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THE RELATION OF STRUCTURE TO THE SPREAD OF EXCITATION IN THE FROG'S SCIATIC TRUNK

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In the past a great many attempts have been made to explain quantitatively the spread of current and of excitability about electrodes on a frog's nerve trunk in terms of the electrical properties of a simple cable consisting of a resistant (and capacitative) cylindrical membrane separating a conducting core from a conducting outside medium. We have shown (Rashbass & Rushton, 1949*b*) that in at least three important respects the excitability of the sciatic trunk does not behave as it would if this were so. For the excitation does not always arise at the cathode, the excitability does not fall away exponentially on either side of a single electrode, nor are the results the same when the nerve is stimulated by bipolar electrodes as by the symmetrical tripolar arrangement. In the present paper we attempt to show how these results can be explained in terms of the electrical properties of the structures of the nerve trunk.

In 1926 Bishop, Erlanger & Gasser drew attention to the appreciable electrical resistance (and capacity) of the nerve's connective tissue sheath, and suggested that 'many of the properties of the nerve as usually measured may be in fact the property of this non-nervous structure'. Although since then various workers (see Discussion) have given differing significance to the part played by the epineurium, its contribution to the spatial characteristics of nerve has not been extensively investigated. In the first part of this paper we show that when the epineurial connective sheath has been removed, the nerve follows closely the expectations of the simple cable theory, and in the second part we show by what property the connective tissue brings about those deviations from the simple theory which are exhibited by the unstripped nerve.

Part 1. The spread of excitation in stripped nerve

Methods

Dissection. The sciatic nerve of the frog was dissected out from the cord to the end of the peroneal branch, the tibial branch being ligatured and cut short, but the branches to the hamstrings left rather long. The nerve was placed on the stage of a dissecting microscope and illuminated

from above. The ligatures on the central and the cut tibial branch were held down by clips, keeping the main trunk under a slight degree of tension. The removal of the epineurium, performed under the microscope, was carried out entirely by means of two steel needles with sharp points. These were used to cut the sheath either by compressing it downwards against the microscope stage, or outwards one needle against the other. The first incision of the sheath was made in the fork between the hamstring branch and the main trunk. The cut was extended right round the trunk, dividing the sheath in two, and then a longitudinal slit was made down to the bifurcation at knee level. The epineurium could then be removed from 15 to 20 mm. of the thigh region.

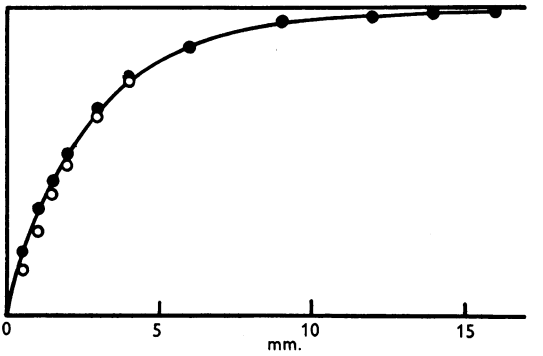

Fig. 1. Excitability curve of stripped nerve. Abscissae: interpolar length. Ordinates: excitability (=reciprocal of current strength for $\frac{1}{4}$ maximal response).

Experiment. The nerve, prepared in this fashion, was embedded from knee level upwards in a cylinder of agar jelly, and set up in the apparatus which has been previously described (Rashbass & Rushton, 1949*b*). In the majority of experiments where the stripping and embedding of the nerve were accomplished without mishap, these operations resulted in no appreciable reduction of the maximum action potential recorded from the peroneal branch. For this and other reasons to be mentioned later, we believe the nerve to be quite healthy despite these manipulations. The rest of the experimental procedure, which was the same as in our previous experiments on unstripped nerves, consisted in measuring the threshold for bipolar and symmetrical tripolar stimulation with various electrode separations.

Results

It will be recalled that the simple cable theory requires that the excitability falls away on either side of a single pole according to the exponential curve $e^{-x/\lambda}$, where x is the absolute distance away from the pole and λ is the space constant. Thus excitation will always occur at the cathode, and by the Superposition Theorem, bipolar and symmetrical tripolar thresholds will be the same (Rashbass & Rushton, 1949*b*). If then the excitability curves are scaled to have a maximum value 1, they should both coincide with the exponential $1-e^{-x/\lambda}$. Now the results of one experiment upon a large Swiss variety of *Rana temporaria* are plotted in Fig. 1. Dots show the bipolar excitability; circles, the symmetrical tripolar excitability. The curve $1-e^{-x/\lambda}$ is also drawn where $\lambda=2{\cdot}8$ mm., chosen to give the best fit. The curve fits the bipolar excitability closely, but the tripolar points are seen to lie below the curve for interpolar distances less than 4 mm., though the divergence is very small when

compared with the corresponding curves (Fig. 2) taken from the same nerve before removal of the epineurium (see Part 2). Latency measurements, in the experiments in which they were determined, showed that at small electrode separations, the point of excitation was still displaced from the cathode, but to a rather less extent than when the connective tissue sheath was intact. These results indicate, therefore, that the nerve, after removing the epineurium, behaves very much more like a simple cable than before.

There are several factors which may contribute to the discrepancies which remain. (*a*) The connective tissue is never entirely removed. However efficiently the main sheath is dissected away, there always remain septa of connective tissue which pass among the nerve fibres in the trunk, subdividing it into smaller bundles. These bundles Tasaki (1939*a*) found to affect current distribution, and they may, in part at least, contribute to the slight divergence

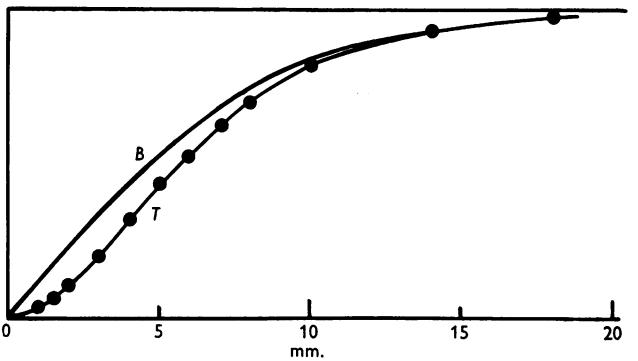

Fig. 2. Excitability of the nerve before stripping plotted as in Fig. 1. *B*, bipolar stimulation; *T*, symmetrical tripolar stimulation.

between the two excitabilities of Fig. 1. (*b*) Though the change in potential gradient at the electrode is sharp (Fig. 2, Rashbass & Rushton, 1949*b*) it is not perfect, so that the tripolar stimulus is physically not precisely the superposition of two dipolar ones. (*c*) The fibres in the centre of the nerve are surrounded by others, whose effect would be to enhance (*a*). We believe this contribution to be very small (see end of Part 2). (*d*) Probably the most important and certainly the most interesting consideration arises from the fact that fibres are only excitable at the nodes of Ranvier (Kato, 1934, 1936; Tasaki, 1939*a*, *b*). It is thus clearly insufficient to consider simply at which point in a cable the maximum depolarization occurs, we must consider which *node* is most depolarized. This distinction is not important in unstripped nerve since here the excitability alters only gradually with distance. But in the experiment of Fig. 1, we should certainly expect that the excitability relations of each nerve fibre would depend significantly upon the proximity of its nearest node to the cathode. Moreover, the variation among fibres in this respect

will account qualitatively for the observed deviation from the simple cable expectations.

For, in bipolar excitation with the anode close to the cathode, though the excitability will be maximal exactly at the cathode, it will fall away much faster towards the anode than away from it, consequently the majority of the nodes will be excited in the region just extrapolar to the cathode. This will account for the observed latency shift, and the inexactness of comparing bipolar and tripolar excitations by superposition. But the tripolar conclusions are more striking. As the two anodes approach the cathode on either side, they diminish the cathodal stretch of nerve, and sharpen the peak of excitability. Distant nodes become relatively less easily excited, and eventually are excluded from the cathodal stretch entirely. A proportion of the nerve fibres will in this way become inexcitable. Thus three conclusions follow when the anodes are close. (i) The tripolar curve will lie below the bipolar (Fig. 1). (ii) The divergence will be more, the greater the size of action potential selected as index of excitation. (iii) There will be a reduction in the maximal action potential obtainable owing to the exclusion of some fibres.

These three phenomena have been regularly observed. But clearly many factors besides the distribution of nodes may enter into their interpretation.

Asymmetrical tripolar stimulation

Tasaki (1939*b*) has stimulated a single nerve fibre through three electrodes X, O, Y applied to consecutive nodes. Simultaneous current pulses through X and Y could be independently adjusted with regard to strength and direction, the sum of the currents returning through O. For arbitrary values of the current x (through X) the value of y (through Y) was found which was just threshold. Then y plotted against x in the usual way resulted in a triangle, each straight line corresponding to excitation at one of the three nodes. In our previous paper (1949*b*) we performed the same experiment upon the whole (unstripped) nerve, and, for interelectrode distances of 1 or 2 mm., got results quite different from Tasaki's.

It now seems natural to explain the difference in terms of the connective tissue sheath, for we have seen in Figs. 1 and 2 above how by stripping, we change the symmetrical tripolar curve very nearly to the expectations of the simple cable theory. Moreover, we have shown that the Superposition Theorem, applied to the curve of Fig. 2, will accurately predict our non-triangular results with unstripped nerve, and Rushton (1928) proved that the simple cable theory always predicts a triangle, such as was found by Tasaki.

We have therefore repeated upon stripped nerve the experiments of the previous paper, and Fig. 3 shows the results upon the same nerve which yielded Figs. 1 and 2, the interelectrode distance $XO=OY$ being 1, 2, 3, or 4 mm. As before the observed results are plotted above the 45° line ($x=y$);

below it is plotted the expectation from the simple cable theory when $\lambda = 2{\cdot}8$ mm., as found in Fig. 1. The shape of these triangles therefore is completely determined. If observations fitted this theory exactly, the points above the line would be precisely a reflexion of the curve below. In fact, there are two kinds of deviation: (*a*) In the first quadrant (where both X and Y are

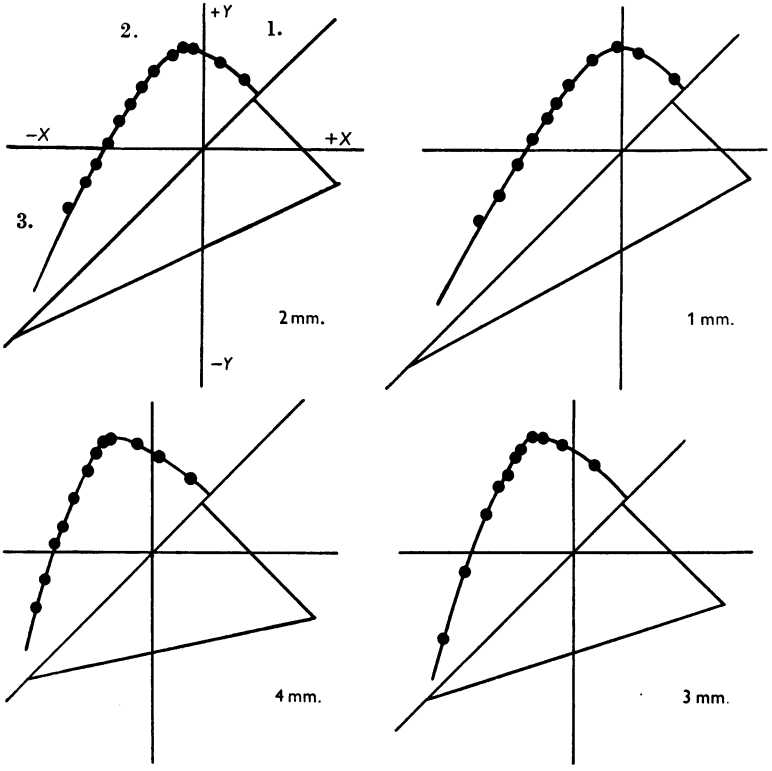

Fig. 3. Results of stimulating the stripped nerve with tripolar electrodes with distance $OX = OY = 1$, 2, 3, 4 mm. respectively. Current through X plotted against Y for threshold. Only the experimental points above the 45° line are shown. Below are the theoretical results calculated from the simple cable theory. Upon the 2 mm. curve the axes and quadrants are labelled according to the usual convention.

anodes) the threshold is higher than expected. This we have already related to the distribution of nodes about the cathode, for it is the case of anodes close on either side of the cathode. (*b*) In the second quadrant, the corner of the triangle is rounded off, and the threshold is lower than expected. In theory this corner is where excitation arises simultaneously from electrodes O and X. When O and X are close together, therefore, this region will correspond to a specially long cathodal stretch of nerve, and the decline of excitability from the two peaks at O and X will be more gradual than usual. Thus the same

factors in node distribution which raise the threshold in region (*a*), lower it in region (*b*). Residual connective tissue, of course, will equally account for the deviations from the simple cable results.

Anodal effects in stripped nerve

Anodal effects do not concern the main purpose of this paper so long as we are satisfied that the excitability concerned is the cathodal 'make' and not the anodal 'break', and also that no impulse arising at the cathode fails to reach the recording leads on account of anodal block. Both these effects are negligible in the ordinary way, but they are greatly enhanced by stripping the nerve. As

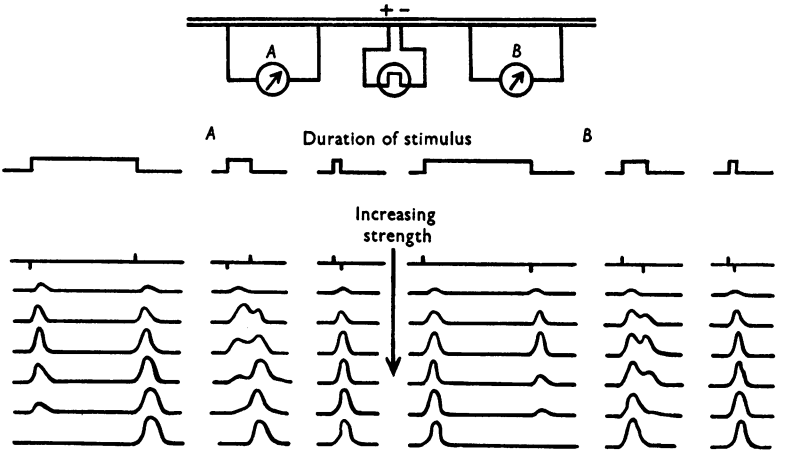

Fig. 4. Diagrammatic representation of stimulation and conduction block at the anode in stripped nerve, for currents of various strengths and durations.

the phenomenon is in itself interesting and possibly important, and since it certainly may affect the technique and interpretation of experiments with stripped nerve, we shall digress somewhat to discuss it.

Fig. 4 represents the matter diagrammatically. The left half of the figure indicates the results when the anode lies between the cathode and the recording leads; the right half, when it does not. Each half contains three columns of records corresponding to stimuli of, say, 10, 2, 0·5 msec. duration. Each column contains in diagrammatic form the action potentials seen with stimuli of various strengths. Consider first the results with 10 msec. duration of stimulus. In this case the action potential at 'make' is easily distinguished from that at 'break' because the latter arrives 10 msec. later on the cathode ray traverse. Moreover, if the instant of 'make' remains fixed on the traverse, and the duration of the stimulus is altered, the first or 'make' wave remains fixed, but the second wave moves with a constant latency behind the instant of

'break'. It will be seen from Fig. 4 that the thresholds for 'make' and 'break' are about equal, and the two waves increase to maximal in much the same way with increasing strength. But if the record is taken on the anodal side (left) the 'make' wave begins to decline soon after the maximal has been reached, and indeed sometimes before it, expecially when the electrodes are placed close together. A somewhat greater stimulus will reduce the response to zero. At this stage, by taking the record from the other end of the nerve or by reversing the polarity of the stimulus, we still obtain a full-sized action potential. So it is plain that the nerve has been maximally excited (for *A* fibres), and that all these have been blocked at the anode. The unusual susceptability to anodal block with stripped nerve is mentioned by Tasaki (1939*a*). Precisely the same thing happens in the reverse direction with anodal 'break' excitation. Here the interposition of the pole which had been the cathode produces a block which is altogether similar to the anodal block just considered. It is likely that some polarization of the thin silver-silver chloride electrodes contributed to the 'break' effects, but such polarization would of course apply equally to unstripped nerve which does not behave in this way.

As the duration of the stimulus is decreased, the threshold for 'break' rises faster than that for 'make' (as is well known); otherwise the results are the same. But it will be appreciated that when the duration of the stimulus is reduced to 0·5 msec. it is by no means obvious whether the excitation observed is cathodal 'make', or anodal 'break'. It is possible, however, to make certain of this by several methods. Latency measurements will decide between anode and cathode when the electrodes are well separated. The triangular results of Fig. 3 distinguished clearly which is the stimulating electrode and whether at 'make' or 'break' (for the isosceles triangle for 'break' is the other way up, with apex in the first quadrant and base in the third). Finally we may gradually increase the duration of the stimulus until the make and break waves are separate. It is usually easy to see that the wave to be analysed shows a smooth transition to one and not to the other of the final pair of waves.

We have applied such tests to the measurements described in the earlier part of this paper, and we are satisfied that all the thresholds discussed there are 'make' thresholds. Anodal block, too (with one possible exception) played no part. For only in symmetrical tripolar excitation was it necessary to place the anode in the path of conduction, and here it was found that the half-anode did not usually diminish the maximal action potential until the stimulus was raised considerably above maximal. Hence there was certainly no blocking at $\frac{1}{4}$ maximal. The exception was when the two anodes were close on each side of the cathode. We have already spoken of the anomally of this case, which deviates from the simple cable theory, with a fall in the maximum response obtainable. This phenomenon could be due to the distribution of the nodes, it could be due to anodal block and there could be yet other factors.

In seeking a cause for the low threshold at which these classical anodal effects appeared, we considered three possibilities. (*a*) The nerve is in bad condition. (*b*) Our Ringer has a composition different from that of the interstitial fluid. (*c*) The effect is due in some way to the sharp change of excitability about the anode in stripped nerve. We were unable to obtain evidence in support of any of these suggestions in a variety of exploratory experiments.

(*a*) In support of the idea of a poor condition of nerve is the suspicion one naturally has of sheath-strippers however careful they say they are, and in fact our phenomenon did seem to increase a little with time. Against this are the following observations: (i) the maximum action potential (recorded from the unstripped peroneal nerve) was not reduced by stripping the sciatic nerve for a distance of 2 cm. about the excitation site; (ii) a stripped nerve left overnight in Ringer at room temperature, was found to be excitable next morning; (iii) stripping the nerve neither increased the refractory period nor diminished the maximum frequency of a tetanus which the nerve could conduct.

(*b*) The unstripped nerve is bathed by interstitial fluid; our phenomenon might be due to the change in this environment. It clearly was not due to embedding in agar jelly, for the results occurred equally when the stripped nerve was stimulated in Ringer or air. Against environmental significance are the following observations: (i) however long an excised unstripped nerve is allowed to equilibrate with Ringer, it never shows these properties; (ii) we were unable by applying fluids of different ionic compositions to restore a stripped nerve to 'normal' excitability.

We have not considered non-diffusible constituents.

(*c*) This paper leads to a precise idea of how removal of the epineurium changes the space distribution of current and excitability in nerve at anode and cathode, but the enhancement of anodal effects does not seem to follow as a logical consequence. Whether it follows as a physiological consequence could be established with certainty by applying to stripped nerve the external potential distribution which normally occurs in the interstitial region (see Part 2). We have not done this accurately, and rough approximations have given inconclusive results.

Conclusions to Part 1

Our former experiments upon unstripped nerve showed that the results would not fit the simple cable theory. Now we see that by removing the epineurium, we remove these discrepancies nearly entirely. Such as remain could well be due to residual connective tissue and the nodal structure of the nerves.

The cable to which the nerve approximates has a space constant $\lambda = 2{\cdot}8$ mm. (Fig. 1). This value was obtained in an experiment where the nerve resistance was very high compared with that of the surrounding agar-Ringer, hence 2·8 mm. is the 'characteristic length' of the most excitable fibres. This means that (conductivity of sheath per mm.) × (resistance of core per mm.)

$$= 1/(2{\cdot}8)^2 = 0{\cdot}13 \text{ mm.}^{-2}.$$

This value is about the average of our results with Swiss frogs. The English frogs (which were smaller and more emaciated) gave values of λ between 2·0 and 2·5 mm.

Part 2

In this paper our prime object is to study why the unstripped nerve exhibits a distribution of excitability so different from the expectations of the simple cable theory. In Part 1 we have seen that removal of the connective tissue sheath also removes most of the deviation from the simple cable theory. This proves that *in situ* most of the deviations were due to the presence of the epineurium, and the question now arises as to the nature of the epineurial influence. It is conceivable that the action is simply by holding the fibres close together as in a tight net, or some degree of impermeability may preserve a chemical environment for the nerve fibres different from Ringer's fluid. But by far the most likely suggestion is that the epineurium is somewhat resistant to the current which must flow across it when the nerve fibres within are excited.

This may be tested by inserting an electric probe into the interstitial fluid beneath the epineurium. For, if the sheath has electrical resistance, some fraction of the applied potential will be recorded across it, but if the resistance is negligible, as maintained by Lorente de Nó (1947*a*, *b*), so will be the recorded potential difference. As it turns out, the potential drop across the epineurium is far from negligible, but we have a much more stringent test than this to satisfy if we are to be assured that the epineurial resistance is not merely one factor but *the* factor which accounts for the deviation from the simple cable theory. For there is only one distribution of interstitial potential which will explain the observed excitability results, and unless we find that the interstitial fluid has this theoretical value of the potential at every point, it cannot afford a complete explanation.

The experiment, then, consists in essence of three kinds of measurements: (*a*) The spread of excitability about the cathode. This is best obtained by the symmetrical tripolar curve (Fig. 2). (*b*) The potential of the interstitial fluid beneath the epineurium, recorded directly from an inserted wire. Here it is best to apply the current through a pair of electrodes 1 mm. apart, so that the greatest possible fraction of the applied potential contributes to our measurement. (*c*) The nerve is now stripped and λ is measured as already described in Part 1.

Method

The whole set of experiments was always made upon the same nerve. The accuracy required is very high, and could only be achieved by a good deal of attention to detail. The best experiments were made upon large Swiss frogs (*R. temp*), whose sciatic-peroneal nerves were dissected out and set in a jelly rod as described in our previous paper (1949*b*), the epineurium remaining intact. The nerve was set up in the usual stimulating apparatus, and bipolar and symmetrical tripolar excitability curves obtained. The nerve was now removed from the apparatus, and the agar was gently cleaned away (which was not difficult) preparatory to a wire being introduced beneath the epineurium. The wire was of fine enamelled silver s.w.g. 42. One end was scraped bare for connexion to the amplifier, the other cut sharply across and insulated with a minute smear of 'durofix'.

Some 5 mm. from this tip the insulation was scraped away for a distance of 0·5 mm., and immediately before insertion into the nerve, the following insulation test was carried out.

A glass tube filled with Ringer's fluid had an electrode at each end so that a current pulse might be sent through the fluid. The middle of the tube was perforated to admit an Ag.AgCl recording electrode connected via a cathode follower to one input of the amplifier. The other input also via a cathode follower was connected to the fine wire to be tested whose bare silver surface, 5 mm. from the tip, was also chlorided. This wire was gently passed down the tube until the applied current pulse gave zero output. Usually this condition was when the bare area of the wire was exactly level with the other recording electrode, and in this case the wire was accepted (though the justification is not conclusive). Otherwise the specimen of wire was rejected.

It is important not to use the bare tip of the wire rather than the more complicated method just described, for in early experiments where this was done, the recorded potentials proved quite unreliable in many cases. We believe that this was because the sharp tip tended to pierce the epineurium either during the insertion or in subsequent manipulation, and even a partial entry would give a misleading result of the kind which we obtained. The more complicated method has the double advantage that the bead of 'durofix' over the tip removes its sharpness, and the potential is measured from a place which is usually well in the body of the nerve.

The insertion was performed under a dissecting microscope. A hole was torn in the epineurium of the tibial branch by a sharp needle, and the wire, held in forceps covered with soft material to protect the enamel, was pushed in and passed up the nerve within the sheath. The nerve rested on a mirror so the wire could always be seen through the epineurium either directly or reflected from below. Slight bending of the nerve helped to guide the wire tip more or less along the centre of the trunk. The wire was passed up until the bare patch was seen to lie about the middle of the branch-free thigh region. It was tied in this position by a ligature round the tibial branch near the point of entry of the wire.

The nerve with interstitial electrode was now again set in jelly, and the former experiment precisely repeated. This was to observe how far the normal excitability curve was changed by the insertion of the wire, its continued presence, and the other manipulations described. The change was usually small, and when it was not, the experiment was rejected. The nerve in its jelly rod is now carefully removed, and the apparatus prepared for measuring the interstitial potential.

The arrangement here is to send the current between two electrodes fixed at about 1 mm. apart, and to move the nerve along so that the bare part of the wire is at various distances from the electrodes. The nerve was attached at either end by Ag.AgCl hooks to an insulating framework held in a rack and pinion so that the jelly rod could be displaced as required correct to 0·1 mm. Unfortunately, two electrodes close together are very sensitive to irregularities both of moisture and contact as the jelly rod moves across them, and at first readings were quite unreliable. The difficulty was overcome by two procedures:

(*a*) Good contact was secured by casting for the nerve rod a jelly tunnel set around the electrodes. This was done before the nerve was moved into position. A glass rod 1 mm. in diameter was threaded through the wire loops which formed the electrodes, and a drop of hot agar-Ringer placed over the loops and rod. After setting, the rod was carefully withdrawn and the portions of jelly extrapolar to the electrodes cut away. Within this little agar tunnel the moist agar rod slid easily and with good fit, and the contact was fairly uniform as judged by passing a fixed current pulse through the electrodes in the jelly, and measuring the potential difference appearing between the two silver hooks supporting the ends of the nerve.

(*b*) The error remaining was reduced as a result of the observation that, though a change in moisture at the electrodes could still alter somewhat the potential difference both between the two hooks, and also between the internal wire and one of them, it changed these in nearly the same proportion. So it was only necessary to express the interstitial potential as a percentage of the potential difference between the hooks, to obtain a figure almost independent of irregularities in contact. In this way we believe that the readings of the interstitial potential were exact to about 1% of the potential difference of the stimulating electrodes. Readings were always repeated in

the reverse sequence, and the whole set was rejected unless every reading had been repeated within a tolerance of 2%.

Measurements were made as follows. The interstitial electrode was led to the grid of a cathode follower whose twin was connected to one of the hooks at the end of the nerve. A change-over switch allowed the other hook to be connected instead. Both readings were taken for each setting of the nerve, and the percentage calculated. The current applied was a square wave of the same duration as that used in the experiments upon excitability, but of strength very much below threshold. An accurately measured fraction of the applied current was added to the cathode circuit of one of the cathode followers so that there was null output in the amplifier. This proved a very accurate and convenient method of measuring the interstitial potential, for the setting of this fraction is independent both of the current strength applied to the nerve (as we verified provided that it was not strong enough to produce an action potential) and to the linearity of the amplifier. And since the null condition is established at the amplifier's input, a great sensitivity may be used to record the balance without any danger of overload.

After the interstitial potential curve had been measured and confirmed, the nerve was removed from the apparatus, and the internal wire withdrawn and its insulation retested. The nerve was then cleaned of jelly, and treated as has already been described in Part 1 of this paper.

Theory

In Part 1 we have seen that stripped nerve behaves very much in accordance with the simple cable theory, and the fibres excited in Fig. 1 could be regarded as cables with $\lambda = 2{\cdot}8$ mm. having an excitability at each point proportional to the membrane depolarization. Let us now assume that in the experiments of Part 2 (which were made upon this same nerve before stripping) we are dealing with the same fibres, behaving in the same way. Then, since the difference in excitability seen in Fig. 2 is not to be attributed to a change within the fibres, it must be due to a change outside. This means that the presence of the epineurium has modified the potential distribution applied to the fibres. Can we calculate what modification would be required to produce Fig. 2? Certainly this can be done, but it is not precisely what we require.

If the interstitial potential calculated is to be directly comparable with that actually measured by the internal wire, we need to have the stimulus the same for both cases. The direct potential measurements were made using electrodes placed 1 mm. apart, consequently we need to analyse the spread of excitability around electrodes so placed. Now this is easily found from Fig. 2, for both theory (Rashbass & Rushton, 1949*b*) and experiment (Rashbass, 1949) have shown that the required excitability (the slot excitability curve) is given exactly by the differential of curve T, Fig. 2. This differential is plotted in Fig. 5, curve B, whose ordinates therefore show the excitability of the nerve at various distances on the cathodal side of an electrode pair placed at $x = \pm 0{\cdot}5$ mm. Now since excitability is proportional to membrane depolarization, the ordinates of curve B give the potential difference across the membrane at each point on a cable of $\lambda = 2{\cdot}8$ mm. There is only one external potential distribution which will give this membrane potential distribution, and we proceed to find it.

Let V_1 = interstitial potential at x, which we require to evaluate.

V_2 = potential at x in the axis cylinder of the fibres which are excited.

$1-\psi(x)$ = tripolar excitability curve T, Fig. 2 scaled to a maximum of unity.

r = resistance of axis cylinder per mm.

$\frac{1}{R}$ = conductivity of axon sheath per mm.

$\frac{R}{r} = \lambda^2 = 7{\cdot}8$ mm.^{2}.

$-a$ = arbitrary constant.

Now we have seen that the ordinates of curve B, Fig. 5, are proportional both to

$$\frac{d}{dx}[1-\psi(x)],$$

and to

$$\frac{V_2-V_1}{R} = \frac{1}{r}\frac{d^2V_2}{dx^2},$$

therefore

$$a\frac{d\psi}{dx} = \frac{V_2-V_1}{\lambda^2} = \frac{d^2V_2}{dx^2}; \tag{1}$$

therefore

$$V_2 = a\int_0^x \psi\, dx,$$

if we define as zero the potential at $x=0$. But from (1)

$$V_1 = V_2 - a\lambda^2\frac{d\psi}{dx}$$

$$= a\left[\int_0^x \psi\, dx - \lambda^2\frac{d\psi}{dx}\right]. \tag{2}$$

Now $\psi(x)$ is plotted in curve A, Fig. 5. It is simply T, Fig. 2, upside down, and still scaled to a maximum of unity. Since ψ and λ^2 are exactly known, the value of V_1/a may be determined absolutely from equation (2) for all values of x.

The differentiation and integration of $\psi(x)$ was performed arithmetically as follows. Values of $\psi(x)$ measured up on curve A, Fig. 5, were tabulated one below the other for $x = 0{\cdot}5, 1{\cdot}5, 2{\cdot}5 \ldots$ mm. in this order. Suppose that at $x=(n-\frac{1}{2})$ the ordinate value in the table is N, then we obtain $\left(-\frac{d\psi}{dx}\right)$ for $x=n$ by subtracting from N the value which lies immediately below it in the table. Also $\int_0^x \psi\, dx$ will be given by adding to N all the figures in the table which stand above it.

The curves B and C, Fig. 5, show the values of $\left(-\frac{d\psi}{dx}\right)$ and $\int_0^x \psi\, dx$ respectively calculated from the experimental curve A by this method. The unit of length is taken as the cm. in order that the representation may be at a convenient scale. Since $\lambda^2 = 0{\cdot}078$ cm.$^2 = \frac{1}{13}$, we must add to C, $\frac{1}{13}$ of B in order to obtain the required interstitial potential V_1/a.

Now it will be recalled that when the interstitial potential was directly measured, it was expressed as a percentage of the difference between the two ends of the nerve. Thus if V_1/a is to be plotted upon the same scale, the arbitrary constant a must be chosen so that the potential difference between the ends of the nerve amounts to 100.

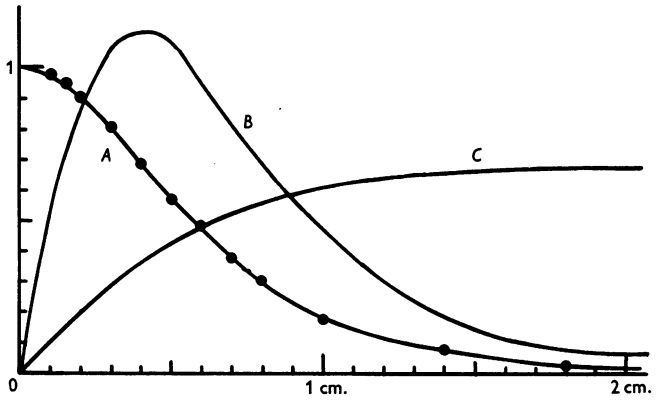

Fig. 5. Curve A is a replot upside down of T, Fig. 2; B is the negative differential of A; C is its integral. Unit of length = 1 cm. Curve A plots the distribution of excitability about an isolated cathode at O; B about an electrode pair at $\pm 0{\cdot}5$ mm.

Results

Since conditions are symmetrical, it is only necessary to represent the potentials in the various layers to the right of $x=0$, for those to the left will be equal and of opposite sign if we define zero potential as that at $x=0$, the mid-point between the electrodes. The curves of Fig. 6 are plotted in this way. They all pass through O and are scaled to have a value of 50 for large values of x. The electrodes are situated at $x = \pm 0{\cdot}5$ mm.

Curve V_0 shows the potential of the jelly outside the epineurium, and is a replot of Fig. 1 (Rashbass, 1949). We do not require it for our analysis, and it is included merely to complete the picture of the potential distribution in the nerve layers. Curve V_2 is the calculated potential in the axis cylinder of those fibres which are excited. It is a replot of curve C, Fig. 5. Curve V_1 shows the interstitial potential calculated as described above, e.g. by adding to C Fig. 5, $\frac{1}{13}$ of curve B.

It must be emphasized that the predicted interstitial potential V_1 has been calculated without any reference at all to the internal electrode. If our ideas were wrong, the actual interstitial potential might be any reasonable curve passing through O and running asymptotic to the 50 horizontal. If the sheath resistance were negligible, for instance, the curve would coincide with V_0. The actual potential measurements, however, are given by the black and white

circles of curve V_1, Fig. 6. The white circles are correctly placed, but the black ones belong to the left half of the symmetrical curve. They have been replotted in their corresponding places on the right side in order to show the degree of symmetry obtaining in this nerve, and to allow bothh alves to be compared with the computed curve V_1. It is seen that the curve falls only slightly below the points.

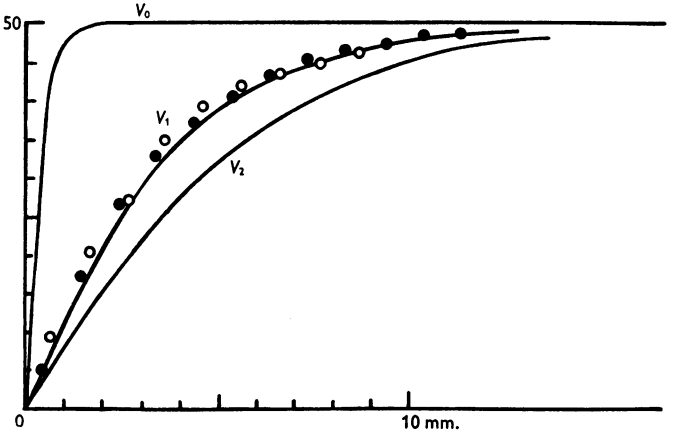

Fig. 6. The distribution of potential in the various layers of nerve to the right of an electrode pair situated at $\pm 0{\cdot}5$ mm. V_2 potential of core, a replot of C, Fig. 5. V_1, interstitial potential $= C + B/13$ (Fig. 5). V_0, surface potential. Black and white circles show the interstitial potential directly measured.

If it had not been possible to measure the interstitial potential directly, and we had been forced to rely entirely upon the calculations from excitability, we should have hesitated to accept a potential distribution where a far greater drop occurs across the epineurium than across the myelin. But the close confirmation by direct measurement leaves little doubt that the distribution of Fig. 6 is a fact. It also strengthens the theory which led to this agreement.

Is the resistance in the epineurium?

The epineurium holds the nerve fibres tightly packed in the trunk, so that it is reasonable to suppose that the fibres in the centre are screened by those nearer the outside. Stripping off the sheath certainly allows the fibres to separate, and so would abolish this screening. Is it certain, then, that what we have called the resistance of the epineurium is not in fact the resistance of the peripheral layer of nerve fibres, held compact by a quite permeable connective network? We incline to the view that epineurial resistance is the principal factor, for the following reasons:

(*a*) Fibre screening is only tenable if it is assumed that both excitability and potential measurements are made near the centre of the trunk. But it would

be surprising if the current excited easiest those fibres which were most screened from it. Moreover, potential measurements appeared to be the same no matter whether the bare recording area were deeply buried in the nerve, or so close beneath the epineurium that details of the scratches on the wire could be seen in the microscope.

(*b*) On several occasions a little slit was torn in the sheath just opposite the bare area of the wire when this was deeply situated in the trunk (one can always obtain the locality by measuring back from the visible tip). The potential of the wire which was initially at V_1, Fig. 6, jumped at once to V_0, and the potential curve measured in these conditions followed curve V_0 almost exactly. This effect of sheath puncture is naturally most marked at small extrapolar distances where the potential across the sheath, directly measured, is seen suddenly to fall to about 1% of its former value.

(*c*) In the early experiments when the sharp tip of the fine wire was left bare, it sometimes happened that the terminal portion got bent sideways so that the point stuck into the epineurium, raising it from the side of the nerve like a little tent on its pole. The recorded potential in such a case was about half-way between curves V_0 and V_1. Nothing but epineurial resistance can explain the potential drop in this case. And a low value is to be expected, because the stretching of the sheath and perhaps some penetration by the point may substantially diminish the resistance at the very place where it is measured.

Validity of the experiment

We have found this experiment very hard to perform owing to its delicate manipulations, its many accurate determinations, and the overall uniformity essential to the analysis. There is danger in such a case of prejudging the answer by being more ready to accept as 'good' those experiments which fit well, for one can generally find grounds for questioning the validity of results which turn out discrepant.

In order to avoid this, we considered all our principal sources of error and set a certain standard of performance. If that was not attained, this part of the experiment was at once rejected. If it was attained, this part was accepted, come what may. We have already mentioned the insulation test for the fine wire, and the criterion of nerve damage when the wire was inserted. All determinations were repeated in reverse order. The interstitial potential must be entirely reproduced within 2%, the thresholds within 5% and so on. The hardest test to satisfy was the uniformity of the epineurium as measured by the symmetry of the interstitial potential curve. Fig. 6 shows a nearly perfect symmetry, for the white dots and the transposed black dots practically fall upon the same curve. We never saw another curve as symmetrical as this, and usually they were quite bad. Now, the whole argument of this experiment is based upon the assumption of the uniformity of the sheath, and therefore

asymmetrical nerves could never be used either to prove the theory or to disprove it. All such experiments, therefore (and that is the majority) had to be rejected, in the sense that they could not be used to compare accurately the two methods of obtaining the interstitial potential. They could invariably be used to establish that the interstitial potential has a distribution like curve V_1 and quite unlike curve V_0.

In the course of this work our standards became more and more exacting as we discovered new sources of error and developed a better technique. It became rarer to succeed in passing all the tests, but the successful results approached closer to those we have described. It therefore appears that the curves of Fig. 6 represent the results to which all the experiments converge as their errors are progressively eliminated.

Only three experiments actually passed all the tests in their final form and the one here described was technically best, and also gave the closest correspondence between the calculated and observed interstitial potential. The other two were nearly as close, the deviation going hand-in-hand with sheath asymmetry. Thus though our valid experiments are very few, we believe that they are in fact valid experiments.

Discussion

The idea that the epineurium may have an appreciable resistance is an old one and based on three kinds of evidence—histological, pharmacological and electrical. The evidence from structure is simply that the nerve fibres are known to be surrounded by a denser outer sheath, the epineurium, and more numerous finer sheaths, the perineuria and endoneuria, and this raises the question whether such sheaths may affect the passage of electrolytes, but does little to answer it.

Diffusion. According to various authors, the epineurium is considered to be, or not to be a barrier to diffusion because of the rate of action of ions, narcotics, etc., applied to the nerve in various states of dissection. Tasaki (1939*b*), see also Kato (1936), found that narcotics applied directly to a node acted completely within 1 sec. or so, and we have witnessed the same thing in experiments by Huxley and Stampfli investigating the action of ions on frogs' single nerve fibres (unpublished as yet). A drop of sugar solution on the node abolished conduction, and a drop of Ringer restored it instantly and regularly. The action of these agents on unstripped nerve is some hundreds of times as slow, but it cannot be argued from this that the sheath is a barrier to diffusion and must have electrical resistance. There are two processes which contribute to the penetration of substances in solution, and since it is important to distinguish them, let us take a simple physical example.

A glass cylinder is half filled with $CuSO_4$, and above this is carefully run in isotonic Na_2SO_4 so there is a sharp line of demarcation between the two

solutions. Now diffusion is the penetration of solute to all parts of the solvent due to thermal agitation without any movement of the solvent. It would take some days to reach equilibrium in this example. Obviously the result could be achieved in a few seconds by vigorous shaking. In that case it is the agitation of the solvent which distributes the solute. Suppose that the solutions were set in jelly, the diffusion would be more or less unchanged, but, of course, shaking the jelly would not hasten mixing. Now the importance of this is that both *diffusion* and what we shall call *mixing* contribute to the penetration of substances, but only diffusion contributes to the electrical conductivity. So care must be exercised in arguing from penetration to electrical conductivity.

From these considerations we see that there is nothing inherently contradictory between Lorente de Nó's observation (1947*a*, p. 21) that 0·11 M solution KCl produces conduction block in 15 min. and his statement that the sheath does not act as a diffusion barrier (p. 23). For though the process takes perhaps a thousand times as long as it would by the direct application of the ions to a node, this might be explained simply by the absence of mixing. A stripped nerve set in jelly might show the same thing. It is harder to accept his conclusion that the action is so rapid that the sheath cannot possibly be a diffusion barrier. Indeed if this were so, we should expect that the outer layer of nerve fibres would be paralysed by a solution as strong as 0·1375 M within the first second, but no change can be detected for about a hundred times as long as this (p. 23).

A more direct approach is obtained by comparing results (*a*) with sheath intact, and (*b*) when removed either by dissection or by using the spinal roots. Rice & Davis (1928) found that chloral hydrate acted faster upon nerve in the region of cut branches, and paralysed the trunk more rapidly the more connective sheaths were cleaned away. Feng & Gerard (1930) found that methylene blue stained the sheath but would not penetrate, and that slitting the sheath permitted entry. They found that sheath-slitting accelerated conduction block by KCl, NaCN, $CaCl_2$, or glucose some ten times. For instance, KCl with intact sheath took 15 min.; without sheath less than 2 min. This striking result, however, is no evidence that the sheath is a *diffusion* barrier. It is undoubtedly a barrier to mixing, and the experiment does not prove that it is more.

The effect of mixing could easily be avoided by comparing stripped and unstripped nerves as in Feng & Gerard's experiments if both the nerves were set in jelly (rather thinner than in our experiments). Another way is to compare the penetration in the trunk and in the spinal roots, since the latter have hardly any epineurium but are probably held together compactly enough to stop much mixing. Bishop (1932, p. 182) in a footnote states that the same concentration of KCl acts much more quickly upon the roots than the trunk, and attributes this to the slow penetration through the frog's epineurium. Lundberg (1948) finds the same relation in the cat, where the penetration in

the roots was about 1000 times as fast as in the trunk. Since mammalian nerves have a thicker epineurium than frogs', and far more perineurial sheaths, Lundberg's results are not directly comparable with those from the frog, but they certainly favour the view that connective tissue is a barrier to diffusion.

Most of the evidence as to diffusion of reagents through the epineurium has been inconclusive because it has arisen as a complication in experiments directed to quite a different end. But it is hard to avoid the conclusion that the sheath is to some extent a diffusion barrier. Obviously a convincing experiment must secure penetration without mixing, must test the penetration by nerves of similar susceptibility, and must secure conditions of diffusion which are sensitive to sheath resistance—probably with a high concentration of reagent and a short penetration time. It is doubtful whether such a method of obtaining the epineurial resistance would be easier or more reliable than direct electrical investigation.

Current distribution. There can be very few of those working upon the relation of electricity to nerve activity who have not wondered how far the connective tissue might not contribute to the relations studied. In the literature, doubts usually come to expression when observations diverge from theory, but little is generally done to substantiate the question. Bishop *et al.* (1926), on the other hand, in their analysis of factors distorting action potential records, found the epineurium to be highly polarizable, as shown by comparing the potential distribution in the normal trunk either with the stripped trunk or the spinal roots. They express so clearly the implications of this, that it is surprising that physiologists have continued to neglect it. Bishop (1928*a*, *b*) developed the matter further, Cole & Curtis (1936) found that the transverse impedance of nerve was largely removed by careful stripping the sheath, and Tasaki (1939*a*) observed that the chronaxie of a single nerve fibre diminishes when the epineurium is removed, and then shortens still further when the single fibre is quite isolated.

These definite indications of epineurial influence are not seriously challenged by the great mass of work where a rather good correspondence has been observed with the predictions of the simple cable theory. The authors are usually content with establishing a formal relation, and make no pronouncement about the properties of the sheath. It is otherwise, however, with Lorente de Nó, who has recently expressed himself very strongly upon the matter.

'This fact (α), together with the other fact (β) that no electrotonic potentials can be produced after nerves have lost their core-conductor properties, constitutes conclusive evidence to warrant the statement that the existence of the connective tissue sheath may be ignored in the analysis of potentials recorded from the surface of nerve' (1947*a*, p. 13).

This statement leaves us no alternative but to attempt some discussion of the evidence, for not only is this quite contrary to our conclusions, but also our results, if accepted, would cast some doubt, we fear, upon many of Lorente de Nó's conclusions. It is certain that in the few pages that follow we shall do scant justice to his two volumes of interwoven argument. We can but state that we have tried to focus upon the impact point of our conclusions with his, and to appreciate as well as we can the line of his argument, in which we have been much assisted by a helpful and explanatory letter.

The fact which we have labelled (β) in the statement above constitutes by itself conclusive evidence if it is certain that the agents which abolish the core-conductor properties of axons are without effect upon the epineurium. If we do not know what the agents do to the epineurium, it is no evidence at all. Examples of loss of electrotonus are given (Lorente de Nó, 1949*a*, p. 405; *b*, pp. 174, 184) due to the action of K^+, iodoacetamide, and Cu^{++}. There is no physical evidence as to the action on the epineurium. The belief that its resistance did not fall to zero rests upon subsequent histological examination (osmic acid) where the membrane was seen to be normal. We are not histologists and no doubt underestimate what the microscope can reveal to one highly skilled in this science. But if the foregoing experiment had been done with a fine wire beneath the epineurium, and if the directly measured sheath potential had been seen to fall to zero, we should certainly accept this conclusion rather than the one derived from histology. Now the results of the suggested experiment may be reached without in fact performing it. For initially there is certainly a polarization potential across the epineurium, as we have invariably found, and at the end there is no polarization potential anywhere, as Lorente de Nó has found. It therefore seems plain that the epineurium suffers a resistance change without modification of histological structure.

The fact which we have labelled (α) is as follows. When a square current pulse is passed down a long uniform stretch of nerve, the potential recorded from two surface points in the middle region is at every instant proportional to the current (1947*b*, p. 13, fig. 11). From this it is reasonable to conclude that all the longitudinal conductors are ohmic (non-polarizable). It gives no information whatever about the sheaths through which the current passes radially because there *is* no current passing radially within range of the recording electrodes. We were therefore puzzled to know how this experiment could constitute Lorente de Nó's conclusive evidence that epineurial resistance is negligible. He has kindly indicated to us his line of argument, which again involves the histologist's viewpoint. It depends upon appreciating that the fine connective network—the fibrillenscheide of Key and Retzius—which surrounds the myelinated fibres throughout the trunk, has its fibres continuous with the epineurium. It is argued, therefore, that the absence of any polarization by the longitudinal current shows this network to be of non-polarizable

material, and hence that the epineurium, which is of the same material, is also non-polarizable.

This argument appears to be open to criticism of two kinds:

(i) *Electrical.* The observation of fact (α) proves that the longitudinal conductors have an ohmic resistance. If the fibrillenscheide is not part of the longitudinal conductor, it is impossible to argue anything about its non-polarizability. If it is part, its resistance must be ohmic. Suppose that it is not zero, then Lorente de Nó's argument leads at once to the result that the epineurium also has resistance. Thus the argument can hardly constitute conclusive evidence for the opposite opinion.

(ii) *Histological.* Before we can argue from the absence of polarization by longitudinal currents to the non-polarizability of the fibrillenscheide, we must be sure that these sheaths are in a condition to be polarized by this current, namely that they run transversely across the trunk, and are without gaps through which the current could by-pass them. But the fibrillenscheiden do not have this structure at all. Key & Retzius (1876, pl. VII (man), pl. IX (frog)), Cajal (1928, fig. 11, p. 63), Laidlaw (1930, fig. 1), de Rényi (1932, figs. 14, 15, pp. 1398–9), Nageotte (1932, fig. 3, p. 199), all agree in describing an exceedingly fine network closely investing the neurilemma, and as incapable of being polarized by a longitudinal current as the epineurium itself. From the descriptions of these authorities it would appear that longitudinal currents exhibit no polarization because no nerve structure exists for such a current to polarize. It is impossible, therefore, to argue from non-polarizability by longitudinal currents to any property of the epineurium.

Since a longitudinal current cannot and does not polarize anything, we may inquire what is the nature of Lorente de Nó's longitudinal polarization. From his mathematical treatment of it, it must be a vector with the dimension of potential gradient and direction longitudinal. It is generated within the nerve core in some way by current flow, but the author states definitely that it is not the polarization of core structure by current flowing longitudinally (1947*b*, p. 12). But it is by no means clear how current flowing radially could produce polarization longitudinally, nor why this should be directed up the nerve rather than down, at any given point. 'Under conditions such as these it is indeed regrettable that the experimental observations do not throw light on the mechanism underlying the longitudinal polarization of the nerve fibres' (1947*a*, p. 100). 'Since the longitudinal electromotive forces that appear in the core of the nerve fibres are not accessible to direct measurement, the only possibility of establishing their existence consists in demonstrating that the predictions of "equation (2)" do not agree with the experimental results' (1947*a*, p. 246).

Now, 'equation (2)' is a relation derived from the simple cable theory which we have seen does not in general apply to unstripped nerves such as were used in Lorente de Nó's experiments. When our nerves were stripped they appeared

to satisfy pretty well the predictions of 'equation (2)', and if this happened with Lorente de Nó's nerve, removal of the sheath would also remove his 'longitudinal polarization'. We believe this likely on analytical grounds.

Consider his fig. 1 (1947*a*, p. 3), which plots the distribution of the demarcation potential along the nerve. The curve is seen to resemble our curve *A*, Fig. 5, which plots the distribution of excitability about the cathode—the single-pole excitability curve (Rashbass & Rushton, 1949*b*). In both cases an exponential is to be expected upon the simple cable theory, and in both cases we observe the same kind of deviation from expectation. The resemblance is more than coincidental, for the formal identity of the two curves may be established and that independent of any special assumption as to structure.

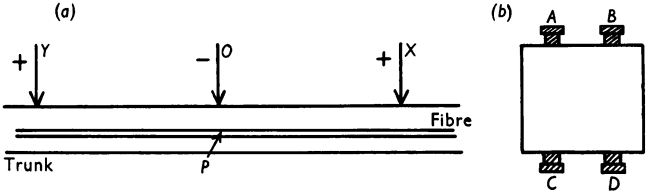

Fig. 7.

Fig. 7 (*a*) shows a nerve trunk arranged for symmetrical tripolar stimulation, and within, one of the nerve fibres excited. This is all the structure that is assumed. We shall now show the relation between the spread of excitability and the spread of demarcation potential, by applying Rayleigh's Reciprocity theorem. This theorem may be stated simply as follows. The box shown in Fig. 7 (*b*) contains any kind of electrical network in which the Superposition theorem holds, and the wires from terminals *A*, *B*, *C*, *D* connect to four points anywhere in the network. A current i sent in through AB will generate a voltage V between C and D. Then the theorem states that if this same current i is sent through CD, it will generate the same voltage V between A and B.

Now curve *T*, Fig. 2, shows the symmetrical tripolar excitability for various interelectrode distances x. The ordinate of this curve $1-\psi(x)$ represents therefore the depolarization produced across the axon membrane at *P*, Fig. 7, by a fixed current pulse applied through the anodes (*X* and *Y*) for various values of x. But, by the Rayleigh theorem, if a fixed current sent in through (*X* and *Y*) and out through *O* produces at *P* a potential difference $1-\psi(x)$ across the membrane, then a fixed demarcation current across the membrane at *P* will produce a potential difference of $1-\psi(x)$ between *O* and (*X* and *Y*). Or, measuring the demarcation potential between *X* and a distant point to the right (as in Lorente de Nó's fig. 1), we obtain the curve $\psi(x)$ which is plotted in *A*, Fig. 5. The similarity of these two curves is therefore by no means fortuitous.

Now it is precisely to account for the fact that the demarcation curve is not exponential, that Lorente de Nó first introduces the postulate of longitudinal polarization. We have just seen that another interpretation is possible, for the epineurium whose resistance exactly explains the shape of curve *A*, Fig. 5, also leads us to expect the same shape for the demarcation potential curve. But we may go further. Since the formal equivalence of excitability spread and demarcation spread does not depend upon any assumption as to structure, the correspondence occurs equally in stripped nerve. Now we have seen (Fig. 1) that by stripping, we reduce the excitability spread to the theoretical exponential, hence the demarcation spread will also in this case be a simple exponential. If this reasoning is valid, therefore 'longitudinal polarization' will be abolished by stripping off the epineurium.

It will be observed that the foregoing formal argument neglects the foll owing: (i) that the type of fibre excited at one quarter maximal response may not be the same as that responsible for the demarcation potential; (ii) that one of the nerves compared was in jelly and the other in air; (iii) that the demarcation potential was probably produced by a method which also damaged the epineurium; (iv) that the Superposition theorem does not always hold exactly for nerve.

It is plain that many of the phenomena which Lorente de Nó attributes to longitudinal polarization could not be explained simply by taking into account the resistance of the sheath. But since some of the results seem to be completely explicable in this way, and since longitudinal polarization itself is not a very precise concept, we feel doubtful whether it is the most fruitful way of explaining the others.

CONCLUSIONS

This paper, together with the four which preceed it, form a connected study of the distribution of excitability in frog's nerve, and we have now cleared up most of the points left outstanding in our former work. Starting by proving that when anode and cathode lie close together, excitation does not arise at the manifest cathode but at some point 3 mm. away (Rushton, 1949), we investigated the distribution of excitability in this case (the slot excitability curve), and found that it did not fall away from the cathode along an exponential curve, but had the form shown in *B*, Fig. 5, with its maximum 3 mm. extrapolar (1949*a*). We now see that the shape of this curve is exactly that required by the simply cable theory as applied to the axons themselves, and Fig. 6 shows clearly how it comes about that depolarization ($=V_1-V_2$) is maximal at 3 mm. extrapolar. Stimulation by a single pole (1949*b*) gave the distribution of excitability shown here in *A*, Fig. 5, which is exactly the negative integral of *B* (Rashbass, 1949). If this curve has been determined, we may predict the excitability of each point on a uniform nerve for any given distribution whatever of the stimulus, by application of the Superposition theorem (1949*b*).

If a stripped nerve has any given potential applied to the outside the potential of the core may be found most simply by the graphical method of subtangent analysis (Rushton, 1937). If the external potential curve is analysed to the right with subtangent λ and the curve so obtained then analysed to the left in the same way, the resulting curve gives the potential of the core at each point. Each analysis takes about a minute to do.

Now a bundle of uniform nerve fibres contained in a uniform epineurium may be treated in a similar fashion. In this case there are two constants λ_1 and λ_2, and the subtangent analysis must be performed in both directions with each subtangent. If this is done upon the external potential distribution V_0 of the present paper, by choosing λ_1 and λ_2 suitably the curve C, Fig. 5, may be obtained with an accuracy within the thickness of the ink line. It follows that we may obtain the core potential of the fibres concerned in Fig. 5 for any given external potential distribution whatever, by analysing this given curve using subtangents λ_1 and λ_2. The excitability curve is, of course, the second differential of the core potential.

The validity of the analysis does not depend upon the assumption that all the fibres in the nerve are uniform (which is certainly not true). It depends upon the fact that a suitable choice of λ_1 and λ_2 does exactly generate the single pole excitability curve A, Fig. 5, and hence it must also give all the results which could be obtained by applying to A the Superposition Theorem. It will therefore give the right answer, but one cannot safely argue back to the significance of λ_1 and λ_2.

The localization of excitation in uniform nerve follows at once from the foregoing, for threshold excitation will arise at the place where the excitability curve has its maximum. The maximum is always rather flattened, and the sharpest condition easily realized is when a cathode has an anode close on either side. The excitability curve in this case is the differential of curve B, Fig. 5, and even here the nerve is catelectrotonic for 3 mm. on either side of the cathode, however close the anodes may be. The excitation site will be displaced away from an isolated cathode when the anode approaches within 10 mm., the relation being shown in (Rashbass & Rushton, 1949*b*) Fig. 3, curve L. If the nerve is not uniform because of the nodes or other factors, the excitation site may be some distance from the exact maximum on the rounded excitability hump. Plotting according to the method of Fig. 3. (present paper) probably gives the most accurate information as to the precise excitation site.

Electrotonic potentials

It is plain from Fig. 6 that it is not safe to assume either that the potential difference between the outside (V_0) and the nerve core (V_2) is all occurring across the nerve membrane, or that all the current which flows down the core passes back outside the epineurium, for there is also interstitial current. It is true that this nerve, mounted in jelly, is not quite comparable with a nerve in air as used in measurements of electrotonus. However, though we have not thoroughly analysed the latter condition, Rushton has demonstrated to the Physiological Society (unprinted) two significant observations. (*a*) A fine wire was inserted beneath the epineurium, and the potential difference was recorded between this and a distant point of nerve when a square polarizing current of 2 msec. was applied in the usual way. If the bare area of the fine wire was exactly under the cathode (within 0·2 mm.) there was zero electrotonic

potential recorded, but a large action potential appeared if the polarizing current exceeded threshold. Electrotonic potentials of either sign could be obtained by displacing the fine wire one way or the other in relation to the polarizing electrode. The potential across the epineurium at the cathode was of course very large. It will suffice here to say that an investigation of the potential distribution on either side of the epineurium in those experiments is entirely consistent with the expectations of this paper, and may be calculated approximately from the double cable theory. (*b*) When the epineurium is stripped off, electrotonus is very small. Measured between the cathode site and a distant point when the stimulus was just sufficient to give a maximal action potential, the electrotonus was less than 5% of the spike height. This confirms the impedance measurements of Cole & Curtis (1936), where, after careful removal of the sheath, the transverse resistance was found to drop to one-fifth of its previous value.

It is clear that the presence of the epineurium is a formidable complication to measurements of electrotonus. Preliminary work to analyse this was discontinued when we found how hard it was to obtain a nerve with a uniform sheath. Such analysis is in any case rather academic, since investigations of electrotonus in future are bound to be conducted upon stripped nerve.

Nearly a quarter of a century ago Bishop *et al.* appreciated very clearly the essential content of this paper, and it is appropriate to end by quoting their conclusions (1926, p. 607).

'Further, our experiments tend to show that the sheath polarization is a large component of the "escape", and analogously, must produce distortion of the nerve's own potential as recorded. It, together with the high longitudinal resistance of nerve, tends to mask any change that may occur in the polarizability or permeability of the nerve with excitation. It also tends to make the effective contact of an electrode with the nerve, however narrow, broader than the actual electrode surface. Finally it must profoundly affect electrotonus, as measured, in the same manner as it affects escape. . . .Thus most of the artifacts in the nerve records discussed above reduce to effects of the presence of, or alterations in the condition of, a structure which is not nerve at all. We cannot avoid the suspicion that much of the work on nerve resistance and permeability, electrotonus and polarization, liminal gradients, etc., needs reinterpretation in the light of the possible effects of a highly polarizable resistance being interposed between the nerve axon and the electrode.'

Summary

1. A method is described for removing the epineurium from the frog's sciatic (p. 110) which appears not to damage the majority of the nerve fibres (p. 117 (*a*)).

2. After stripping, the bipolar and tripolar strength-length curves (Fig. 1) are found both to fit closely the curve $1-e^{-x/2\cdot8}$. Before stripping this nerve, the curves were as shown in Fig. 2.

3. The small discrepancies which remain after stripping may be due to residual connective tissue, or to the distribution of the nodes of Ranvier about the cathode.

4. Fig. 3 shows the results of asymmetric tripolar stimulation. The curves approximate to the triangle obtained by Tasaki when electrodes were applied to three consecutive nodes.

5. Anodal 'break' excitation and the anodal block of conduction are much increased by stripping so that these phenomena appear at current strengths low enough to complicate 'make' threshold measurements (see diagram, Fig. 4). We cannot explain this.

6. If the epineurium has a resistance, the potential of the interstitial fluid within will be quite different from that applied to the outside of the nerve. Part 2 describes an experiment to find out whether the difference between Figs. 1 and 2 is due entirely to this cause. It may be determined as follows:

(*a*) It is possible to calculate uniquely what the interstitial potential must be to account for the curves of Figs. 1 and 2. The result is curve V_1, Fig. 6.

(*b*) The interstitial potential may be measured directly by inserting a fine electric probe beneath the epineurium. The results are the black and white dots which lies close to curve V_1, Fig. 6.

7. The curves V_0, V_1, V_2, of Fig. 6 show respectively the potentials of the exterior, the interstitial fluid, and the core of the fibres excited in Figs. 1 and 2. There is generally a greater potential drop across the epineurium than the myelin.

8. Lorente de Nó's view that the epineurium does not affect current distribution is discussed. His arguments are criticized, and his 'longitudinal polarization' shown to be largely a manifestation of the polarization of the sheath which he had neglected.

9. The bearings of epineurial resistance upon the electrophysiology of nerve are summarized.

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