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SUBTHRESHOLD POTENTIALS IN MEDULLATED NERVE

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The mode by which impulses are generated in medullated axons has been the subject of much controversy. There are two conflicting views: (*a*) According to the schools of Erlanger and Kato (see e.g. Blair, 1938; Tasaki, 1940) the impulse in a normal medullated nerve fibre starts at the point of stimulation in an all-or-none fashion, and a subthreshold electric stimulus produces only local changes which are directly proportional to the applied current intensity. (*b*) Opposed to this is the view that the nerve membrane can be partially excited, and that a weak non-conducted response is elicited by subliminal electric stimuli (Rushton, 1937; Katz, 1937, 1939*a*, *b*).

The supporters of the first view claim that there is no sign of a local action potential, nor of any non-linear reaction of the nerve to applied stimuli, until the stimulus is strong enough to elicit a transmitted response. The supporters of the second view have presented evidence which indicates that the excitatory process at the cathode increases in a progressive, non-linear fashion as threshold is approached.

The state of the controversy was reviewed by Katz (1939*b*). The evidence for a non-conducted, graded, response was complete in the case of non-medullated nerve (Hodgkin, 1938), but not of medullated nerve, where the direct demonstration of a local action potential had not been possible. It is true that with an integrating sensitive galvanometer and high-frequency alternating stimuli, a local negative potential, of non-linear characteristic, was observed, but its time course was not known. It might conceivably have been a slow permanent depolarization rather than a summation of brief changes as required by the excitability measurements (Katz, 1937).

A further attempt has been made to throw light on this problem, with a method based on the following observation. If a brief shock was applied to a medullated nerve trunk, through two electrodes only 1–2 mm. apart, there was evidence indicating that a relatively large local response was set up at the cathode which spread to either side and soon overpowered the anelectrotonic

effect at the other stimulating lead (Katz, 1937, 1939*a*). From an analysis of excitability curves on the frog's sciatic nerve (Katz, 1937, Fig. 11) a brief local response was predicted, its duration at room temperature being a little over 0·5 msec. If a recording lead were placed half-way between these stimulating leads, one might hope to balance the applied potential and the electrotonic polarization effect sufficiently to detect a small local action potential.

METHODS

Most experiments were made on sciatic nerves of English *Rana temporaria* using a stretch of nerve, proximal to its main division, which is practically free of branches. In some experiments, peroneal or phalangeal nerves were used. The nerves were soaked for ½–1 hr. in oxygenated Ringer's solution and then mounted in a moist paraffin-wax chamber on adjustable platinum electrodes. In some experiments, non-polarizable electrodes were used (Ag/AgCl connected with the nerve by a 1% Agar/Ringer tube and cotton-wick). The thin phalangeal nerves were set up in paraffin oil to prevent drying.

Stimulating and recording electrodes were arranged as shown in Fig. 1. All electrodes were adjustable, the position of lead *C* being specially controlled by a fine screw drive, which was used to obtain optimum electric balance.

The stimulating and recording apparatus were the same as described by Katz & Schmitt (1940). The stimulus consisted of a thyratron controlled condenser shock (0·05 μF discharging through a resistance pad of about 70 Ω). The current strength was varied by two resistance pads (a coarse and fine control), the output of which was calibrated with a galvanometer. The shock was applied to the nerve via a shielded air-cored transformer (making the stimulus diphasic) and a reversing switch (Fig. 1). Single or low-frequency shocks (2–3/sec.) were used and the local potential changes recorded by a balanced resistance-capacity coupled amplifier and cathode-ray oscillograph, previously described. The oscillograph deflexion due to a rectangular input voltage, applied through sciatic nerve and platinum electrodes, reached 50% of its maximum in 12 μsec. and 90% in 35 μsec. In Fig. 1, a balanced input is shown, and the earth applied to a centre-tapped potentiometer across the stimulating leads; this, however, was not essential, the result being unchanged if recording lead *C* was earthed.

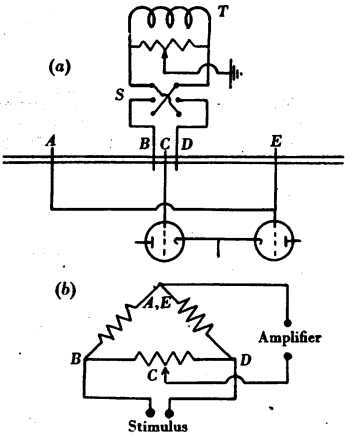

Fig. 1. Arrangement of stimulating and recording electrodes. (*a*) *A*, *C*, *E*, recording leads; *B*, *D*, stimulating leads. All electrodes are adjustable, *C* being provided with a fine control. Distances: *AC* and *CE* about 15 mm. each; *BD*, 1–2 mm.; *T*, secondary transformer coil; *S*, reversing switch. (*b*) Equivalent bridge circuit (neglecting reactances).

RESULTS

If a weak shock, e.g. 0·2 threshold, is applied and electrode *C* adjusted to give the least shock artifact, a small residual potential change is obtained, which is due to an unbalanced remainder of stimulus and electrotonic potential. It has a very rapid initial component, due to the brief applied potential (which is distorted by the stray capacities of the recording apparatus), followed by a slower and smaller potential change which is largely due to polarization in the

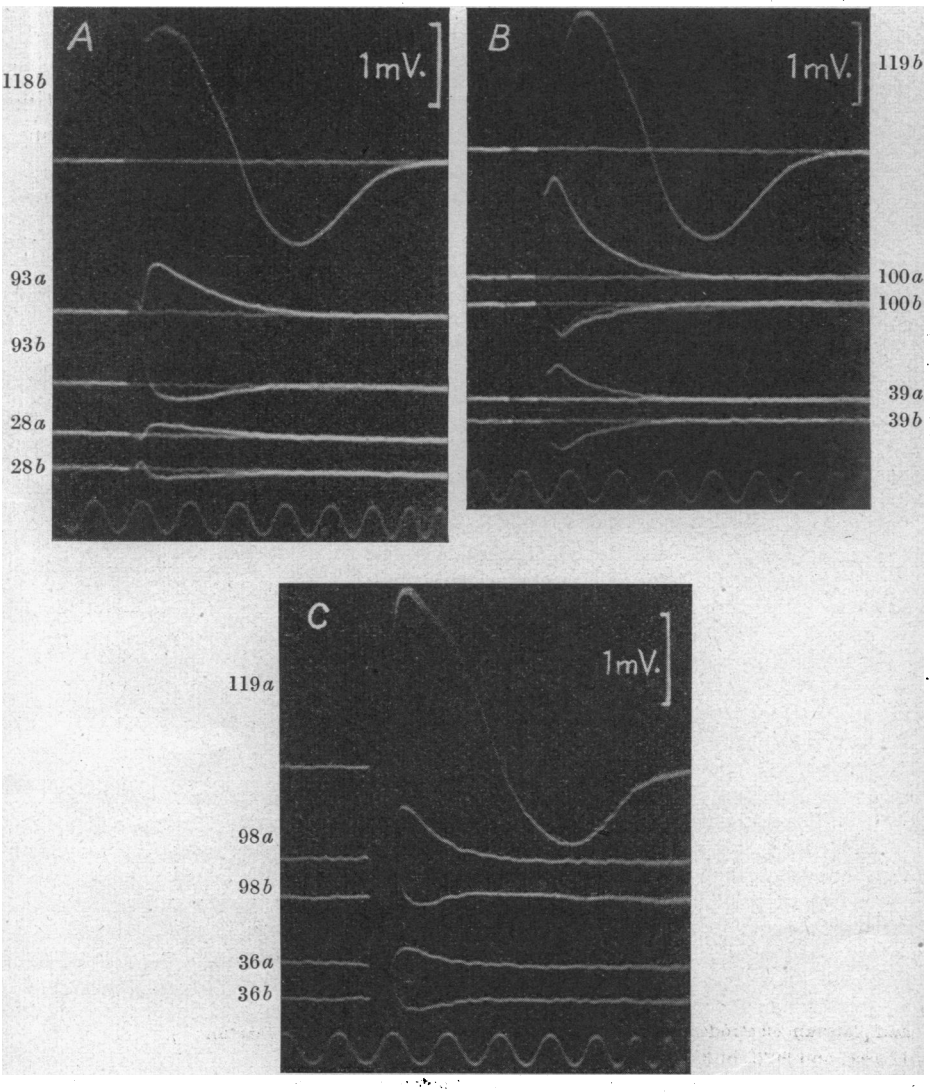

Fig. 2. Potential changes in frog's nerve due to brief shocks, recorded with set-up of Fig. 1. Negativity at lead *C* gives upward deflexion. *A* and *B*, sciatic nerve at 24° C. Two different positions of lead *C* (cf. Fig. 1). Several sweeps are superimposed. Opposite polarities of shocks are indicated as (*a*) and (*b*). Strength of shocks in relative units (the strength at which least diphasic spike was seen is taken as 100). *A*, successively from above: 118 (*b*), 93 (*a*), 93 (*b*), 28 (*a*), 28 (*b*). *B*, 119 (*b*), 100 (*a*) (subthreshold), 100 (*b*) (threshold, a small diphasic spike appears occasionally), 39 (*a*), 39 (*b*). *C*, another sciatic nerve at 23·5° C. Single sweep records, from above: 119 (*a*), 98 (*a*), 98 (*b*), 36 (*a*), 36 (*b*). Time-base, 5 kcyc./sec.; voltage scale, 1 mV.

nerve. The initial artifact lasts about 0·1 msec., sometimes less, depending upon the effectiveness of the balancing arrangement. After that, the records can be measured accurately and scrutinized for any 'non-linear' negative potential change. The crucial test is whether, on reversing the shock, the residual potential recorded at *C* reverses symmetrically, or whether there is an asymmetrical change which has the characteristics of a local response.

The results are illustrated in Figs. 2 and 4. It is seen that with weak stimuli, of 0·3 threshold and less, there is an almost perfect symmetry of the local

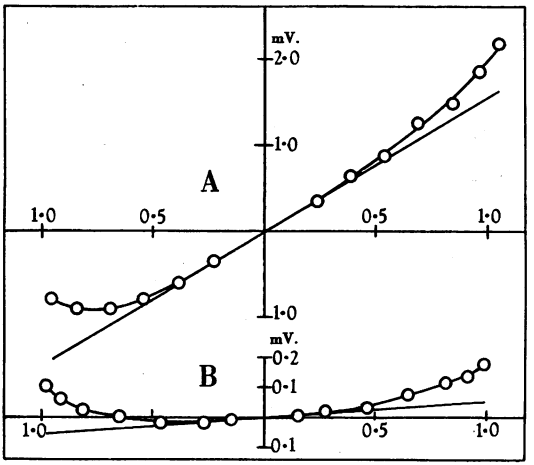

Fig. 3. Local potential changes in frog's sciatic nerve. *A* and *B*, two typical experiments, with different degrees of 'shock balance'. Ordinates: local potential change at a given brief interval after the shock (0·1–0·15 msec.), negativity plotted upwards. Abscissae: shock intensity, in relative units, threshold of least diphasic spike being taken as unity.

potential changes, while with stronger, but still subliminal shocks, a marked asymmetry appears which is due to a progressively increasing local depolarization.

If the potential is measured at a given time after the beginning of the shock and plotted against shock intensity, a characteristic curve is obtained which is approximately linear over a small range of weak intensities and, from about 0·3 threshold bends *up* towards increased negative potential independently of the direction of the current. This suggests that, from about 0·3 threshold onwards, a non-linearly increasing depolarization is added to the unbalanced remainder of shock and electrotonic potential. This was verified by slightly altering the position of lead *C*, thus causing a variation in the size and shape of shock and electrotonic potential, without affecting that of the superimposed non-linear depolarization (cf. Fig. 6). Hence, the slope of the linear portion in Figs. 3 and 4, *A* is quite arbitrary and depends merely upon the adjustment of the balancing electrode: with perfect balance, the initial slope of the curve

would be zero. If we extrapolate this linear portion, we may use it as a base-line from which to measure the size of the non-linear effect. In seventeen experiments on the frog's sciatic nerve, at about 20° C., the mean size of the local depolarization at threshold strength was 0·37 mV., varying between 0·1 and 0·75 mV.

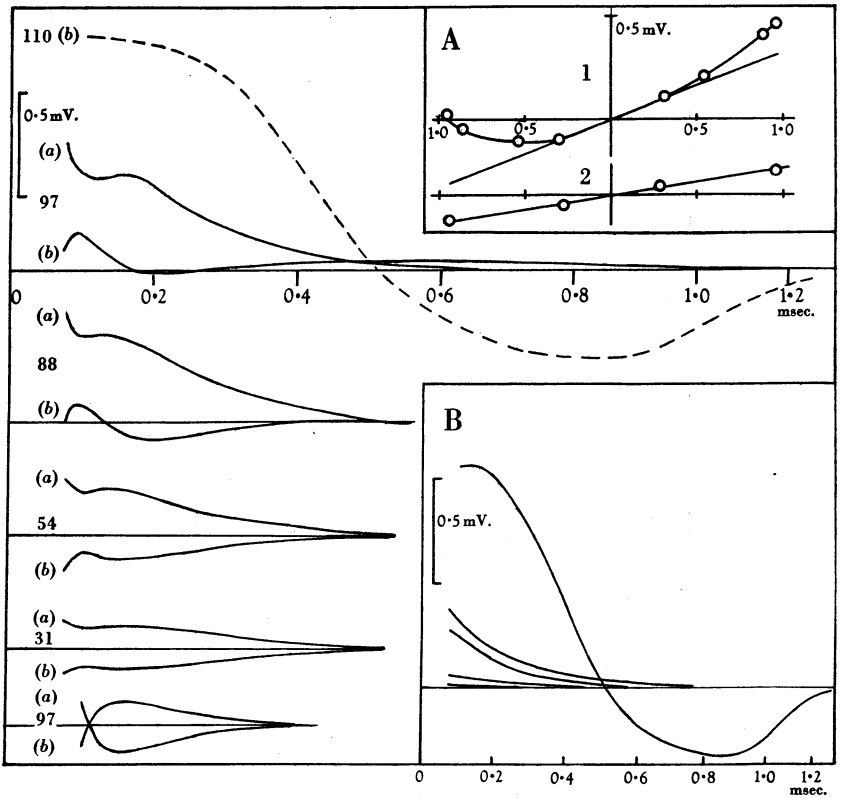

Fig. 4. Sciatic nerve at 23·5° C. Potential changes with equal and opposite shocks. Strength and polarity of shocks indicated as in Fig. 2. From above: 110 (*b*) (broken line); 97, 88, 54 and 31, (*a*) and (*b*) each. Lowest record: strength 97 repeated after crushing. Inset *A*, local potential changes plotted as in Fig. 3, at 0·16 msec. after beginning of shock. A 1: normal nerve; A 2: after crushing. Inset *B*, mean potentials, with equal and opposite shocks. Successively from above: 110; 97, 88, 54, 31. After crushing, the mean potentials become indistinguishable from the baseline.

It is clear that this local negative potential change is of the same origin as that previously recorded with a slow, integrating galvanometer (Katz, 1937, Fig. 16). In both cases, the negativity became measurable above about 0·3 threshold and increased progressively with stimulus strength. The galvanometer records, however, did not permit size or time course to be determined;

the integrated negative potential, with subthreshold alternating current at 5000 cyc./sec., was equivalent to a permanent potential change of about 50–100 μV.

To find the duration of the local depolarization, the mean of two potential changes, with shocks of equal strength and opposite sign, must be determined, as shown in Figs. 4 and 5. In most experiments, the initial part of the curve was obscured by shock artifact, and only its falling phase could be determined. The decline is approximately exponential with a half-time of about 115 μsec. (This low value applies to a small interelectrode distance, and a temperature of 23° C.) In the case of Fig. 5, a local depolarization of 0·3 mV. is obtained which rises to a peak in 65 μsec. and declines with a half-time of 105 μsec. These values are slightly too large because of a small lag introduced by the amplifier (see 'Methods'). The time course may be compared with that of the 'local response' predicted on the basis of excitability measurements (Katz, 1937, Fig. 11). It was indicated there, that for small interelectrode distance (1–2 mm.) and at room temperature, the local response with a 95% threshold stimulus rises to a peak in 80 μsec. and falls with a half-time of 115 μsec. With a stimulus of 85% threshold, the rising phase would be shortened to about 60 μsec. (cf. Katz, 1937, Fig. 4, *A*).

It was usually found that with increased stimuli, the local depolarization increased in size as well as duration (Figs. 4 and 6), which again agrees with the earlier analysis of excitability measurements (Katz, 1937).

Extensive checks were made to reveal sources of error which might vitiate these results. The non-linear depolarization is abolished by deep alcohol narcosis, or by crushing the nerve, and a strictly linear artifact only remains (Fig. 4). Other routine tests were made (varying the amplification; reversing amplifier input or output leads to check against non-linearity in the recording system; varying the position of the earth point; altering the position of the recording leads to obtain different degrees of unbalance; using non-polarizable electrodes), but they failed to reveal any significant fault.

The question arose whether the depolarization was, in fact, a subliminal and non-conducted change, or possibly due to fibres which were blocked or cut between the recording leads, or for some other reason did not give a diphasic action potential.

In the first place, however, the 'minimum threshold' indicated by the sudden appearance of a diphasic spike, is usually well defined, and can be determined within about 5% of stimulus strength, even on the whole sciatic nerve and with moderately high amplification (see Fig. 2). The critical threshold value can then be verified by using higher gain. At amplifications of about 10^6, the 'threshold play' of single, or a few synchronous, spikes can be seen in many sciatic nerves quite clearly and leaves no doubt as to the critical threshold of the most excitable fibre, or group of fibres. As the 'non-linear

effect' becomes measurable with stimuli of little more than one-third of that strength, conducted impulses can hardly be involved.

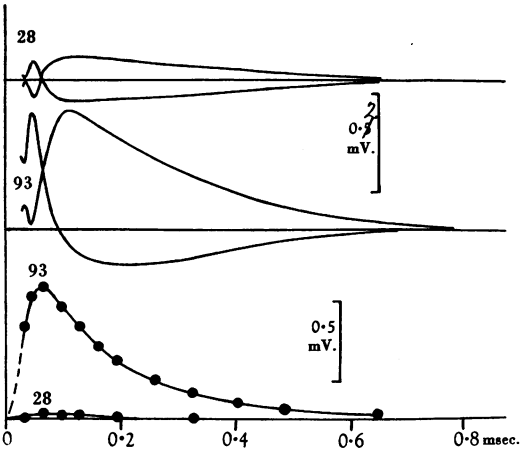

Fig. 5. Sciatic nerve, 24° C. From above: local potential changes with shock intensities 28 and 93, and mean potentials.

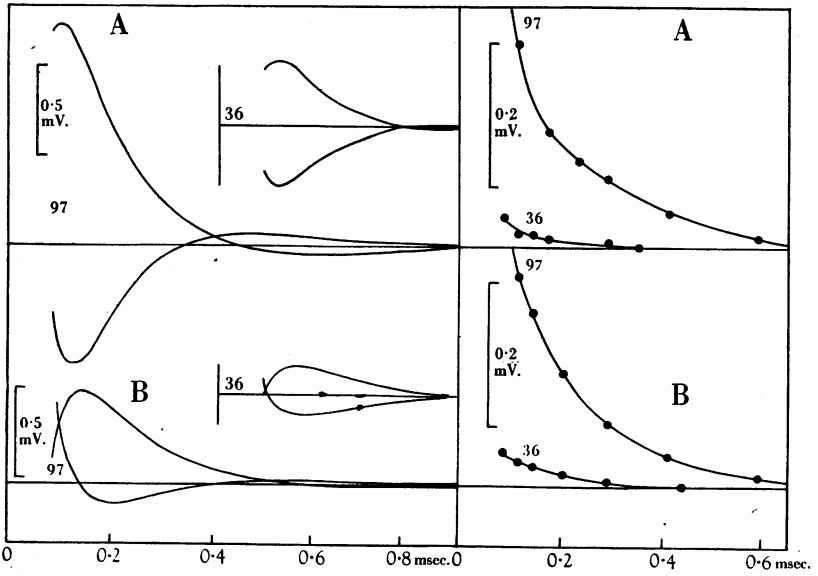

Fig. 6. Effect of varying the balancing position of lead *C* (cf. Fig. 1). Left part of figure shows recorded potential changes with strength 97 and 36. Different balancing positions in *A* and *B*. Right part shows the respective mean potentials, which are not appreciably affected by altering the balancing point.

Moreover, the non-linear negativity shows a typical spatial decrement: if the distance between lead *C* and the stimulating electrodes is increased to

about 2 mm., the local depolarization at *C* is reduced to about two-thirds, and if the distance is made greater than 6 mm., it is abolished.

One may, therefore, conclude that in the frog's sciatic nerve, subliminal stimuli produce a potential change which has the characteristics of a local response and which, in its electric sign, its non-linear growth and relation to threshold strength, in its time course and spatial spread, corresponds with the local responses described in earlier papers.

Several experiments were made on the peroneal and phalangeal branches of the sciatic nerve, but these gave much less clear-cut results. In two experiments on peroneal nerves, a local response of 0·3 and 0·1 mV. was obtained. In two out of six experiments on phalangeal nerves, a non-linear depolarization was seen with certainty (0·1 and 0·05 mV. respectively); in the other cases, local responses were either absent or so small as to be of doubtful significance.

This discrepancy between different nerves may be due to the fact that the aggregate spike potential of the phalangeal nerve is much smaller than that of the sciatic (e.g. at twice threshold strength, one obtains 3–4 and 15–20 mV. respectively) and that the phalangeal nerve contains relatively few large fibres (Blair & Erlanger, 1933) whose thresholds, moreover, are widely separated. It is true that the minimum transmitted response in the thin phalangeal nerve is fairly large and discrete, giving an all-or-none spike of about 0·25 mV., but a 10 or 20% stronger shock may be needed to bring in more fibres. In the sciatic nerve, although the threshold response is very small, a large number of axons are brought in at a 10 or 20% greater intensity, giving a much larger spike, and all these fibres are responsible for a portion of the subliminal 'local response'. As the applicable stimulus intensity is limited by the fibres of lowest threshold, the chances of seeing a local response in a compound nerve are small unless it contains many, fairly uniform, large fibres, whose individual thresholds are not too far apart. The local response due to one or two large fibres only, giving a spike of 0·25 mV., may be no more than 20 μV., and this is not large enough to be recorded with certainty. That this explanation is probably correct was suggested by an experiment on a sciatic nerve which contained a few unusually excitable fibres, their threshold being separated by a large step from the rest of the nerve. In this nerve, only a very small local response could be seen, as the applicable stimulus strength was limited by the threshold of the hyperexcitable fibres and, therefore, was very weak relative to the majority of the fibres. The matter has been discussed at some length, as it has probably some bearing on Blair's (1938) consistent failure to observe a local response in the frog's phalangeal nerve.

DISCUSSION

The result of this investigation is in complete agreement with the findings previously described (Katz, 1937, 1939*a*) and shows that the non-linear increment of excitability at the cathode is accompanied by a brief depolarization of the same temporal and spatial characteristics. The size of this local response, relative to the full-grown spike, cannot be determined in multi-fibre preparations, but it is probably rather less than 10% of the individual fibre spike.

The present observations are in contrast with statements by Blair (1938) and Tasaki (1940) disclaiming the existence of any non-linear effect of subthreshold stimuli on medullated axons. Blair worked exclusively on the frog's phalangeal nerve where the chances of observing a local response are small. He used a common recording and stimulating lead of about 2 mm. effective width (cf. Blair & Erlanger, 1936), which would reduce the local potential changes owing to electrotonic decrement.

Even so, Blair's consistent failure to observe any non-linear effect might have had something to do with his particular method of testing for it (Blair, 1938, Fig. 2). By *halving* the intensity of a near-threshold shock and *doubling* the amplifier gain, he obtained two records, which matched well during their later part, but differed during the initial shock artifact, which was consistently larger with the weaker shock and higher gain. Blair dismissed this discrepancy as insignificant and ascribed it to an artifact. This may be justified, but as an alternative possibility, the discrepancy may have been due to a small calibration error causing the shock to be a little more than one-half, or the amplifier gain slightly more than double, which would significantly affect his argument.

Tasaki (1940) emphatically denied the existence of a local response, though it is not clear on what particular piece of evidence that statement was based. In a more recent paper, Tasaki & Takeuchi (1942) admit the possibility of a partial excitation of the nerve membrane, but claim that according to 'theoretical considerations' the accompanying potential would be too small to be seen. No calculations are given to support this statement which seems to depend upon faulty speculations. Working on an isolated frog's nerve fibre, Tasaki & Takeuchi (1942, pp. 765–8) actually record potential changes which have the characteristics of a local response (non-linear increase up to threshold; duration about 0·5 msec.; size nearly one-sixth of a 'uni-nodal' spike which, at threshold, takes off from it; abolished by urethane). Tasaki & Takeuchi conclude, however, that this cannot be a local response, because according to their theory, the latter should be immeasurably small. As an alternative, they suggest that the subthreshold potential change is due to 'action currents of the connective tissue' or perhaps of some invisible sympathetic fibres, but one may be permitted to wonder whether the authors themselves take this strange argument very seriously. Invisibly small residual nerve fibres would neither

have the required low threshold, nor give a fair-size action potential, compared with the isolated motor axon; and connective tissue which gives action potentials indistinguishable from a local response of the nerve, still remains to be discovered. If not their interpretation, their experimental result itself, obtained on a skilfully dissected single motor axon, certainly falls into line with all the other positive evidence of a non-conducted response and is, in fact, an excellent confirmation.

Tasaki & Takeuchi (1942, Fig. 2) measured a 20% lowering of threshold at an adjacent node of Ranvier, accompanying the local potential change. They regard this as unduly small and believe that there is no connexion between potential and excitability change. Considering, however, that the local action potential was less than one-sixth in amplitude, and less than one-eighteenth in potential-time area, than the spike, it is difficult to follow their argument.

An interesting feature of the local response is its relation to stimulus intensity (Fig. 3). There is no definite point at which the local response starts, but merely a small initial curvature which becomes noticeable at about 0·3 threshold. Apparently we are dealing, not with a membrane response of critical threshold, but with some continuous non-linear function of the nerve membrane which eventually, at propagation threshold, leads to a self-regenerating or 'chain' reaction (cf. Appendix).

Local depolarization is not the only non-linear effect produced by subthreshold stimuli. It has been shown (Cole & Baker, 1941; Katz, 1942) that the impedance of nerve and muscle is a continuous, non-linear function of the applied current, and that the membrane conductance increases at the cathode as the resting potential is diminished and, vice versa, decreases at the anode. It is natural to suppose that the two non-linear processes, viz. changes of membrane conductance, or ion permeability, and of subthreshold potential, are somehow related. A simple reaction by which they might be linked, and which might lead to a progressive chain-reaction, has been outlined elsewhere (Katz, 1942).

It must be pointed out that the presence of a non-ohmic membrane resistance which is reduced at the cathode does not, *in itself*, lead to an excess depolarization. On the contrary, a mere reduction of resistance at the cathode would cause the catelectrotonic potential to be *smaller* than the anelectrotonic. One would have to assume some secondary change (cf. Katz, 1942, p. 180) to find a causal connexion between the two phenomena.

SUMMARY

1. Blair (1938) and Tasaki (1940) have insisted that the nerve impulse in normal medullated axons is generated in an all-or-none fashion, that the effects of subliminal shocks are strictly proportional to their intensity, and that a local response to a subthreshold stimulus does not exist.

2. The matter has been re-examined by recording the subthreshold potential changes in the frog's sciatic nerve, resulting from brief single shocks. Using a small distance between the stimulating electrodes and a bridge-like arrangement of the recording leads, it is possible to reduce shock artifacts and electrotonic potentials sufficiently to search for a non-conducted response.

3. With weak shocks, up to about one-third of threshold, the local potential change (i.e. unbalanced remainder of shock and polarization potential) increases linearly with shock intensity. Above that strength, a small local depolarization is observed which increases more than linearly as the stimulus intensity is raised. At threshold, the amplitude of this local negative potential is about 0·35 mV. (mean of seventeen experiments) in the sciatic nerve. This potential is abolished by killing or deeply narcotizing the nerve.

4. The time course of this depolarization is brief: at 24° C. and with an interelectrode distance of 1·5 mm. it rises to a peak in about 65 μsec. and falls approximately exponentially, with a half-time of about 110 μsec. This compares well with the predicted time course of the 'local response' previously obtained by analysis of excitability curves.

5. The results confirm previous findings indicating the existence of a local response to subliminal stimuli in medullated nerve. The experimental basis of contrary statements in the literature is discussed.

It is a pleasure to thank Professor A. V. Hill for helpful discussion and criticism, Mr J. L. Parkinson for his invaluable technical assistance and Mr B. C. Abbott for 'reconditioning' the electronic equipment.

APPENDIX

To illustrate what is meant by 'partial excitation' and the 'chain reaction' mentioned above, the following model may be considered (Fig. 7). The membrane element r ('ion permeability') is assumed to vary as a function of the potential p across the membrane and to have the non-linear characteristic shown in Fig. 8, A. All other components have fixed values. The resting membrane has a resistance r_0 and a potential difference p_0. It is clear from Fig. 7 that any change of r will be followed automatically by a change in p, while—according to Fig. 8, A—any change of p will also cause a change of r. At a certain point of the curve, depending upon its attaining a critical slope, the process becomes 'explosive'.

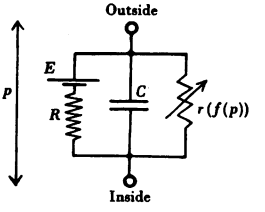

Fig. 7. Electric model of a membrane element.

Let us consider the case of a very brief shock, by which the membrane charge is instantly altered and then left to itself, without any external current flowing. The rate at which the membrane potential changes, after the shock, is then given by

$$-C\frac{dp}{dt}=\frac{p}{r}+\frac{p-E}{R}, \qquad (1)$$

where r is a function of p. To simplify calculations, we assume R to be large compared with r. We then obtain $p_0 = Er_0/R$, and from equation (1)

$$C\frac{dp}{dt} = \frac{p_0}{r_0} - \frac{p}{r}. \tag{2}$$

In Fig. 8, B, dp/dt is plotted as a function of p. It is seen that as the membrane potential p is reduced below the resting value p_0, it tends, at first, to

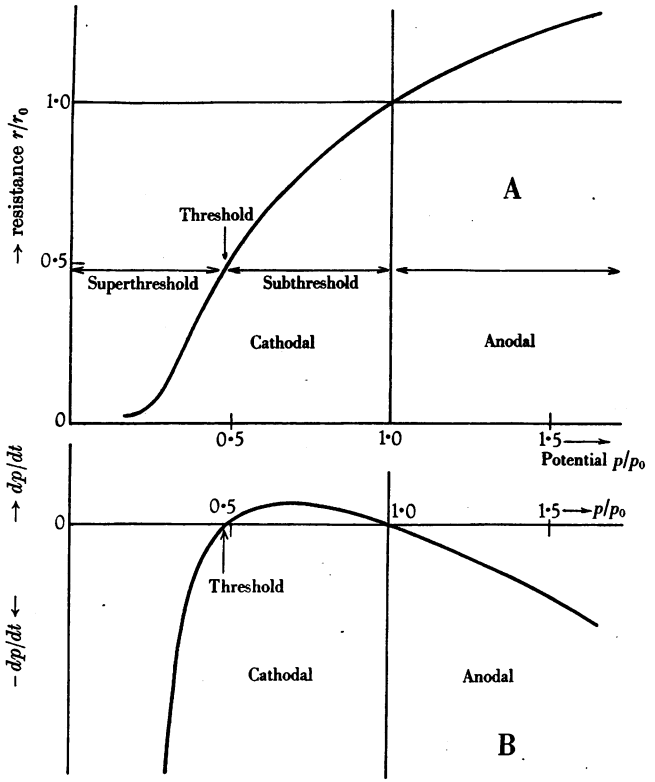

Fig. 8. *A*, assumed relation between r and p; *B*, rate of potential change dp/dt plotted against p.

return to the resting level. Beyond a certain point, this return becomes slower; at a critical point ('threshold'), the depolarization maintains itself and, a little beyond it, becomes progressive. If the membrane potential has been raised above the resting level (at the anode), it always tends to return to it.

Equation (2) can be integrated, e.g. by using an empirical formula expressing r as a function of p (Fig. 8, *A*) and operating in small successive steps. One then obtains a family of curves showing the potential changes following brief shocks of various intensities (Fig. 9). It is clear that these curves are very similar to those described by Hodgkin (1938) and Katz (1937).

Needless to say, the present scheme suffers from the usual limitations of an *ad hoc* model, in that it illustrates only one particular aspect of electric excitation. It takes no account of recovery or of steady state phenomena, nor of the fact that the action potential is greater than the resting potential (Hodgkin & Huxley, 1945).

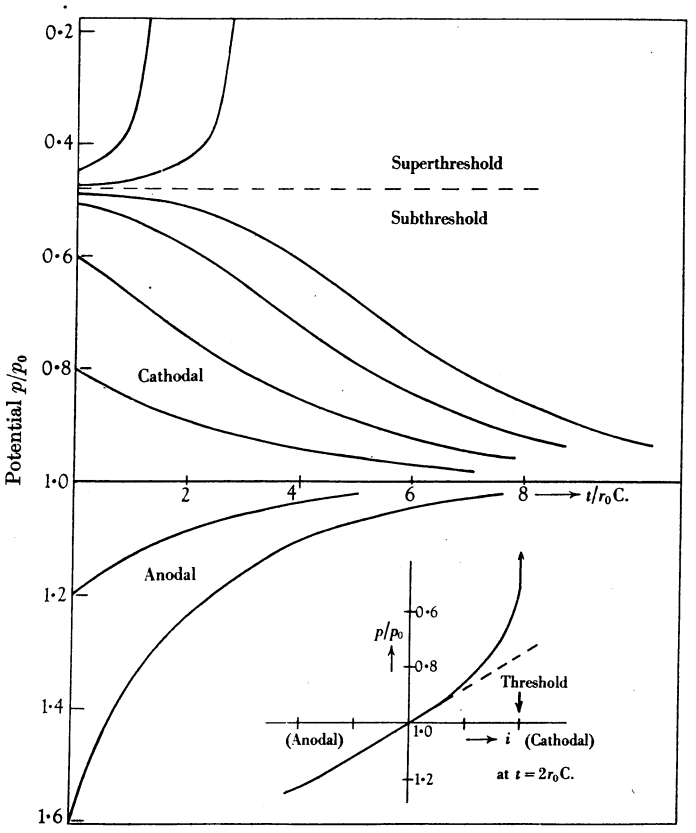

Fig. 9. Time course of membrane potential following brief shocks of various intensities. Inset, potential change, at a given time interval ($t = 2r_0 C$) plotted against relative shock intensity.

There is another objection. Impedance measurements with alternating current (Cole & Baker, 1941; Katz, 1942) have shown that the membrane resistance decreases at the cathode, when an applied stimulus approaches threshold. But Fig. 9 (inset) shows a relation between voltage and current which curves upward, and this would seem to require that the membrane resistance, as a whole, should increase at the cathode. As Fig. 9 agrees with the data obtained by Hodgkin (1938) and others, there seems to be a curious conflict between the two sets of observations. It should, however, be borne in mind: (*a*) that the relation between an applied current and a transient potential

change is not the same as an impedance measurement, made during the potential change, with alternating current of relatively high frequency, and (*b*) that, in the case of Fig. 9 (inset), *i* refers to a brief initial pulse and *p* to a potential change measured after a certain interval, so that little light is thrown on the value of the membrane resistance. Nevertheless, there remains an apparent discrepancy which has not been satisfactorily resolved.

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