, however, that it works in the required direction and is of the right order of size to explain the observed effects.

#### IV—THE COMPARISON OF $k$ CALCULATED FROM THE INTENSITY-FREQUENCY RELATION WITH ALTERNATING CURRENT, WITH $k$ CALCULATED FROM THE STRENGTH-DURATION (VOLTAGE-CAPACITY) CURVE

A crucial test of the theory from which equations (1) and (6) were deduced was to compare the value of  $k$  calculated by equation (6) from

experimental data with alternating current, with that deduced from an observed strength-duration (voltage-capacity) relation. In the former,  $I^2$  was plotted against  $n^2$  on the high side of the optimum frequency, and  $k$  and  $I_0$  were calculated from the linear relation obtained. (The procedure is illustrated in fig. 3b and fig. 9b.) In the latter,  $\log_{10} E$  was plotted against  $\log_{10} RC$  and the best fit of the theoretical relation obtained by the procedure described elsewhere (Hill, 1936b, p. 441). This gave  $k$  and  $E_0$ . In both cases a minimal steady muscular contraction was used as index of constant response of the nerve, the condenser discharges being one-way at about 50 per second.

A very complete system of "reverses" eliminated the effects of any progressive change in the preparation during an experiment. Most experiments began and finished with alternating current. First a series of readings was made at various selected frequencies, increasing from about the optimum upwards; this series was then repeated in the reverse order at the same frequencies. Next a series of about nine readings was made with condensers, for the  $E-RC$  relation, usually from  $RC = 10$  msec. to  $RC = 20 \mu\text{sec}$ . This series also was then repeated in the reverse order. Finally a second complete series of readings (with "reverse") was made with alternating current.

At every frequency  $n$ , therefore, of the alternating current there were four readings of the intensity  $I$ , suitably spaced in time to ensure the elimination of any effect of progressive change in the preparation. The mean value of  $I$  at each  $n$  was used in the calculation. Similarly for each  $RC$  with the condenser discharges there were two values of  $E$ , of which also the mean was taken. The consistent results obtained were doubtless due, in part, to this systematic elimination of the effect of any gradual alteration in the preparation during an experiment.

No attempt was made to determine  $k$  by a single pair of readings, as in "chronaxie" measurements; a whole curve was always recorded.

The main series of experiments was made during February and March, 1936, at various temperatures from  $16.5^\circ$  to  $27^\circ$  C. In every experiment, at least a reasonably good linear relation between  $I^2$  and  $n^2$  was obtained with alternating current, permitting no doubt of the value of  $k$ : similarly the  $E-RC$  results fitted the theoretical curve with the same order of accuracy as was described elsewhere (Hill, 1936b).

Table VII gives a summary of these results. The mean of 18 values of  $k$  from alternating current is  $0.44$  msec.: that of the corresponding values of  $k$  by condenser discharges is  $0.48$  msec. In some experiments the difference is well beyond the limits of experimental error; we suggest that in these, for some unknown reason, the alternating current and the

TABLE VII—COMPARISON OF  $k$  (AND  $I_0$ ) BY ALTERNATING CURRENT AND BY CONDENSER DISCHARGES

Temperature °C.	$k$ (A.C.) msec.	$k$ (condensers) msec.	$I_0$ (A.C.) μA.	$I_0$ (condensers) μA.	$I_0/I'_0$	Remarks
16.5	0.46	0.52	1.21	1.28	0.94	—
18	0.51	0.56	1.30	1.31	0.99	—
20	0.47	0.48	1.41	1.64	0.86	—
19	0.49	0.53	1.37	1.44	0.95	—
19	0.42	0.33	2.48	2.82	0.88	$8 \times Ca$
19	0.28	0.38	2.53	2.33	1.08	$8 \times Ca$
20	0.67	0.69	0.82	0.93	0.88	—
20	0.57	0.62	1.11	1.04	1.07	—
22	0.31	0.45	3.77	3.26	1.16	$8 \times Ca$
21	0.46	0.50	2.73	3.12	0.87	$8 \times Ca$
21	0.57	0.48	—	—	—	$8 \times Ca$
23	0.13	0.16	2.93	3.86	0.76	$8 \times Ca$
21	0.58	0.51	1.35	1.83	0.74	$8 \times Ca$
22	0.52	0.71	0.89	0.85	1.05	—
22.5	0.32	0.32	3.54	3.26	1.09	$8 \times Ca$
23	0.55	0.52	0.99	1.42	0.70	—
25	0.35	0.50	1.64	1.79	0.92	—
27	0.275	0.385	1.80	1.72	1.05	—
Mean	21°C.	0.44	0.48	0.94	—	—

condenser discharges were exciting different fibres in the nerve. On the average, however, the agreement is so close as to leave no reasonable doubt that the same quantity is being measured by both methods. The ratio of  $I_0$  (crest) deduced from the alternating current readings to  $I_0'$  deduced from condenser readings has a mean value of 0·94, but the individual readings are rather variable and the standard deviation of the mean is  $\pm 0\cdot03$ . The approximate equality of  $I_0$  and  $I_0'$  was referred to in § III above.

The series of experiments in Table VII was made under conditions carefully standardized as the result of long experience; a number, however, of earlier experiments had given the same result, and since these were made under a variety of conditions their evidence adds weight to the conclusion from the others. For example, in December–January, 1934–35, using the Cambridge oscillator and the electric response of the nerve as index, the results given in Table VIII were obtained.

TABLE VIII

Temperature ° C. ....	Room	20	21	19	6
$k$ (A.C.) msec.....	0·30	0·58	0·38	0·51	0·93
$k$ (condensers) msec. ....	0·35	0·54	0·32	0·59	0·89

Later, in May and June, 1935, with the Muirhead oscillator and the electric response of the nerve as index, the results given in Table IX were obtained.

TABLE IX

Temperature ° C. ....	6	19	6
$k$ (A.C.) msec.....	0·80	0·40	0·72
$k$ (condensers) msec. ....	0·92	0·38	0·62

The mean ratio of  $k$  (by A.C.) to  $k$  (by condensers) in these eight experiments is 1·01, which confirms the conclusion from the experiments in Table VII that there is, on the average, no significant difference between the  $k$ 's measured by the two methods.

According to Coppée (1934*b*; 1934*c*, p. 20) the chronaxie is equal to one-third of the period of the "isopotential frequency", the latter being defined as the frequency (above the optimum) for which the threshold (root-mean-square) is equal to the constant current rheobase. The crest value of such a current is  $\sqrt{2}$  times the rheobase. We have found above (§ III) that, for repetitive constant current pulses, the observed rheobase  $I_1$  is equal to the true rheobase  $I_0$ . Hence if  $I$  is the "isopotential current", from equation (6) (the frequency being well above the optimum),

$$I^2/I_1^2 = I^2/I_0^2 = 2 = 1 + 4\pi^2k^2n^2.$$

Therefore  $4\pi^2k^2n^2 = 1$  and  $k = 1/2\pi n$ . Putting the chronaxie =  $0.693k$  (Hill, 1936b, p. 442), we obtain

$$\text{chronaxie} = 0.110/n.$$

According to Coppée it is  $0.33/n$ . Coppée, however, did not use repetitive current pulses, but a single current, and we have found above (§ III) that this may have a considerable effect. If his observed rheobase was too high, say twice as high as it would have been for repetitive pulses, then his "isopotential current" also was twice as high and  $I^2/I_0^2$  instead of being 2 was really 8. This would give  $4\pi^2k^2n^2 = 7$ , from which  $0.693k = 0.29/n$ . The last figure agrees approximately with the number he gives, viz.,  $0.33/n$ . It is difficult, however, to believe that his rheobase can have been so much too great; some additional cause is required to explain the divergence. Possibly the wave form of his oscillator was not good enough. (Cf. his fig. 5, p. 245, 1934a.)

There is no particular significance in it, but it is interesting to note that the stimulus of minimum energy (per impulse started) is the same as Coppée's "isopotential current". The energy per stimulus (neglecting alterations of impedance with frequency) is proportional to  $I^2/n$ , i.e. (from equation (6)), to  $(1/n + 4\pi^2k^2n)$ . This is a minimum when  $n = 1/2\pi k$  or  $k = 1/2\pi n$  as we found above. Thus  $k = (\text{period of stimulating current of minimum energy})/2\pi$ . This may be compared with the cases of stimulation (1) by condenser discharges and (2) by constant current pulses: for (1),  $k = RC$  for minimum energy: for (2),  $k = (\text{pulse duration for minimum energy})/1.26$  (Hill, 1936b, p. 443).

## V—THE EFFECT OF INTER-ELECTRODE DISTANCE ON THE I-n RELATION

*The Effect on k and Rheobase*—We have just shown that  $k$  is the same when determined (a) by condenser discharges, and (b) by alternating current. This might conceivably be due to chance. Temperature has a similar effect on  $k$  obtained by either method, but the effect of temperature is not specific. A more specific means of changing  $k$  is to alter the inter-electrode distance. It was found by Cardot and Laugier (1914) and Cardot (1914), and confirmed by others (see Hill, 1936b, p. 448) that reduction of interpolar length increases the rheobase and decreases the value of  $k$ . Experiments, therefore, were made to find out whether diminished interpolar length has the usual effects on rheobase and  $k$ , when these are obtained by alternating current stimulation.

For this purpose, the I-n relation was determined for two interpolar distances (about 2 and 25 mm. respectively), using either the electric response of the nerve (a constant small galvanometer deflexion, or

TABLE X

(a) EFFECT OF INTERELECTRODE DISTANCE ON  $k$  OF NERVE DETERMINED BY ALTERNATING CURRENT

Temperature, °C.	24	24	20	23	22	23	26	23	20	20	20	20	6	21	23	Mean
Electrode separation, mm.—																
Long	30	27	28	28	27	25	25	25	20	23	22	26	19.5	19.5	25	
Short	1.9	1.9	1.9	2.0	2.0	2.0	2.0	1.5	3.5	3.0	1.7	2.0	1.5	1.5	2	
Time factor $k$ , msec.—																
Long	0.32	0.40	0.89	0.40	0.84	0.41	0.39	0.45	0.42	0.40	0.32	0.82	0.42	0.33	0.49	
Short	0.26	0.22	0.28	0.24	0.32	0.22	0.23	0.23	0.26	0.23	0.23	0.59	0.26	0.16	0.27	

The first eight experiments were made with muscle response as index; the remainder with nerve action current.

(b) EFFECT OF INTERELECTRODE DISTANCE ON  $k$  AND  $\lambda$  DETERMINED BY ALTERNATING CURRENT

Temperature, °C.	21	23.5	24.5	27.5	28.5	26	28.5	26.5	28	28.5	28	28.5	26	26	26.5	Mean
Distance, mm.																
Long	19.5	19.5	22	22	22	15	15	22	22	22	22	22	25*	25*	25*	22
Short	1.5	1.5	2	2	2	2	1.5	1.8	2	2	2	2	2	2	2	1.9
$k$ , msec.																
Long	0.42	0.33	0.64	0.34	0.27	0.37	0.28	0.52	0.32	0.39	0.41	0.37	0.37	0.37	0.39	
Short	0.26	0.16	0.22	0.17	0.125	0.25	0.15	0.21	0.175	0.17	0.28	0.28	0.25	0.25	0.25	0.21
$\lambda$ , msec.																
Long	6.3	6.6	3.8	4.3	3.9	4.3	3.5	3.0	3.3	2.4	4.1	2.9	4.0	4.0	4.0	
Short	6.6	5.3	4.6	4.3	3.7	4.1	2.9	3.4	3.0	3.3	4.6	3.3	4.1	4.1	4.1	

\* 8 mm. of far end crushed.

occasionally a constant fraction of maximal response determined at each frequency) or a small tetanic muscle response, as index of nerve excitation. Three stimulating electrodes were used, the "fixed" point being placed nearest to the muscle or to the lead-off electrodes. In these experiments it was impracticable to injure the nerve between the stimulating leads: impulses, therefore, originating from the two "variable" stimulating points could not be avoided.

Typical results are shown in fig. 6 and Table XI, and a summary in Table Xa and b. The threshold intensity at low frequencies was always higher for short interpolar length; the threshold ratio between small and great distances diminished with increasing frequency. The time factor  $k$ , determined, as described in § IV, from the  $I^2 \cdot n^2$  relation in a low frequency range on the high side of the optimum, was always smaller for the short interpolar length. Using 2 mm. and 25 mm. distances respectively, the ratio  $k(\text{long})/k(\text{short})$  averaged 1.85 in the experiments

TABLE XI

EXPERIMENT OF 4 APRIL, 1936, AT 23° C., 19.5 MM., AND 1.5 MM.  
INTERELECTRODE DISTANCES. SMALL ELECTRIC RESPONSE OF NERVE  
AS INDEX.

Frequency, per second .....	30	50	70	100	150	200	300	500
<hr/>								
Threshold intensity, $\mu\text{A.}$ (r.m.s.)								
Long .....	2.9	2.6	2.4	2.4	2.4	2.5	2.8	3.5
Short .....	11.6	10.0	9.3	8.8	8.6	8.6	8.7	9.5
<hr/>								
Frequency, per second .....	700	1000	1500	2000	3000	4000	5000	6000
<hr/>								
Threshold intensity, $\mu\text{A.}$ (r.m.s.)								
Long .....	4.1	5.2	6.8	8.6	11.9	15.5	19.5	22.9
Short .....	10.2	11.7	13.1	14.9	18.5	23.4	28.3	33.0

EXPERIMENT OF 30 APRIL, 1935, AT 20° C., 20 MM., AND 3.5 MM. INTER-ELECTRODE DISTANCES. SMALL ELECTRIC RESPONSE OF NERVE AS INDEX

Frequency, per second .....	100	300	500	800	1000	1300
<hr/>						
Threshold intensity, $\mu\text{A.}$ (r.m.s.)						
Long .....	1.22	1.58	1.97	2.67	3.19	3.89
Short .....	2.38	2.66	3.05	3.69	3.99	4.59
<hr/>						
Frequency, per second .....	1600	2000	2500	3000	4000	5000
<hr/>						
Threshold intensity, $\mu\text{A.}$ (r.m.s.)						
Long .....	4.73	5.79	7.31	8.89	12.2	16.4
Short .....	5.21	5.84	6.82	7.92	10.0	12.7

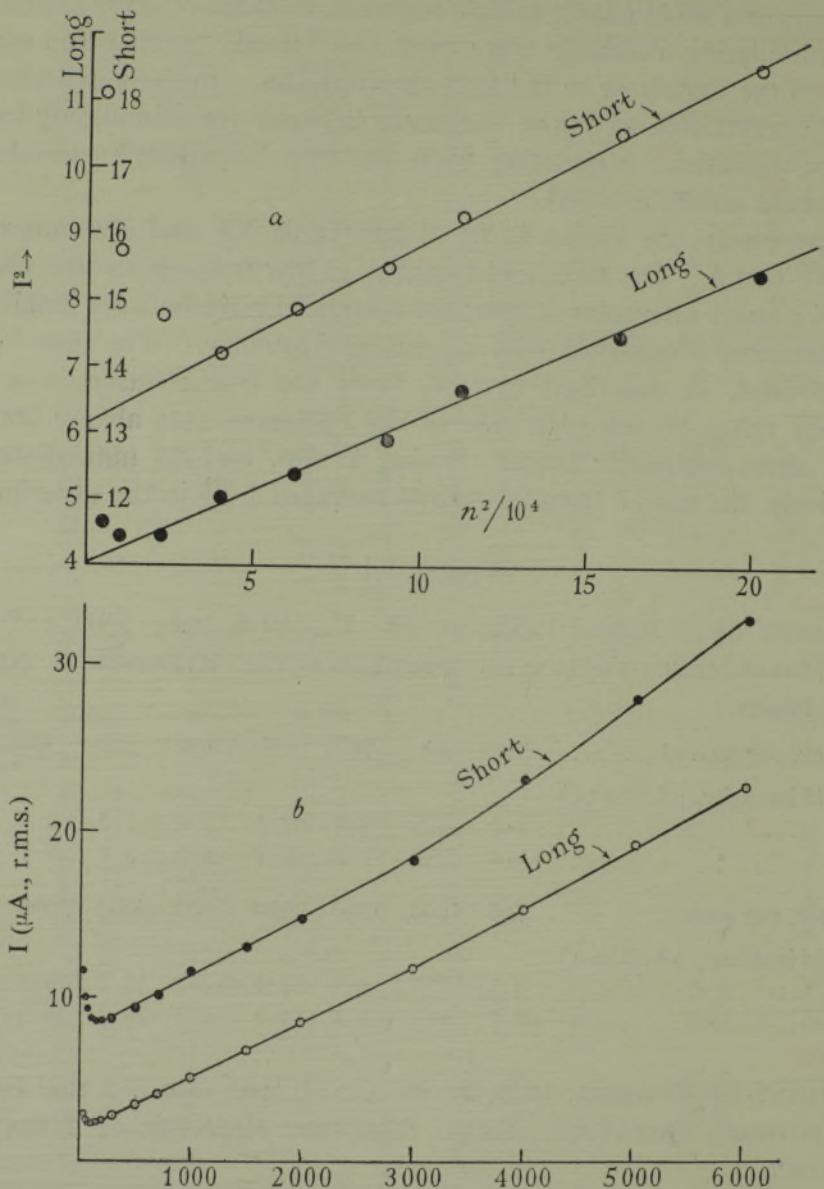


FIG. 6.—Effect of interpolar length, in alternating current stimulation, on (a) the  $I^2 - n^2$  relation at lower frequencies, (b) the  $I - n$  relation at higher frequencies. (a) 21 March, 1936:  $26^\circ$  C.; interelectrode distances, 25 mm. and 2 mm.; small muscle tetanus as index.  $k$  (long) = 0.39 msec.:  $k$  (short) = 0.23 msec. (b) 4 April, 1936:  $23.5^\circ$  C.; interelectrode distances, 19.5 mm. and 1.5 mm.; small constant nerve response as index.

of Table Xa; with 2 mm. and 20 mm. (means) it averaged 2·0 in those of Table Xb. Bouckaert found (Hill, 1936b, p. 449) that  $k$  determined by condenser discharges was about twice as great for 15–20 mm. as for about 1 mm. interpolar distance.

The curves obtained at small inter-electrode distance were apt to show certain deviations from the theoretical relation, as is the case also with condenser discharges. Using a threshold muscle response as index, a "double" curve was often found when fitting the theoretical log E-log RC curve (Hill, 1936b) to the observed values. The  $I^2-n^2$  relation sometimes showed distinct curvature (the slope diminishing with increasing frequency) even at frequencies just beyond the optimum.

Occasionally the  $I-n$  curves for small and great interelectrode distance crossed at some intermediate frequency (see Table XI). In these cases, the ratio of threshold intensities for short and long interpolar length was less than 2 at the optimum frequency (usually being greater than 3) while at high frequencies  $I$  (short)/ $I$  (long) became less than unity. When applying one-way pulses or one-way condenser discharges with a fixed stimulating cathode for all distances used, the corresponding phenomenon was never observed. With alternating current, however, one cannot avoid the possibility of exciting at both electrodes (since, for the present purpose, the variable electrode region could not be injured), and the variable electrode region may have different excitabilities for "short" and "long". If, by chance, the "variable" stimulating point for the small distance had a higher excitability than the two other points, the crossing of the  $I-n$  relation shown in Table XI is easily understood.

We conclude that the effects of interelectrode distance on rheobase and  $k$ , when these are determined by alternating current, are the same as when they are derived from condenser discharges: which adds to the conviction that the quantities obtained by the two methods are identical in nature.

*The Effect on  $\lambda$* —In § VI below the value of  $\lambda$  determined by alternating current on the low side of the optimum frequency is shown to be the same as that derived from exponentially increasing current. The effect of electrode separation on the value of  $\lambda$  determined by exponentially increasing current has been examined by Schriever (1932) and by Solandt (1936a). Schriever measured what he called the "*Einschleichzeit*", which (Hill, 1936a) should bear a constant ratio to  $\lambda$ . In five experiments at 30 mm. and at 3 mm. respectively, the mean ratio of his times was 1·25 (1·12 to 1·35); the mean ratio of the "chronaxies" was 1·62 (1·45 to 1·73). Solandt in eight experiments with long (30 mm.) and with fairly short (4–6 mm.) distances found no regular difference in  $\lambda$ . In six

experiments with 30 mm. and 1-2 mm. the mean ratio  $\lambda$  (long)/ $\lambda$  (short) was 1.3 (1.25 to 1.41). According to Bouckaert the ratio of the  $k$ 's should be about 2. The effect of interpolar length on  $\lambda$  is clearly far less than on  $k$ .

It was desirable to verify this conclusion with alternating current, and 12 experiments were made (Table Xb) in which both  $\lambda$  and  $k$  were determined from observed data using equations (5) and (6), (a) for an electrode separation of about 20 mm., and (b) for one of about 2 mm. The method was to employ three stimulating electrodes (two pairs) as described above, and to find the threshold I for a number of frequencies  $n$ , from 30 to about 1000 cycles per second. At each  $n$ , I was determined for short and for long interpolar distance, and when the series had been completed with increasing frequencies a reverse series was made, and the mean value of I at each  $n$  taken for the calculation. A minimal electric response was used as index of constant excitation.

As usual  $I^2$  was plotted against  $1/n^2$  and  $n^2$  respectively, on the low and the high sides of the optimum frequency, and so  $\lambda$  and  $k$  determined. With the short interpolar distances, sometimes deviations from the linear relations occurred at the very low (30/sec.) and at the higher ( $> 500/\text{sec.}$ ) frequencies, in the sense that at these the observed points lay *below* the lines. These anomalous points were disregarded and the values of  $\lambda$  and  $k$  calculated from the good linear relations found over the rest of the range. If attention had been paid to them its effect would have been to make  $k$  still smaller, and  $\lambda$  larger, for short interpolar length, and so to emphasize the chief conclusion we shall draw, viz., that  $\lambda/k$  is increased by a reduction of interpolar length.

The results are given in Table Xb. At an average temperature of 26° C., the values of  $k$  for 20 and 2 mm. separation were respectively 0.39 and 0.21 msec., while the values of  $\lambda$  were 4.0 and 4.1 msec. Thus  $\lambda$  determined by alternating current is unaffected by interelectrode distance within the range 2-20 mm., while  $k$  is doubled. In the experiments of Table Xb the mean value of  $\lambda/k$  for 20 mm. distance is 10, for 2 mm. distance 20. This will be referred to in § VIII below.

The experiments of Table Xb were very consistent and left no doubt of the result. Experience has convinced us that of all methods of determining the time constant  $\lambda$  on rapidly accommodating nerve, the best is that based on equation (5) employing alternating current below the optimum frequency. This may be due partly to the fact, illustrated in fig. 1, that threshold excitation occurs with alternating current when the threshold is normal, *i.e.*, in the just unaccommodated nerve. Whatever the reason, we are inclined to lay much more weight on the present results

than on those of Solandt and of Schriever, and to attribute such effect of diminished interpolar length as they found with exponentially increasing currents, to some consistent complicating factor. The conclusion we draw is that, while  $k$  is considerably affected by diminished interpolar length,  $\lambda$  is almost, or altogether uninfluenced.

The effect of electrode separation in changing  $k$  without changing  $\lambda$  can be illustrated in another way, as in fig. 6. This represents the fourth experiment of Table Xb. The observed value of  $I$ , divided by an interpolated value of  $I_{op}$ , is plotted against  $\log n$ , for 22 and for 2 mm. separation respectively. For the 2 mm. the optimum frequency is 186 cycles

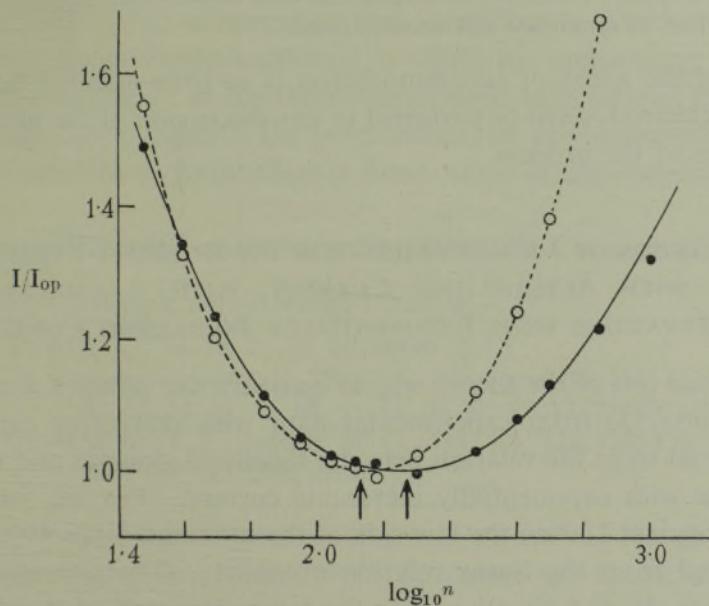


FIG. 7—Relation between threshold alternating current and frequency for short (full circles, full line) and for long (hollow circles, broken line) interelectrode distance, 2 mm. and 22 mm. respectively, 27.5° C. The threshold at the optimum frequency is taken as unity for plotting in each case; actually it was for 22 mm. 1.91  $\mu$ A, for 2 mm. 6.97  $\mu$ A. The arrows represent the optimum frequencies, 186 and 135 respectively.

per second; from this  $k\lambda = 0.73 \times 10^{-6}$ , agreeing with the product of  $k$  and  $\lambda$  of Table Xb. For the 20 mm.,  $n_{op} = 135$ ; hence  $k\lambda = 1.39 \times 10^{-6}$ , agreeing approximately with the product of  $k$  and  $\lambda$  from the table.

The difference of optimum frequency is immediately evident from fig. 7. So also is the difference of shape, which (fig. 2) depends on the value of  $k/\lambda$ . Comparison of fig. 7 and fig. 2 shows at once that  $k/\lambda$  is considerably greater for 22 mm. than for 2 mm. separation. A similar figure could be

drawn for each of the experiments of Table Xb. The increase of optimum frequency with diminished interpolar length is a sign that  $k\lambda$  is less: the broadening of the curve, a sign that  $k/\lambda$  is less. This is only another expression of the fact that  $k$  is diminished while  $\lambda$  is little, if at all, affected.

According to Coppée (1935c, p. 32), the optimum frequency was not modified appreciably by bringing the electrodes nearer (0·1 mm. must surely mean 1·0 mm.); but the "isopotential frequency" and the "flatness" of the I-n curve were increased. The former would mean that  $k\lambda$  was unaltered; the latter that  $k/\lambda$  was diminished. This could be true only if  $k$  became less and  $\lambda$  proportionally greater with reduced electrode separation; the latter is contrary to our experience. Some disturbance probably affected Coppée's result, for he admits that with shorter inter-polar distance his I-log  $n$  curve lost its symmetry and became tilted.

The fact that the speed of accommodation is so little affected by distance between electrodes will be pertinent in any discussion of the physico-chemical nature of the process.

## VI—THE COMPARISON OF $\lambda$ CALCULATED FROM THE INTENSITY-FREQUENCY RELATION WITH ALTERNATING CURRENT, WITH $\lambda$ CALCULATED FROM OBSERVATIONS WITH EXPONENTIALLY INCREASING CURRENTS

Another crucial test of the theory was to compare the value of  $\lambda$  calculated by equation (5) from experimental data with alternating current, with that deduced from the relation between threshold strength and time-constant of rise with exponentially increasing current. For the former,  $I^2$  was plotted against  $1/n^2$  on the low side of the optimum frequency, and  $\lambda$  was calculated from the linear relation obtained. (The procedure is illustrated in figs. 3b and fig. 9b.) For the latter, the method described by Solandt (1936a) was employed. The results have been given already by Solandt (1936b, Table IV). In order to obtain a high optimum frequency, and so to make the determination of  $\lambda$  by alternating current more accurate, the preparation was used at a rather high temperature (average 25·6° C.) and had previously been soaked for 1 to 5 hours in a Ringer's solution containing eight times the normal concentration of calcium. The value of  $\lambda$ , therefore, was small. In the experiments with alternating current a minimal steady tetanus of the muscle was taken as index of constant response in the nerve: in those with the exponentially rising currents, a minimal twitch was used. In 12 experiments the mean value of  $\lambda$  with alternating current was 4·6 msec., with exponential currents, 4·9 msec. The agreement is so good as to leave no reasonable doubt that the same quantity is measured by both methods.

## VII—THE OPTIMUM FREQUENCY

In a large number of experiments extending over a year the optimum frequency for alternating current stimulation was determined in frogs' nerves under a variety of conditions. In most of these experiments the Muirhead oscillator was used, but in some, particularly those with a low optimum frequency, the photoelectric arrangement described in § II. In some experiments muscle response, in others nerve response, was used as index of constant excitation. In every case the optimum frequency was determined by plotting  $I$  against  $\log n$  (see figs. 2 and 3 above).

The results are given in Table XII. They are divided into groups according to the temperature and according to whether the Ca-concentration of the Ringer's solution in which the preparation was soaked was normal or high. If the three mean results with normal Ringer's fluid are plotted,  $\log n_{op}$  against the temperature, an approximately straight line is obtained (fig. 8) from which a mean value of the optimum frequency for

TABLE XII—OPTIMUM FREQUENCIES, CYCLES PER SECOND

## I—After soaking in normal Ringer's solution—

- (a) At 6° C.: 10, 19, 21, 24, 30; mean 21.
- (b) At room temperature, 16–22° C., average about 18½° C.: 40, 40, 42, 45, 48, 50, 54, 54, 54, 59, 60, 62, 63, 63, 69, 75, 77, 77, 80, 81, 81, 85; mean 62.
- (c) At 24 to 31° C., average 28° C.: 115, 123, 123, 128, 136, 140, 140, 144, 155, 170, 186, 195, 206; mean 151.

## II—After soaking in Ringer's solution containing high Ca (4 to 10 times normal)—

- (b) At room temperature, 17–23° C., average 19½° C.: 76, 93, 95, 95, 102, 103; mean 94.
- (c) At 30–34° C., average 31½° C.: 178, 191, 210, 224, 245, 245, 270; mean 223.

the particular frogs and Ringer's solution used can be read off at any temperature. At 10°, 20°, and 30° C., respectively these interpolated values are 29, 71, and 186 cycles per second. An approximate  $Q_{10}$  is 2.5.

The effect of temperature on the optimum frequency was shown by v. Kries (1884) who found that in "warm" nerve it might be more than 200, in "cold" nerve less than 50, by Achelis (1930) and by Coppée (1934c, p. 40). According to the latter the  $I$ - $\log n$  curve is simply translated to the right, *i.e.*, without change of shape, by a rise of temperature. This would mean that  $\lambda/k$  was unaffected by temperature. We have no direct evidence on this point in a single preparation at different temperatures, but in our experience the lower values of  $\lambda/k$  were far more frequent at the higher temperatures.

The two mean results with high calcium are shown in the same figure. At room temperature the effect of calcium in raising the optimum frequency is considerable. Two special experiments were made to verify this. In one, a preparation at 21° C., after soaking 2 hours in normal Ringer's fluid, gave  $n_{op} = 40$ ; after a further  $5\frac{1}{2}$  hours in 10 times normal Ca it gave  $n_{op} = 95$  at 23° C. In the other at 22° C., after soaking  $4\frac{2}{3}$  hours in normal Ringer's fluid,  $n_{op}$  was 77; after a further 4 hours in 10 times normal Ca it was 102 at 23° C. At about 30° C., however, the effect of high calcium appears to be negligible. According to Solandt (1936a), a high Ca concentration considerably diminishes  $\lambda$ , and since

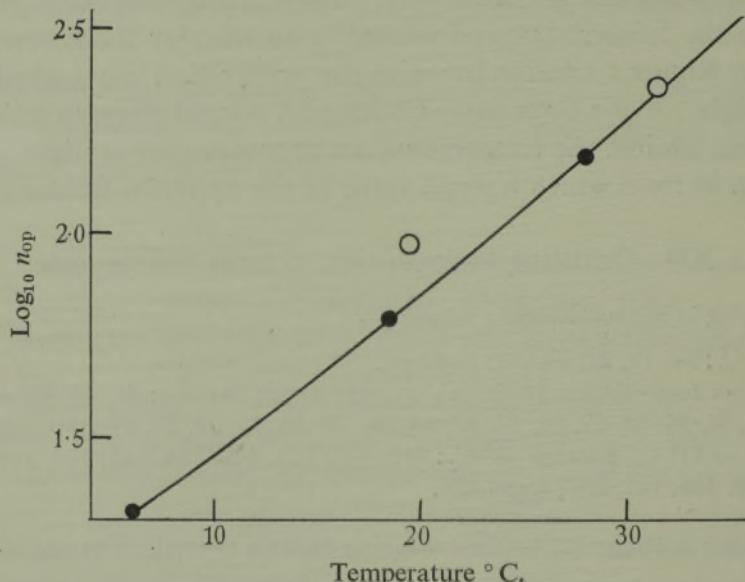


FIG. 8—Effect of temperature on the optimum frequency in alternating current stimulation. Mean results of Table XII. ● normal Ringer's solution; ○ nerve treated with high Ca.

(equation (2))  $n_{op}$  is inversely proportional to  $\sqrt{\lambda}$ ,  $n_{op}$  is increased. At a high temperature, however,  $\lambda$  is already so small that a high Ca apparently does not diminish it further.

A value of  $n_{op} = 62$  for room temperature gives, from equation (2),  $k\lambda = 6.6 \times 10^{-6}$ . A mean value of  $k$  for this temperature is about 0.4 msec., so that the corresponding value of  $\lambda$  should be about 16 msec. This is well within the normal range found by Solandt (1936a) at room temperature with exponential currents. The value of  $n_{op} = 94$  for room temperature with high Ca-nerves corresponds to  $k\lambda = 2.9 \times 10^{-6}$ , which with  $k = 0.4$  msec. requires  $\lambda = 7.2$  msec. This is in the range of small values of  $\lambda$  found by Solandt for nerves with high Ca. A value of

$n_{op} = 151$  at  $28^\circ\text{ C.}$  in normal nerve corresponds to  $k\lambda = 1.11 \times 10^{-6}$  which with  $k = 0.25\text{ msec.}$  requires  $\lambda = 4.4\text{ msec.}$  A value of  $n_{op} = 223$  at  $31\frac{1}{2}^\circ\text{ C.}$  in high Ca nerves corresponds to  $k\lambda = 0.51 \times 10^{-6}$ , which with  $k = 0.2\text{ msec.}$  requires  $k = 2.5\text{ msec.}$  At the other extreme, a value of  $n_{op} = 21$  at  $6^\circ\text{ C.}$  corresponds to  $k\lambda = 57 \times 10^{-6}$ , which with  $k = 1.0\text{ msec.}$  requires  $\lambda = 57\text{ msec.}$  These are all values which experience has led one to expect as usual.

The behaviour, therefore, of the optimum frequency, with respect to changes of temperature and of calcium concentration, is what we should predict from the known effects of these on  $k$  and  $\lambda$ , and its absolute value corresponds to reasonable values of those quantities.

An attempt was made to obtain a very low optimum frequency by increasing the value of  $\lambda$  by depriving the nerve of ionized calcium. The best way of decreasing the Ca-ion concentration is to soak the nerve in an appropriate citrate solution (soaking a nerve in low calcium solution takes a very long time), and we have found a clear relation to exist between the value of  $\lambda$  observed by exponential currents and the Ca-ion concentration calculated from the formulae of Hastings, McLean, Eichelberger, Hall, and Da Costa (1934); this will be described in a later paper.

A nerve was soaked in citrate-Ringer's solution and then stimulated with the photoelectric arrangement described in § II. The latter was necessary since the optimum frequency expected was very low. The results were very complicated. There were signs of the usual  $I-n$  relation, but superimposed on this were effects which we could only attribute to repetitive response of the nerve to each cathodic half-wave. Katz (1936*b, c*) has shown that when accommodation is very slow a long series of action currents may occur when a constant current is passed through a medullated nerve, and we imagine that something similar must have happened with the long-lasting waves of the alternating current at low frequency. It proved impossible to determine the optimum frequency with any certainty, and the experiments were not continued.

An attempt was made also to find the optimum frequency of the human ulnar nerve. This proved to be below the range in which the wave form of the oscillator, with its amplifier, could be trusted, and the photoelectric arrangement did not give enough current. We suspect, moreover, that repetitive response of the slowly accommodating nerve was affecting the result. According to Solandt (1936*a*), a normal value of  $\lambda$  in human ulnar nerve is  $70\text{ msec.}$  This, on the analogy of frog's nerve, is sufficient to give repetitive response to a constant current of rather low intensity. We had embarked on the experiment without making a calculation which would have shown it to be useless. With  $k = 0.35\text{ msec.}$

and  $\lambda = 70$  msec. the calculated value of the optimum frequency is  $n_{op} = 32$ , and with such a large value (200) of  $\lambda/k$  it is necessary (fig. 2) to go far below the optimum before the rise of threshold in that region can be measured. With an apparatus capable of giving an alternating current of several milliamperes at (say) 50 volts, and of pure enough wave form from (say) 2 to 500 cycles per second, the phenomena of optimum frequency could probably be demonstrated in human nerve—but only provided that, at the lower frequencies, repetitive response to each cycle did not spoil the result. Such apparatus was not available and would have had to be specially constructed, and, moreover, the hope of avoiding repetitive response was not great, so the experiments were discontinued.

### VIII—THE RELATION BETWEEN THE TWO TIME-CONSTANTS OF EXCITATION

In fig. 2 it is shown that the theoretical form of the  $I$ -log  $n$  curves, on either side of the optimum frequency, depends on the ratio of the two time-constants, being more pointed (rising more steeply on either side of the optimum) the less the value of  $\lambda/k$ . Coppée (1934c, p. 16) was aware of this variation, and used as a measure of the flatness ("aplatissement") of his curves the width of the  $I$ - $n$  (not  $I$ -log  $n$ ) relation at twice the minimum threshold height. Let  $n_1$  and  $n_2$  be the frequencies for threshold  $2I_{op}$ , then Coppée measured the flatness of his curves by the factor  $a = (n_1 - n_2)/n_{op}$ . He states that for the frog's sciatic nerve  $a$  varies from 3·5 to 8, for the non-neural part of the sartorius of frog and toad from 11 to 13, for the foot of a snail from 3·5 to 8.

Putting  $I/I_{op} = 2$  in equation (4'), and solving for  $n/n_{op}$  we find

$$a = (n_1 - n_2)/n_{op} = \sqrt{3(\lambda/k + k/\lambda)} - 6.$$

From this the following values of  $a$  can be calculated:—

$\lambda/k$	2	3	5	10	20	40	60	100
$a$	3·67	4	4·65	6·02	8·14	11·22	13·63	17·5

The values of  $a$  given by Coppée correspond to the following values of  $\lambda/k$ :—

Frog's sciatic nerve .....	$\lambda/k = 1\cdot6 - 19\cdot5$
Sartorius .....	$\lambda/k = 38\cdot5 - 54$
Snail's foot .....	$\lambda/k = 1\cdot6 - 19\cdot5$ .

The variation is wide. The higher figure for nerve is well within our normal range; the lower figure, however, is completely outside it: we have never observed a value of  $\lambda/k$  of less than 7·3. Moreover, the results

given in Coppée's Table I for frogs' nerves allow the value of  $k$  to be calculated from the chronaxie ( $= 0.693k$ ), and the value of  $k\lambda$  to be calculated from the optimum frequency (equation (2) above), from which  $\lambda$ , and so  $\lambda/k$ , can be determined. The results are given in Table XIII.

TABLE XIII

$k$ , msec. ....	0.23	0.245	0.36	0.29	0.24	0.22	0.25	0.245	0.22
$\lambda$ , msec. ....	3.4	6.1	7.0	8.0	4.7	10.6	6.5	4.9	2.6
$\lambda/k$ ....	15	25	19	28	20	48	26	20	12

The values of  $\lambda/k$  lie well within our normal range: not one of them approximates to the 1.6 calculated from Coppée's  $a$ . We suspect that some error must have affected the latter. Further, Coppée (1936c, p. 18) states that in his experiments the period corresponding to the optimum frequency was 30–60 times the chronaxie: from this it can be calculated that  $\lambda/k$  lay between 11 and 44. In any case, all his results confirm Solandt's (1936a) conclusion that the value of  $\lambda/k$  is highly variable.

That  $\lambda/k$  is variable under the conditions of the present experiments with alternating current is shown by the fact that we have obtained values of  $\lambda/k$  from 7.3 to 45. It would be easy to get larger values than 45, but in order to have a high optimum frequency we have intentionally worked at a high temperature and very often with a nerve previously soaked in a high Ca-solution, both of which tend to give a low value of  $\lambda/k$ . In normal (spring) nerves, after soaking for a few hours in normal Ringer's fluid, we have found values at about 28° C., ranging from 10 to 27: after soaking for 24 hours, one of 45. In high-Ca nerves at about 32° C. we have found values from 7.3 to 20. On one occasion at 23° C. a high-Ca nerve gave  $\lambda/k = 34$ . A normal nerve at 17° C. gave a value as low as 17.

High values of  $\lambda/k$  at low temperature mean a low optimum frequency and a nerve accommodating slowly, conditions which practically ensure repetitive response to each cathodic half-wave on the low side of the optimum frequency. With a large value of  $\lambda/k$  the curves of fig. 2 are so blunt that one would have to go to very low frequencies to obtain any accuracy in the determination of  $\lambda$ . It is unprofitable, therefore, to work with alternating current on frogs' nerves in this region of very low frequency: which is the reason why we have not obtained values of  $\lambda/k$  much larger than we have actually found. Even within our range, however, of values of  $\lambda/k$  the variation is so wide (more than sixfold) that it is impossible to maintain that (in Lapicque's words, quoted by Fabre, 1934) "Toute constante de temps obtenue sur un organe excitable au moyen d'un

*courant de forme quelconque est proportionnelle à la chronaxie de cet organe*". Monnier in his theory (1934) assumed  $\lambda/k$  (his  $\tau_2/\tau_1$ ) to be constant and equal to 6. Fabre also originally followed authority in supposing that his "*constante linéaire*" is proportional to the chronaxie, and gave a formula connecting them; later (1931, 1934) he came to the conclusion that the former can vary independently of the latter. In this we confirm him:  $\lambda/k$  in frog's nerve, according to our experience, may have any value between about 7 and almost infinity.

#### IX—THE AGREEMENT OF THE I-n RELATION WITH THEORY

Many experiments have been made in which the relation between threshold intensity and frequency was determined. A few of them are recorded in Table XIV. These have been chosen to represent several different conditions. In the first two a minimal steady muscle response was used

TABLE XIV—THE RELATION BETWEEN I AND n ON BOTH SIDES OF THE OPTIMUM FREQUENCY

*Experiment of 9 January, 1936*—Muscle nerve preparation of Hungarian *Rana esculenta* after 3 hours 10 minutes in Ringer's solution with  $6 \times \text{Ca}$ , at  $32.2^\circ\text{C}$ . Minimal tetanic response of muscle: clear sharp index, no doubt of reading. Nerve 48,000 ohms, external resistance 952,000 ohms. Series from 30 to 1300 cycles per second and reverse; mean of two readings at each frequency. Note that the thresholds are high because of the calcium.

Cycles per second .....	30	50	70	100	150	225
Threshold, $\mu\text{A. (r.m.s.)}$ .....	8.55	6.1	5.25	4.75	4.45	4.35
Cycles per second .....	350	500	700	900	1100	1300
Threshold, $\mu\text{A. (r.m.s.)}$ .....	4.50	4.75	5.3	5.95	6.6	7.25

In fig. 3b above  $I^2$  is plotted against  $n^2$  for the 9 highest frequencies, and  $I^2$  against  $1/n^2$  for the 8 lowest frequencies. The two relations approximate to straight lines, on the higher and the lower sides respectively of the optimum frequency. As that frequency is approached both relations bend up. The straight portions allow one to read off the values of  $k$  and  $\lambda$  respectively, and also the value of  $I_0$ .

In fig. 3a I is plotted against  $\log_{10} n$ , and a theoretical curve (equation (1)) is drawn with the constants,  $I_0 = 4.10$ ,  $k = 0.1815$  msec.,  $\lambda = 2.91$  msec. The fit is good. From equations (3) and (2)  $I_{op} = 4.36$  and  $n_{op} = 219$ ;  $\lambda/k = 16$ . An exactly similar and equally good experiment was made on the opposite preparation of the same frog. It has been in the high-Ca solution for  $1\frac{1}{4}$  hours only, and the temperature was  $31^\circ\text{C}$ .  $n_{op}$  was 210 cycles per second;  $I_{op}$  was  $2.6 \mu\text{A. (r.m.s.)}$ ;  $k$  was 0.204 msec. and  $\lambda$  was 2.82 msec.

*Experiment of 17 January, 1936*—Muscle nerve preparation in normal Ringer's fluid 2 hours, then in chamber  $2\frac{1}{2}$  hours at  $28^\circ\text{C}$ . Minimal tetanic response of muscle as index of excitation. Series from 30 to 1000 cycles per second and reverse; mean of two readings at each frequency.

TABLE XIV—(continued)

Cycles per second .....	30	50	80	130	200
Threshold, $\mu\text{A.}$ (r.m.s.) .....	2.39	1.89	1.67	1.58	1.59
Cycles per second .....	300	400	600	800	1000
Threshold, $\mu\text{A.}$ (r.m.s.) .....	1.745	1.95	2.38	2.91	3.43

In fig. 9b  $I^2$  is plotted against  $n^2$  for the 8 highest frequencies, and  $I^2$  against  $1/n^2$  for the 6 lowest frequencies. The two relations approximate to straight lines, on the higher and the lower sides respectively of the optimum frequency. As that frequency is approached both relations bend up. The straight portions allow one to read off the values of  $k$  and  $\lambda$  respectively, and also the value of  $I_0$ .

In fig. 9a  $I$  is plotted against  $\log_{10} n$ , and a theoretical curve (equation (1)) is drawn with the constants,  $I_0 = 1.46$ ,  $k = 0.355$  msec.,  $\lambda = 3.97$  msec. The fit is good. From equations (3) and (2),  $I_{op} = 1.583$  and  $n_{op} = 138$ ;  $\lambda/k = 11.2$ .

*Experiment of 5 April, 1935*—Sciatic nerve of Hungarian *Rana esculenta*, soaked for a few hours in high-Ca Ringer's solution, at room temperature (about 18° C.). Constant fraction of maximal action current of nerve, observed with sensitive galvanometer. Readings of  $I$ , in arbitrary units, in order given below. Stimulation with photo-cell arrangement. The calculated values are for  $I_0 = 53$ ,  $k = 0.47$  msec.,  $\lambda = 8.2$  msec. From equations (3) and (2),  $I_{op} = 56$ ,  $n_{op} = 81$ ;  $\lambda/k = 17.5$ .

Cycles per second .....	5.66	9.83	34.3	79	148	266
<b>Threshold—</b>						
Observed .....	182	126	61	58	58	67½
Calculated .....	189	117	61	56	58	66
<b>Cycles per second .....</b>						
146	75	59	30.4	6.1		
<b>Threshold—</b>						
Observed .....	61	60	58	63	167	
Calculated .....	58	56	57	63	176	

*Experiment of 27 March, 1936*—Sciatic nerve soaked several hours in normal Ringer's solution, at 28° C. Minimal action current of nerve observed with sensitive galvanometer. Series from 25 to 1000 cycles per second and reverse; mean of two readings at each frequency. By plotting  $I^2$  against  $n^2$  and  $1/n^2$  respectively, as in figs. 3 and 9,  $k$  and  $\lambda$  were determined, and the values of  $I$  calculated from equation (1) with the following constants:  $k = 0.266$  msec.;  $\lambda = 2.52$  msec.;  $I_0 = 1.31$ . From equations (3) and (2),  $I_{op} = 1.445$  and  $n_{op} = 193$ ;  $\lambda/k = 9.5$ .

Cycles per second ....	25	30	40	50	60	70	90	110	130
<b>Threshold I, <math>\mu\text{A.}</math> (r.m.s.)—</b>									
Observed .....	3.60	3.12	2.45	2.11	1.88	1.76	1.61	1.50	1.50
Calculated .....	3.53	3.04	2.44	2.11	1.91	1.78	1.61	1.53	1.49
<b>Cycles per second ....</b>									
150	200	250	300	400	500	700	1000		
<b>Threshold I, <math>\mu\text{A.}</math> (r.m.s.)—</b>									
Observed .....	1.445	1.445	1.455	1.48	1.57	1.73	2.00	2.48	
Calculated .....	1.465	1.445	1.47	1.50	1.60	1.73	2.03	2.57	

The agreement between calculated and observed is good, except at 1000 cycles per second.

TABLE XIV—(continued)

*Experiment of 28 March, 1936*—Sciatic nerve soaked for 24 hours in normal Ringer's solution (14 hours at 6° C.); experiment made at 28° C. Minimal action current of nerve observed with sensitive galvanometer. Series from 25 to 700 cycles per second and reverse; mean of two readings at each frequency. By plotting  $I^2$  against  $n^2$  and  $1/n^2$  respectively, as in figs. 3 and 9,  $k$  and  $\lambda$  were determined, and the values of  $I$  calculated from equation (1) with the following constants:  $k = 0.170$  msec.;  $\lambda = 7.6$  msec.;  $I_0 = 2.72$ . From equations (3) and (2),  $I_{op} = 2.78$  and  $n_{op} = 140$ ;  $\lambda/k = 44.7$ .

Cycles per second .....	25	30	40	50	60	70	80	110
Threshold I, $\mu$ A. (r.m.s.)—								
Observed .....	3.56	3.26	3.09	2.95	2.90	2.85	2.80	2.76
Calculated .....	3.55	3.32	3.07	2.95	2.88	2.85	2.79	2.78
Cycles per second .....								
130	150	200	250	300	400	500	700	
Threshold I, $\mu$ A. (r.m.s.)—								
Observed .....	2.76	2.76	2.80	2.81	2.83	2.98	3.09	3.48
Calculated .....	2.78	2.78	2.79	2.82	2.86	2.96	3.08	3.40

The agreement between calculated and observed is good. *Note*—This is the opposite nerve to that of the preceding experiment (27 March);  $\lambda/k$  had increased from 9.5 to 44.7.

as index of constant excitation of the nerve: in the last three the action current of the nerve itself, recorded with a sensitive galvanometer. Of the first two, one was at 32° C., with a preparation which had been soaked in a Ringer's solution with six times the normal Ca; the other was at 28° C., with a preparation soaked in normal Ringer's fluid. Of the other three, one was at 18° C., with a nerve soaked in high-Ca; the optimum frequency being low, the photoelectric arrangement was used to provide the stimulating current. Both of the others were at 28° C., the first after a few hours and the second (on the opposite nerve) after 24 hours' soaking in normal Ringer's solution. In the first two experiments the observed threshold I is plotted against  $\log n$  (figs. 3 and 9) and a *calculated* curve, drawn from equation (1) and the constants given in Table XIV, is drawn through the points. In the last three experiments the observed I is tabulated against  $n$ , together with a calculated I obtained from equation (1) and the constants given.

The agreement of calculated with observed is, in every case, good. Equation (1), therefore, correctly expresses the relation between threshold and frequency; and we have already shown (§§ IV and VI) that the values of  $k$  and  $\lambda$ , necessary to obtain the agreement, are the same as can be found by other experimental methods.

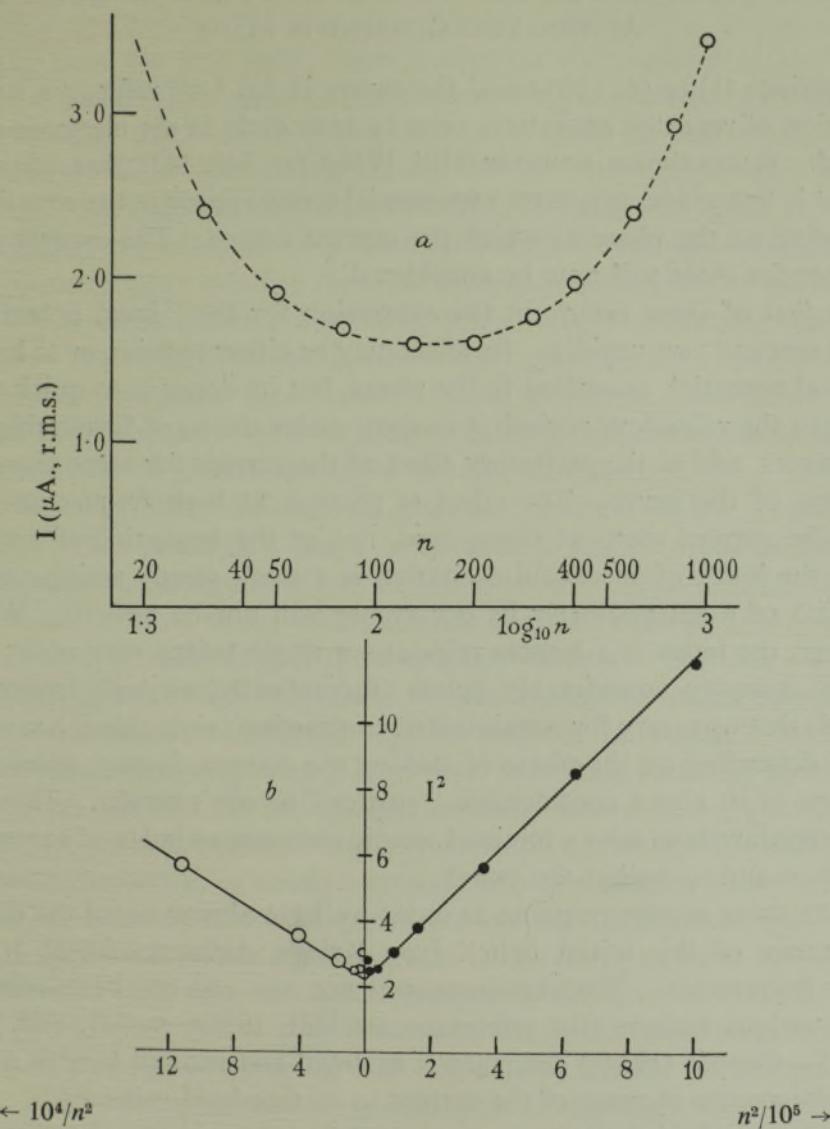


FIG. 9—Experiment of 17 January, 1936; Table XIV. Nerve treated with normal Ringer's solution, at 28° C. Muscle response as index. (a)  $I$  plotted against  $\log_{10} n$ , and a theoretical curve (from equation (1)) with the constants given in Table XIV. (b)  $I^2$  plotted against (to the right)  $n^2$ , and (to the left)  $1/n^2$ , showing linear relations on the high and the low sides respectively of the optimum frequency.

## X—THE INITIAL IMPULSE DEPENDING ON THE PHASE AT WHICH THE ALTERNATING CURRENT IS MADE

Equations (1) to (6) above and the curves in fig. 1 all refer to a steady condition of repeated excitation, once to each cycle at the electrode considered. It was shown, however (Hill, 1936a, pp. 340, 342), that, when the current is first made, two extra exponential terms appear in the equations, depending on the phase at which the current begins. The experimental evidence for these will now be considered.

The *first* of them occurs in the expression for the "local potential"  $V$ . It dies out very rapidly. Its effect may be either to raise, or to lower, the local potential, according to the phase, but its decay is so quick that, owing to the refractory period, it cannot, under the most favourable circumstances, add to the excitatory effect of the current for more than one response of the nerve. The effect is greatest at high frequencies and when the current starts at phase zero, *i.e.*, at the beginning of a wave. When the index of successful excitation is a small steady action-current deflexion of a galvanometer its occurrence will not be noticed. When, however, the index is a muscle response, a single twitch may occur at a current intensity considerably below (theoretically, at high frequency, 50% of) that necessary for a maintained contraction; since this is a random effect, depending on the phase of making the current, it may, unless one is aware of it, give a considerable "scatter" to one's results. The only safe procedure is to take a minimal tetanic response as index of successful excitation and to neglect the twitch.

When using muscle response as index we have always noted the casual appearance of this initial twitch (von Kries's *Anfangszuckung*) at the higher frequencies. The phenomenon is not new and has been referred to by various authors (for references see Hill, 1936a, p. 341, and § XI below). Coppée (1934c) described it in detail and showed how to avoid it by progressive increase of the current to its threshold value.

The nature of this initial response at high frequency is easy to appreciate physically with the aid of fig. 1. At low frequency the local potential  $V$  follows the current practically without phase lag: its maximum cannot be made appreciably greater by beginning the current at any special phase. At high frequency  $V$  lags considerably behind the current, at very high frequency by  $\pi/2$ . The cathodic half of the wave, therefore, has to neutralize the effect of the anodic half before  $V$  begins to rise above  $V_0$ . If the cathodic current begins at, or near, phase zero the neutralization has not to occur, and  $V$  rises higher. This happens to a considerable

extent only with the first cathodic half-wave, so that the excitatory effect of the current is appreciably enhanced only for one wave.

The *second* exponential term occurs in the threshold  $U$ . It dies out less rapidly than the first term, since it involves  $e^{-t/\lambda}$  instead of  $e^{-t/k}$ . Its effect is predominant at low frequencies. Its mathematical expression is complex and need not be given here; it is easier to regard the matter physically. If an alternating current of low frequency begins to run through a nerve at the crest of a wave, *i.e.*, at phase  $\pi/2$ , it will be nearly as effective, *qua* stimulus, as a constant current of the same strength. It will be much more effective than the same current starting at phase zero, which will act as a slowly increasing current allowing the threshold time to rise. Thus with frequencies below the optimum, the twitch threshold—which is practically the constant current threshold if the phase  $\pi/2$  is hit off approximately—will be nearly constant, while the steady tetanus threshold will be greater the lower the frequency.

If, therefore, one “makes” an alternating current of low frequency at random phase through a nerve, and takes a single twitch as index of response, the crest threshold will be found to vary casually, from the high level corresponding to the steady tetanus at that frequency to a low level approximately equal to that for a constant current. The only safe procedure is, as with high frequency, to take a minimal tetanic response as index of successful excitation and to neglect the twitch. The facts, without accompanying theory, have been admirably recorded by Coppée (1934c, pp. 10, 11). In his fig. 3 he correctly shows the  $I$ - $\log n$  relation, for sudden make of current and twitch response, on the low side of the optimum frequency, as nearly a straight line at rheobasic level.

The most exact of our experiments on the  $I$ - $n$  relation were made with a very small steady action current of the nerve as index of constant excitation. With this, the make response is not noticed. Many, however, of our experiments were made with minimal muscle contraction as index, and in these, since we did not employ means (as Coppée did) either of increasing the current gradually or of starting it at phase 0 or  $\pi$ , we always noted, at low frequencies, the casual appearance of the initial twitch at a lower threshold than the steady response. A very simple means was found of avoiding it, *viz.*, to open the short circuit key K (fig. 4) very gently, instead of sharply, to make the current through the nerve. It may be that in this way the current increases gradually to its full value, or starts at phase zero. The muscle is saved from unnecessary stimulation, and the readings are made sharper, if this simple precaution is adopted. The only response observed is then usually the steady tetanus.

## XI—THE INTENSITY-FREQUENCY RELATION AT HIGHER FREQUENCIES

In § IX above equation (1) has been shown to apply rather accurately, within certain limits of frequency, to stimulation of the sciatic nerve of *Rana esculenta* by alternating current. We shall now consider the limits of its application.

At low frequency the limiting factor seems to be the occurrence of repetitive response to each cathodic half-wave of the current; and with nerves accommodating slowly (with large  $\lambda$ ) this means that the  $I\text{-}n$  relation cannot be determined accurately very far along its upward branch to the left. This will prevent the useful application of very low frequency current to slowly accommodating nerves, such as those of mammals or crustacea.

The upper limit to equation (1) appears to be set by the factors which come into play as the period of the current approaches the refractory period. If muscle response be used as index of excitation a further difficulty arises, at higher frequencies, from Wedensky inhibition. We have seen in § X that the twitch response is bound to yield irregular results depending on the phase of making the current; on the other hand, it is almost impossible to obtain a steady tetanic response of a muscle to high frequency stimulation of its nerve. We have made many attempts, using a muscle nerve preparation, to determine the  $I\text{-}n$  relation at higher frequencies, but have been forced to conclude that in this region a threshold steady response of the muscle is not a sufficiently objective index of nerve excitation to give the desired accuracy. There is, in fact, no steady response, but a rapidly declining one, and the decision as to what is a tetanic response and what a twitch depends too much on personal judgment. The only safe method is to avoid the neuromuscular junction altogether, and to work with a threshold response of the nerve itself. All our significant experiments, at higher frequencies, have used as index a very small action current response of the nerve, recorded with a sensitive galvanometer.

In a number of experiments the relation between  $I^2$  and  $n^2$  was determined beyond the range within which it is linear. It is not possible to say very exactly at what frequency the linearity ends; the curve gradually bends away from the straight line, nearly always in a downward direction (fig. 10). Roughly, however, an upper limit to the linear relation can be stated, and for 39 experiments is given in Table XV. Neglecting two interesting and anomalous cases of a very extended relation (to 2000 and 5000 cycles per second), the results can be divided up into four groups, as in the second part of the table. There is clearly an upward trend with

temperature. At 20° C., the upper limit, on the average, would be about 500 cycles per second; at 30° C., about 850. The former corresponds to a period of 2 msec., the latter to one of about 1.2 msec., quantities inside the relative refractory period and approaching the absolute refractory period of the nerve.

The usual type of deviation at higher frequencies from the linear  $I^2-n^2$  relation, a rather exaggerated case for illustration, is given in curve (a), fig. 10. Here at 25.5° C., the  $I^2-n^2$  line is not straight beyond 500 cycles

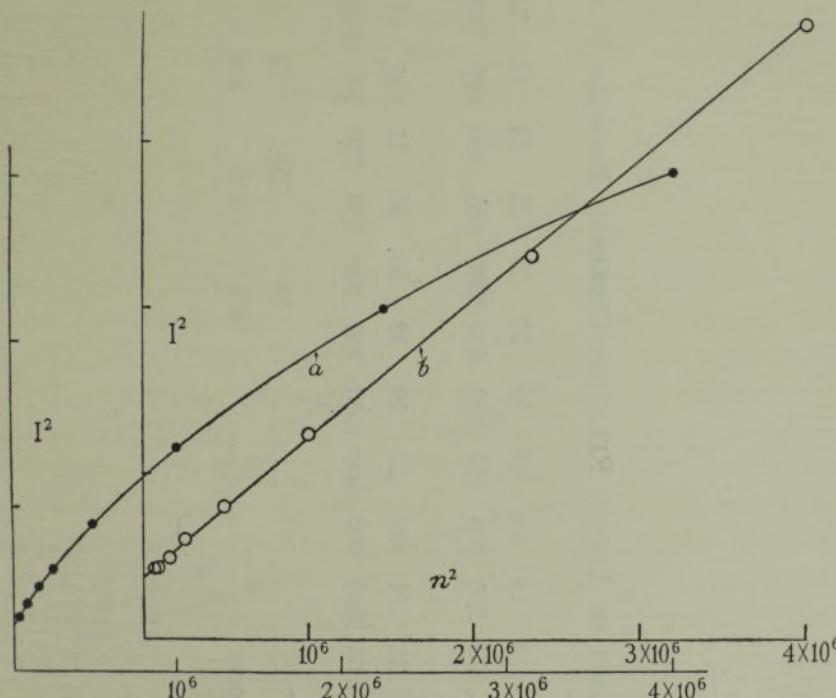


FIG. 10.—The  $I^2-n^2$  relation for threshold stimulation of frog's nerve by alternating current at higher frequency: minimal electric response as index of excitation.  
 (a) 23 March, 1936; 25.5° C.,  $k = 0.375$  msec.; (b) 27 March, 1936; 28° C.,  $k = 0.245$  msec.

per second. For contrast, an unusual case at 28° C. is shown in fig. 10, curve (b). Here the line is straight to beyond 2000 cycles per second. Usually, however, the relations discussed in the earlier part of this paper apply only up to 400–1000 cycles per second according to the temperature; within that range they do apply, and rather accurately. We can give no convincing explanation of the form of the curve usually observed at higher frequencies, but it may be of importance that Harris, Rosenberg, and Sager (1936) found analogous deviations from a linear  $I^2-n^2$  relation at

TABLE XV—UPPER LIMIT OF LINEAR RELATION OBSERVED BETWEEN  $I^2$  AND  $n^2$ 

(a)		Temperature, °C. . .	16	17	17	18	18	18	19	19	20	20	21	22	22	23	23	26	27	
Limiting frequency . .		450	350	500	350	500	530	700	350	500	400	2000	600	500	600	700	700	1000	500	600
Temperature, °C. . .	27	28	28	28	28	29	29	29	30	30	30	31	31	31	31	31	32	34		
Limiting frequency . .	700	500	800	900	1000	5000	900	1000	1000	500	500	850	750	900	1000	1000	1100	1000		
(b) Mean temperature, °C. . . . .												18	22½	28	31					
Mean limiting frequency . . . . .												470	630	820	860					

higher frequencies when using, as index, not response but the attainment of a constant electrotonic potential.

For higher frequencies the plotting of  $I^2$  against  $n^2$  is not satisfactory, because if the higher range is properly represented the lower range is too cramped. A better graphical representation of the results is given at higher frequencies by plotting  $I$  against  $n$ . In fig. 11 four typical experiments are shown. The index of excitation was a very small steady electric response. In all four the  $I$ - $n$  relation is approximately, in none is it

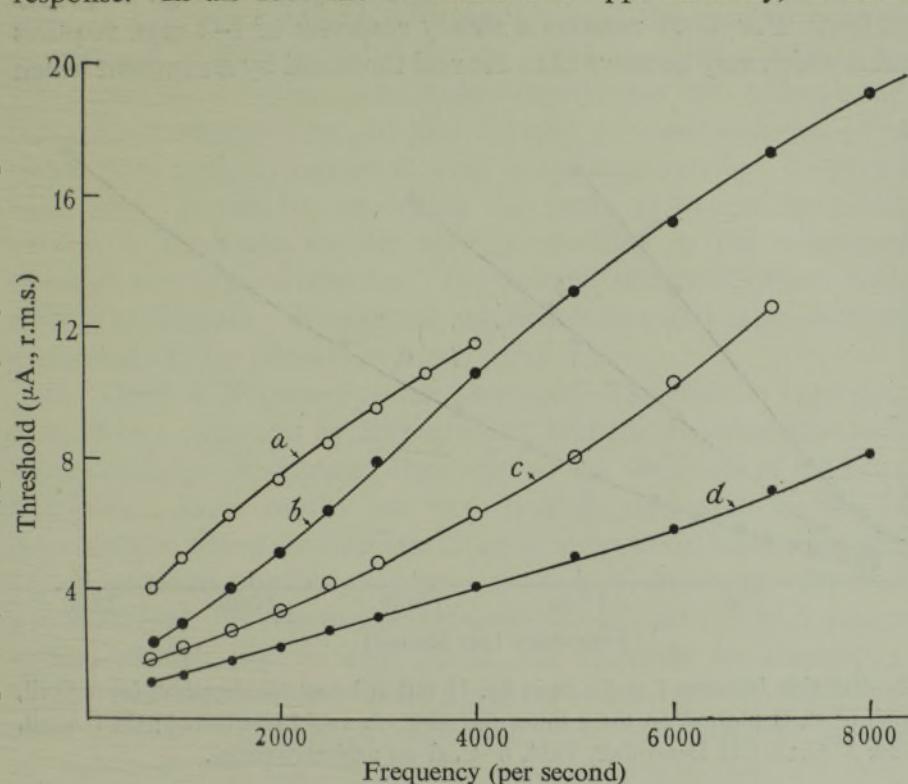


FIG. 11.—Relation between  $I$  and  $n$  for threshold stimulation of frog's nerve by alternating current at higher frequency. Minimal electric response of nerve as index of excitation. (a) 23 March, 1936, 24.5° C.; (b) 27 March, 1936, 28° C., see Table XIV; (c) 28 March, 1936, 28° C., see Table XIV ( $I$  plotted on half the scale, to avoid confusion); (d) 27 March, 1936, 24° C. ( $I$  plotted on one-third the scale, to avoid confusion).

quite, linear. The deviation from a straight line is variable in either direction from one experiment to another. If by chance an exactly linear relation is found, no special significance need be attached to it.

At a low temperature, e.g., at 8° C., or below, regular results of another type are obtained (fig. 12). The  $I$ - $n$  relations bend upwards, the

threshold rising more than in proportion to the frequency. This effect is possibly due, at least in part, to a technical cause. The smallest steady response of the nerve which it is safe to take as an index of constant excitation is 1-2 mm., on the galvanometer scale. At higher frequencies (and 1000/sec. is a high frequency for a nerve at 6° C.) the response is not well maintained owing to the onset of the failure described by Bugnard and Hill (1935*b*, *c*); moreover, the maximal response, so far as it is possible to measure it, appears to be considerably less at high frequency than at low. For both reasons a steady response of 1-2 mm. requires a stimulus which may be more than the real threshold by an amount which

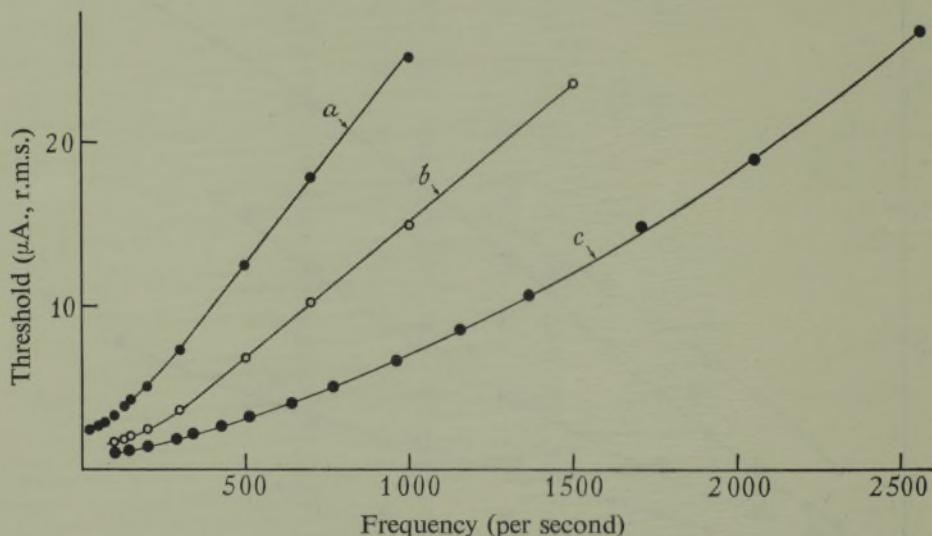


FIG. 12—Relation between  $I$  and  $n$  as in fig. 11 but at lower temperature, (a) 2 April, 1936, 6° C. ( $I$  plotted on three times the scale, to avoid confusion); (b) 1 April, 1936, 5° C.; (c) 21 December, 1934, 6° C. ( $I$  on arbitrary scale).

is greater at higher frequency. This, as a matter of fact, was the effect which, on the basis of the work of Bugnard and Hill, we had expected.

In studying nerve excitation by high-frequency alternating current of sine-wave form we have to take account of several different factors:

- (a) the influence of frequency of excitation as such;
- (b) the smaller excitatory effect of each cathodic half-wave as its duration is reduced;
- (c) the change of current distribution inside the structure of the nerve, or fibre, with alteration of frequency, due to electrical capacity either normally existing or produced by polarization at surfaces or membranes;

- (d) possibly depending on (c) a change over from the excitation of one type or group of fibres to another type or group, as the current distribution alters with frequency.

Of these factors (b) alone is taken into account in deducing equations (1) to (6) above. (a) was examined in detail by Bugnard and Hill (1935a, b, c) for maximal stimulation, using short condenser discharges of constant form but of variable frequency. (c) cannot be properly discussed until we have more definite information about the site and properties of the surfaces or membranes (if they be such) at which excitation occurs. The capacities, however, of biological membranes may be large and their insulating properties high, so that changes of impedance and of current distribution may be important, even at the relatively low frequencies we have used. It will be interesting to study excitation by alternating current in the much simpler systems provided by the non-medullated nerve of marine invertebrates. The possible effects of (d) are still more difficult to estimate. It might be practicable to avoid them altogether by examining the  $I\text{-}n$  relation in single nerve fibres.

*The Effect of Frequency on the Threshold*—The effect of high frequency as such was examined by Bugnard and Hill for maximal stimulation by short condenser discharges; they studied the variation of response with frequency. Their results are not directly applicable to the present investigation, where the response taken as index of excitation was constant, and as small as possible, and the variation of threshold with frequency was examined. It was necessary, therefore, to make special experiments to find whether, and to what extent, the threshold for alternating condenser discharges of very short duration varies with the frequency. A short duration was necessary since the maximum frequency desired was so high that, with the commutator used, not more than 200  $\mu\text{sec}$ . was available for each discharge, and it was thought best to keep the value of  $RC$  short compared with this and with the interval between successive shocks. The duration was so short that it lay almost in the region in which the "constant quantity" relation (Hill, 1936a, p. 317; 1936b, p. 442) obtains. Thus the experiments were directed to finding the effect of frequency as such with alternating condenser discharges of constant duration so short that they could be regarded as effectively "instantaneous".

The method employed was that described by Hill (1934, fig. 1), employing a commutator with 46 segments. This could be driven at any speed up to nearly 100 revolutions per second, and so could give two-way condenser discharges at any frequency  $n$  up to 2000 per second

( $n$  discharges each way). This was used with capacity  $C = 0.005 \mu\text{F}$ . and shunt resistance,  $R_1 = 4000$  ohms. In series with the nerve was a resistance  $R_2 = 50,000$  ohms, which together with the nerve resistance made about 100,000 ohms, and reduced the resultant discharge resistance  $R$  to about 3800 ohms. Thus  $RC$  was just less than 20  $\mu\text{sec}$ . The resistance  $R_1$  consisted chiefly (2800 ohms) of a very fine resistance wire wound non-inductively round the "hot" junctions of a thermopile connected to a Moll galvanometer.

The resistance  $R$  was purposely made rather high and the capacity  $C$  small, because at very high speeds the resistance at the contact of the brush with the commutator might not otherwise be negligible, and there was danger that variable discharges might occur. With  $R = 200$  ohms and  $C = 0.1 \mu\text{F}$ ., for example, which give the same value of  $RC$ , this was very evident. That the arrangement ultimately used was not affected by this error was controlled, for every frequency used, by recording the reading of the galvanometer connected to the thermopile and verifying that this was proportional to the rate of energy liberation  $E^2Cn$  of the stimulus. If the resistance at the contacts on the commutator were an appreciable fraction of the discharge resistance  $R$ , a proportional amount of the energy would be lost at the contacts and the reading of the galvanometer would be less. Since this was not so, the resistance at the contacts was negligible compared with  $R$  and the discharges must have occurred sufficiently nearly from potential  $E$  and with time-constant  $RC$ . It is essential to control the strength and character of the condenser discharges in this way, for otherwise there is serious risk that at high frequency the threshold will appear to be raised owing to the stimuli being, in fact, less effective than they were intended to be.

In most experiments the index of constant excitation was a very small steady action current response (2 mm.) of the nerve, recorded by a sensitive galvanometer. The greatest response of the nerve to a maximal stimulus at the most effective frequency was 150–300 mm. In a few experiments, at the lower frequencies only, a minimal steady muscle contraction was used as index, for a special purpose referred to below. The index response was chosen as small as could be read accurately. A constant fraction of the maximal response at each frequency would have been better but this could not be used since, at high frequency, the maximal response requires a very strong stimulus and this affects the condition of the nerve and subsequent readings of the threshold. With a constant index, the error is least when the index itself is least.

The distant stimulating electrode lay on an injured part of the nerve, so that excitation was at one point only and of frequency  $n$  (not  $2n$ ).

In every determination of the threshold the commutator was set running at a measured speed and then the potential  $E$  was adjusted to give the threshold response desired. The energy reading of the hot wire thermopile was next made, to control the stimulus. The speed of the commutator was then altered,  $E$  was determined again and the energy measured. Thus the threshold  $E$  was determined for a series of frequencies  $n$ .

The results were unexpected. At ordinary (rather high) temperatures (average 27° C.) the effect of frequency on the threshold was negligible, at least within the range 200–2000 double shocks per second. It was not advisable to drive the commutator much faster. The effective range, however, could be increased by cooling the nerve, and 12 experiments were made at about 7° C. Assuming a temperature coefficient  $Q_{10} = 2$ , a frequency of 2000 shocks per second at 7° C. is equivalent to one of 8000 at 27° C. At 7° C., the influence of frequency was rather variable, but the average effect of passing from 60 to 2000 double shocks per second was to raise the threshold by 23%. At 7° C., however, the maximal response was very much less at high frequencies than at low (*cf.* Bugnard and Hill, 1935*b, c*), and the small constant index, therefore, was a larger fraction of maximal. At higher frequencies the increase of response with increase of stimulus strength was rather slow (*cf.* experiment 2 of Table XVI), and we suspect that the effect of frequency on the threshold was really only apparent and due to taking too great an index response at higher frequencies. This could not be avoided without measuring the maximal response at each frequency and taking a constant fraction of it; which would probably have done more harm than good, by altering the condition of the nerve (owing to the strong stimulus necessary) and making the results unreliable. In any case the measurements with alternating current were made in the same way, with a small constant index response, so the two sets of experiments, with condenser discharges and with alternating current respectively, are strictly comparable.

In a low frequency range (45–200 per second) at the higher temperature there was a *fall* of threshold with increase of frequency. This was not large, but nevertheless quite definite, occurring in every one of 12 experiments and averaging 14%. It was not found at a low temperature; possibly it could have been detected in a still lower frequency range. The fall observed at the higher temperature was probably due largely to the fact that in this frequency range the maximal response is greater at the higher frequency, so that the small constant nerve response used as index was a larger fraction of maximal at the lower frequency and therefore required a stronger stimulus. That this was so was indicated

TABLE XVI—EFFECT OF FREQUENCY ( $n$ ) ON THRESHOLD (E, VOLTS) OF TWO-WAY CONDENSER DISCHARGES OF SHORT DURATION ( $n$  DISCHARGES EACH WAY PER SECOND)

		<i>Typical Experiments</i>					
(1) 8.5° C.	Nerve response.						
$n$	137	240	322	479	1110	1900	1915
E	17.7	17.9	18.5	19.3	19.9	19.5	19.2
(2) 8° C.	Nerve response.						
$n$	58	228	315	544	830	1160	1960
E	12.6	13.6	14.4	15.8	16.2	16.7	19
	At $n = 1960$ ,	E, volts	.....	16	17	17.9	19.5
		Response, mm.		0.5	1.0	1.5	2.3
(3) 27° C.	Nerve response.						
$n$	30	60	212	309	654	1160	1940
E	6.85	6.3	5.9	5.8	5.93	5.95	6.1
(4) 28.5° C.	Nerve response.						
$n$	50	188	321	560	845	1178	1975
E	4.7	4.7	4.6	4.6	4.7	4.6	4.6
(5) 25° C.	Muscle response.						
$n$	25	39	68	122	171	372	122
E	10.45	10.3	10.1	10.0	9.9	9.9	10.2
(6) 22° C.	Muscle response.						
$n$	20	37	63	116	210	167	118
E	5.05	4.95	5.0	5.0	5.15	5.05	5.05

#### SUMMARY

- (A) Low temperature, average 7° C., 12 experiments. Nerve response. Percentage rise of threshold E between  $n = 60$  (average) and  $n = 2000$ : 10, 0, 27, 8, 55, 40, 0, 13, 20, 20, 45, 45; mean 23%.
- (B) High temperature, average 27° C., 12 experiments. Nerve response.
  - (i) Percentage fall of threshold E between  $n = 45$  (average) and  $n = 150-250$ : 13, 12, 28, 15, 10, 18, 18, 10, 5, 14, 9, 16; mean 14%.
  - (ii) Percentage rise of threshold E between  $n = 150-250$  and  $n = 2000$ : 3, 3, 10, 0, 0, 0, -5, -7, 0, 3, 3; mean 1%.
- (C) High temperature, average 24° C., 12 experiments. Muscle response. Percentage fall of threshold E between  $n = 25$  (average) and  $n = 150$  (average): 5, 6, 5, 4, 2, 7, 10, 2, 3, 10, 3, 5; mean 5%.

by experiments made with minimal muscle response as index. The effect of frequency on the maximal muscle response is very small in this range (25–150 double shocks per second) so that the index taken was nearly a constant fraction of maximal. With muscle response as index the fall of threshold with increase of frequency, in this low frequency range, was very small, averaging only 5% in 12 experiments. This remaining difference is probably genuine, and of the same nature as the greater difference, described in § III above, between the constant current rheobase and the rheobase measured by repetitive pulses.

The experiments at higher frequencies could not be made with muscle response as index, owing to immediate relaxation of the muscle due to Wedensky inhibition.

We conclude, therefore, that the effect on the threshold of frequency of excitation as such, with alternating condenser discharges of constant short duration, is very small. In the low frequency range the threshold falls slightly as the frequency rises to about 200 double shocks per second. Above that, however, it remains practically constant. Any effect observed in the higher range is probably due to the fact that a constant index response, however small, represents a greater fraction of maximal response at high frequency than at low, and therefore requires a greater stimulus.

We found above (fig. 12) that with alternating current the  $I\text{-}n$  relation at low temperature tends to bend upwards at the higher frequencies. The experiments were made with small constant electric response of the nerve as index; they are comparable, therefore, with the present experiments made with condenser discharges. The bending upwards is probably due to the effect of frequency as such in raising the apparent threshold under these conditions. At high temperature (fig. 11) there was no regular tendency for the  $I\text{-}n$  relation to bend up at the higher frequencies; this corresponds to the absence here of a regular effect of frequency as such on the threshold determined with alternating condenser discharges.

The absence, at higher temperatures, of any regular effect of frequency on the threshold shows that the regular deviation of the  $I^2\text{-}n^2$  relation, for alternating current, from its theoretical linear form cannot be attributed to frequency as such. That deviation can scarcely be due to failure of the simple theory below a certain duration of the individual half-wave, since with condenser discharges the theoretical E-RC relation holds down to very low values of RC. If, for example, the deviation from the linear  $I^2\text{-}n^2$  relation begins at 650 cycles per second, this represents a duration of half-wave of 770  $\mu$ sec., and the E-RC relation has been verified down to about  $RC = 20 \mu$ sec. (Hill, 1936b). We must probably look for the cause of the divergence from theory in the changes of current

distribution inside the nerve, or fibre, with alteration of frequency, due to electrical capacity or polarization as discussed above.

The possibility (*d*) was referred to above (p. 121) that a change of frequency might lead to a change in the current distribution inside a nerve, and so—for a minimal response—to the stimulation of different fibres at different frequencies. That some such random cause produces the varied curves of fig. 11 is suggested by the fact that a single nerve excited at different points at the higher frequencies may produce just such a variety of  $I^2-n^2$  curves. At the lower frequencies, however, the  $I^2-n^2$  relation was consistent (*a*) in being linear up to 500–1000 cycles per second, (*b*) in giving nearly the same value of  $k$  at different points of the nerve, and (*c*) in bending away from the linear relation at higher frequencies as in fig. 10*a*. In six experiments, in which separate  $I^2-n^2$  series were made at each of three points (the other electrode being in a common injured region) the three values of  $k$  lay within the following limits: 0.38–0.41; 0.35–0.36; 0.51–0.59; 0.35–0.48; 0.4–0.5; 0.37–0.47. At lower frequencies, therefore, a consistent relation is found from one point to another, but not at higher frequencies.

The absence of any important effect on the threshold, of frequency of excitation by condenser discharges of very short duration, contrasts, at first sight somewhat strangely, with the rather large effect of frequency, described by Bugnard and Hill (1935*b*, *c*), on the maximal response. Strong high frequency excitation appears to prolong the refractory state, even the impulses which are ineffective, owing to falling in the refractory periods of their predecessors, having an influence in slowing the return of normal excitability. This lengthening of the refractory period causes the nerve to respond less times per second and so diminishes the maximal response. With threshold excitation, however, the situation is different. After one effective stimulus the nerve will be absolutely refractory for a certain interval, then relatively refractory, but its normal excitability will ultimately return, and finally it will respond to the same strength of stimulus as before. Apparently the ineffective shocks which fall during the absolute and relative refractory periods do not prevent—though they may delay—the threshold from returning ultimately to its initial level. The experiments of Bugnard and Hill showed that the return to normal excitability is slowed (the refractory period lengthened) in high frequency excitation by strong shocks most of which fall within the refractory period. Thus the nerve does not respond so often and the maximal response is lessened. The present experiments, however, prove that even if the return to normal excitability is slowed by the weaker shocks used for determining the threshold, it does finally occur; high frequency as such

does not prevent the nerve from continuing to respond, though perhaps at longer intervals, to a given threshold shock. The apparent contrast, therefore, is resolved.

One consequence of the observed fall of threshold in the low frequency range, with rise of frequency as such, must be considered, viz., its influence on the  $I-n$  relation for alternating current in that region, and in particular its effect on the value of  $\lambda$  calculated from the  $I^2-1/n^2$  line below the optimum frequency. Fortunately, the change of threshold with frequency is small; indeed, when minimal muscle response is used as index of excitation almost negligible. The simplest way to test the effect is by trial with actual numbers. Taking, for example, any of the results of Table XIV, let us assume (for simplicity) a given fall of threshold uniformly occurring between 30 cycles per second and the optimum frequency; let us correct for this and plot corrected  $I^2$  against  $1/n^2$ . The result will be (*cf.* figs. 3b and 9b) a line which rises rather less to the left and bends up rather more to the right, giving a value of  $\lambda$  which is slightly greater than originally calculated. The percentage correction in  $\lambda$  will not be more than the percentage change in the threshold over the range considered. This is of little significance. The argument of the preceding Sections of this paper will not be affected.

Since frequency as such has no effect on the threshold above about 200 cycles per second the value of  $k$  calculated from the  $I^2-n^2$  line requires no correction.

*Divergence from the General Theory*—It was disappointing at first, but it is scarcely a matter for wonder, that the divergences from the simple theory discussed in this Section should occur: what was astonishing rather was that the theory agreed with the facts so far and so well. We are inclined, indeed, to believe that in three respects at least the apparent agreement of observation with theory has been slightly improved by favourable chance: (i) the approximate equality of the "true" and the "observed" rheobase, due—it is suggested in § III above—to a balance between electrotonus and accommodation, has probably exaggerated the accuracy of fit of experimental observations to the theoretical voltage-capacity curve (Hill, 1936b); (ii) the effect of frequency as such on the apparent threshold, lowering it more near the optimum frequency for alternating current than at lower frequencies, has given a slightly better linear relation between  $I^2$  and  $1/n^2$ , on the low side of the optimum, than was expected; (iii) the fact that threshold excitation with alternating current occurs (fig. 1) at the moment when the nerve is just unaccommodated has probably eliminated disturbing factors present in other methods of stimulation. It could scarcely be hoped, in any case, that a general

statement which takes no account of the very complex physico-chemical structure of nerve would give a complete description of all the facts. When clear-cut divergence from the theory occurs we may expect to be able to use it to obtain more specific knowledge of the mechanism involved. The facts which fit the general theory cannot provide this knowledge. Such divergences, therefore, are to be welcomed, not for the sake of disproving the general theory, which fits so many of the facts so well that it can scarcely be altogether wrong, but for the sake of elucidating the physico-chemical details of the heterogeneous excitatory mechanism of which the general theory takes no account.

## XII—DISCUSSION

Physiological literature contains many references to the excitatory effects of alternating current. von Kries (1884a) discovered the existence of an optimum frequency, depending on the temperature, and since he published in the same year a paper (1884b) on excitation by slowly increasing current, he probably had in mind the relation between what is now called "accommodation" and the rise of threshold on the low side of the optimum frequency. von Kries also described an *Anfangszuckung* more readily observed at a higher frequency, which was probably due to the rapidly vanishing exponential term referred to in § X above, but which he connected with the *Anfangszuckung* described by Bernstein which may actually have been due to a Wedensky effect.

Nernst's theory of electric excitation was first (1899) applied to the case of alternating current; and von Zeynek (1899) in Nernst's laboratory determined the relation between threshold and frequency for the excitation of human sensory nerves. Nernst in a later paper (1908) discussed alternating current excitation in greater detail, and a vanishing exponential term, analogous to that referred to in § X above, occurred in his equations. Einthoven (1900) examined nerve excitation by alternating current of very high frequency and found enormous currents to be required; from  $6.5 \times 10^4$  to  $10^6$  cycles per second the threshold rose faster than the frequency; near  $10^6$  cycles per second a current of about 10 milliamperes was necessary, enough to heat the nerve  $65^\circ$  C., in one second, whereas with direct current only about 0.6 microampere was sufficient. He stated correctly, contrary to a prevailing error based on a false idea of the specific resistance of living tissue, that the high frequency current travelled throughout the body and not merely on the surface of the nerve.

Wertheim-Salomonsen (1905) employed a singing arc to produce oscillations from  $2.5 \times 10^3$  to  $2 \times 10^6$  cycles per second; he did not

examine the lower range of frequencies; in the higher range he found the threshold to increase more rapidly than the frequency. The singing arc, however, as Nernst pointed out (*see* Reiss, 1907), is unsuited to produce a pure oscillation of the kind required to test any theory. Asher (1923) found the threshold to rise in direct proportion to the frequency in the higher range from  $10^4$  to  $3 \times 10^5$  cycles per second, but he gave few experimental details and reported that even with  $3 \times 10^5$  cycles per second he could obtain smooth tetani from the muscle-nerve preparation of the frog. Some leak, rectification, or other disturbance must have occurred, for otherwise the nerve would have been coagulated by heat by the current necessary. Krüger (1928) worked with frequencies of 400 cycles per second upwards and concluded that the threshold rises faster than the square root of the frequency (Nernst's "Law"). Plotting  $E^2$  against  $n^2$  one obtains roughly a straight line for his lower frequencies, and can calculate a  $k$  of about 0.3 msec. which is of the right order of size.

Lullies (1929, 1930*b*) and Achelis (1930), working with low frequencies on frog's nerve, confirmed the existence of an optimum frequency, while Renquist and Koch (1930) also found it on man. Gildemeister (1930), in a mathematical discussion of Nernst's theory of excitation by alternating current, examined the rapidly decaying exponential term depending on initial phase (*cf.* § X above), and concluded that this would have the greatest effect when the current began at phase zero. Buchloh (1931) examined the influence of initial phase experimentally, and found a small effect at 50–100 cycles per second, but none at 200, 300, and 500 cycles: probably he could not go to high enough frequencies, on the high side of the optimum. Blair (1932) tested an equation similar to equation (6) above, deduced from his theory of excitation, by measurement of the threshold at frequencies between 300 and 40,000 cycles per second. It was necessary to take three or four different values of  $k$ , in the different frequency ranges, in order to obtain a fit; the values of  $k$  deduced from the lowest frequency range were of the right order of size. Coppée's experiments (1934*a*, *b*, *c*) have been fully discussed in various Sections above.

The experiments on frogs' nerves described in this paper fit the present theory very well within a range of frequencies ending usually at 400–1000 cycles per second, depending on the temperature. Above that range, other factors, discussed in § XI, are involved. The papers cited above dealt chiefly with the higher range, and had no "base-line" of a satisfactory theory, applicable to the lower range, from which they could start. Nernst's deduction that  $I$  should be proportional to  $\sqrt{n}$  obviously does not fit the facts. Moreover, at least until recently, the experimental

technique has been difficult, and liable to various errors and disturbances. For a quantitative treatment of the problem most of the older results are of doubtful value. Qualitatively the more important facts were described by von Kries more than 50 years ago.

We doubt whether the experiments recorded hitherto, on stimulation of human nerves and sensory end organs, have much quantitative significance. The large electrical capacity of the skin, and the certainty that capacitative effects in the tissues will affect current distribution as frequency is altered, render deduction from human experiments very difficult. We have made a number of such experiments ourselves, using an amplifier with the oscillator described in § II, and measuring directly the actual current passing into the body with a rectifier and Moll galvanometer; readings were quite easily made and the thresholds were sharp, but the results fitted no particular theory and we could deduce nothing from them. It is not easy to see how changes in current distribution in the tissue, with changes of frequency, can be avoided when membranes of high resistance and considerable capacity lie in all directions in the path of the current. The impedance of the nerve sheath itself may be less at a higher frequency, the current traversing the tissue therefore may more readily flow through the nerve, and so the threshold tends to rise with frequency less rapidly than it should. The stimulation of sensory end organs in the skin might be a more hopeful method of research, except for the presence in their immediate neighbourhood of the large electrical capacity of the skin itself. There can be little security that the current takes the same path at different frequencies, and if not the apparent accuracy of such experiments is illusory. We had hoped that a stable experimental object, viz., the human nerve or sensory organ, would provide rather special opportunities of precise measurement. Actually we have been forced to conclude that the experiments made on frogs' nerves give much more useful information than any which we could obtain on man.

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#### SUMMARY

The theory of nerve excitation by alternating current is discussed. The relation between  $I$  and  $\log n$  should be a symmetrical curve with vertical axis and vertex at the optimum frequency. The optimum frequency

depends on the product  $k\lambda$ , the shape of the  $I$ - $\log n$  curve on the ratio  $k/\lambda$ , where  $k$  and  $\lambda$  are the time constants of excitation and accommodation respectively.

The relation between  $I$  and  $n$  can be considered in two regions, (i) below, and (ii) above the optimum frequency. In (i) there should be a nearly linear relation between  $I^2$  and  $1/n^2$ , from the slope of which  $\lambda$  can be determined. In (ii) there should be a nearly linear relation between  $I^2$  and  $n^2$ , from which  $k$  can be calculated.

Experimental methods are described in detail. The agreement of the theoretical  $I$ - $n$  curve with observation is good, and the values of  $k$  and  $\lambda$  found with alternating current agree closely with those determined by other methods.

The "true" rheobase deduced from alternating current measurements is, on the average, precisely the same as the rheobase observed with repetitive constant current pulses of sufficient duration. The agreement is probably due to a balance between accommodation and electrotonus.

When the inter-electrode distance is reduced,  $k$  is diminished. The effect is the same whether  $k$  is obtained by condenser discharges or by alternating current. A reduction of inter-electrode distance has little, if any, effect on  $\lambda$ .

The optimum frequency increases, with a  $Q_{10}$  of about 2.5, when the temperature rises. Experimental values agree with those calculated from  $k$  and  $\lambda$ .

The value of  $\lambda/k$  is not constant. It varies widely from one nerve to another, it depends upon the Ca-ion concentration, the inter-electrode distance and probably on the temperature. In the present investigation, values from 7.3 to 45 have been observed. By depriving the nerve of calcium far higher values can be obtained, but these are not suited to measurement by alternating current.

The theory predicts the possibility of a single initial excitation by a current below the steady threshold, depending on the phase at "make". This has been verified experimentally, both above and below the optimum frequency.

The effect of frequency as such on the threshold has been investigated with extremely short condenser discharges of constant form but of variable frequency. It is very small. The apparent contrast of this result with the rather large effect of frequency on the maximal response is discussed.

The relation observed between  $I$  and  $n$  with alternating current usually begins to deviate from that predicted by the theory at a frequency of 400-1000 cycles per second, depending on the temperature. This deviation

is not due to the effect of frequency as such, nor can it be caused by failure of the theory to deal with currents of durations corresponding to the frequencies in question. The causes of the deviation are discussed.

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## Further Observations on the Oestrogenic Activity of Synthetic Polycyclic Compounds

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In a former communication (Cook, Dodds, Hewett, and Lawson, 1934) we recorded the oestrogenic activity of a number of polycyclic aromatic and hydroaromatic compounds. The most active substances were found among a series of diols related to the carcinogenic hydrocarbon, 1:2:5:6-dibenzanthracene. We have now extended our investigation to seven new members of this series and have also examined a variety of diols of analogous structure, but containing other ring systems.

Table I summarizes the results obtained in tests for oestrogenic activity carried out with 9:10-dihydroxy-9:10-dialkyl-9:10-dihydro-1:2:5:6-dibenzanthracenes. In none of the new compounds has the activity exceeded that of the most potent compound previously described, namely, the di-*n*-propyl derivative in this series. Among the straight-chain dialkyl compounds this di-*n*-propyl compound represents a peak of activity in a series of which the first (methyl) and higher (*n*-amyl, *n*-hexyl) members are inactive. Two branched-chain compounds (*iso*-propyl, *iso*-butyl) are fairly highly active, but show diminished activity by comparison with the corresponding straight-chain isomerides. This is particularly marked for the *iso*-propyl compound, which is about ten times less active than the *n*-propyl compound. In view of the high order of activity of the *n*-propyl compound, it is remarkable that the allyl