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# CONDUCTION VELOCITY AND MYELIN THICKNESS IN REGENERATING NERVE FIBRES

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All attempts to analyse the compound action potential of nerve in terms of the nerve fibres present depend upon the basic assumption that the largest fibre present conducts with the greatest velocity. While there is no reason to doubt the validity of this assumption, there is as yet no evidence to indicate that fibre diameter, and not some variable correlated with it, is the primary factor determining the conduction velocity of nerve fibres. For example, internodal distance varies linearly with fibre diameter in the nerves of adult animals (Boycott, 1904; Kubo & Yuge, 1938; Vizoso & Young, 1946), and therefore, as Hursh (1939) suggests, can be substituted for the fibre diameter in arguments about the control of velocity. Moreover, the myelin sheath thickness varies with the axon diameter (Sanders, 1946), and thus the total fibre diameter, which is equal to the axon diameter plus twice the sheath thickness, is also related to the axon diameter. Hence the assertion that any of these variables—axon diameter, total fibre diameter, myelin sheath thickness, or internodal distance—controls conduction velocity is consistent with the available experimental evidence favouring a relationship between conduction velocity and fibre diameter. No attempt has been made so far to separate these variables by comparing the conduction velocities of, say, fibres with similar axon diameters but different sheath thicknesses or internodal distances.

During nerve regeneration fibres are found which provide an opportunity of comparing, in the same animal, the conduction rates of normal medullated nerve fibres with those of similar fibres in which the normal relations between sheath thickness, axon diameter and internodal distance have been experimentally altered. The new fibres which invade the distal stump are at first thin and non-medullated, only later acquiring myelin sheaths and increasing in diameter (Young, 1942; Gutmann & Sanders, 1943). As these fibres medullate and increase in diameter during the first 100 days of regeneration, those of the

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proximal stump are progressively reduced; although at the end of regeneration both proximal and distal fibres regain their normal diameters (Greenman, 1913; Gutmann & Sanders, 1943). Sanders (1946) has shown that the myelin sheaths of these shrunken proximal fibres become thickened, the axons being reduced in diameter to a relatively greater extent than the whole fibres. During regeneration, therefore, there is a transitory phase in which the proximal stump contains abnormally small fibres with abnormally thick sheaths. Moreover, Vizoso & Young (1946) have shown that at the end of regeneration all the fibres of the distal stump have internodes of similar length although they are normal as regards size and myelin thickness. In the case of large regenerated fibres the internodal length is therefore considerably shorter than that of fibres of similar diameter in normal nerve.

The experiments described in the present paper were made primarily in order to discover whether abnormal fibres of the above two types conduct at different velocities from the normal fibres from which they are derived and, consequently, what is the effect of a change in myelin thickness or internodal distance upon conduction velocity. The measurements were made upon nerves which had been allowed to regenerate for different times after interruption by crushing with fine, smooth-tipped forceps. This operation interrupts all the fibres but leaves the endoneurium largely intact, with the result that the regenerating fibres undergo little criss-crossing in the scar, and travel mostly down the pathways which they occupied before the injury (Young, 1945). Not only do more fibres succeed in crossing the scar than after nerve suture, but a more complete reconstitution of the nerve trunk occurs after crushing than after other types of injury and nerve repair (Gutmann & Sanders, 1943). The situation is therefore an ideal one for studying nerve regeneration in the absence of complicating factors—such as the shunting of fibres which invariably follows nerve suture.

During these experiments information was also obtained concerning the progressive increase in conduction velocity in the fibres of the distal stump during regeneration, which Berry, Grundfest & Campbell (1944) described as occurring after nerve suture.

## METHODS

*Operative technique*

The animals used were adult rabbits of both sexes. No attempt was made to select animals on a basis of age, race or body weight. Under anaesthesia induced by nembutal (1 gr./kg.) and maintained by ether, and with full aseptic precautions, the peroneal nerve was exposed in the thigh and crushed firmly with smooth-tipped watchmakers forceps. A single firm crush was used, the nerve being held compressed for about 10 sec., this being sufficient to interrupt all the fibres in the nerve (see Gutmann, Guttmann, Medawar & Young, 1942). Two groups of animals were used: (*a*) in those animals from which the data of proximal stump conduction velocity were obtained the nerve was crushed about 1 cm. above the fibula; (*b*) in those which gave data for distal stump velocity the nerve was crushed at the proximal end of the thigh; in a few animals the nerve was crushed in mid-thigh. The majority of animals were treated on one side only, the normal nerve of the opposite side being used as a control where possible, but in a few cases the peroneal nerve was crushed on both

sides. After operation the animals were allowed to survive for various periods before the nerves were excised for conduction velocity estimations. During the period of survival each rabbit was tested twice weekly for the return of reflex spreading of the toes, a reaction which is a reliable estimate of a return of function in muscles supplied by the peroneal nerve (Gutmann, 1942). By comparison of the amplitude of spreading on the operated and normal sides it was possible to make an estimate of the degree of functional recovery.

*Estimation of conduction rate*

All experiments, except a very few noted elsewhere in the text, were made upon excised nerve. The nerves were removed from the animals under nembutal anaesthesia, and soaked in Ringer-Locke solution at room temperature for 5–10 min. This was found to be effective in preventing the repetitive discharge which often occurs with freshly excised nerves (Adrian, 1930). The site of crushing could generally be identified as a slight swelling in the course of the nerve. As great a length of nerve as possible was excised both above and below the site of crushing. 8–10 cm. of nerve was generally obtained. After excision and soaking, clamps were fixed to the ends of the nerve, and the latter transferred to a warm chamber. The two clamps fitted into slots in the chamber and could be separated by means of a screw. With the nerve in position, the clamps were moved apart until the sag of the nerve under its own weight did not exceed 2–3 mm. With the nerve held in this way, the electrodes were brought into position. These were silver wires, the ends of which could be moved along the nerve by means of rods projecting through the walls of the chamber. The chamber was filled with a moist mixture of 95% $O_2$ and 5% $CO_2$ and kept at $37{\cdot}5 \pm 1°$ C. (total range of variation) by means of hot water circulating within the hollow walls.

Stimuli were provided locked to the sweep of the oscillograph time-base by a trigger circuit (Schmitt, 1938). This was coupled by condensers of short time constant (50–100 $\mu$ sec.) to a power valve normally biased to cut off. The output of this valve was connected to the nerve through an internally shielded air-cored transformer (Pumphrey & Rawdon-Smith, 1937). A differential input amplifier of conventional design was used with an earth lead on the nerve near the first recording electrode. In this way the stimulus escape was kept within bounds. The amplifier output was recorded by means of a cathode-ray tube and camera (reduction 3 : 1) in the usual way.

Single sweeps, or more frequently, stationary patterns at 10 per sec. were photographed. This produced no appreciable loss of definition. Time-marking was carried out by a 500-cycle oscillator checked against time-controlled a.c. mains. By keeping the earth and recording electrodes in a fixed position, and moving the stimulating electrodes along the nerve, photographs were obtained of the action potentials at conduction distances decreasing by successive 5–10 mm. steps. In some cases the nerves were stimulated at the peripheral, in some at the central end.

On the photographs of the action potentials the distances from the shock-escape to (*a*) the first inflexion, and (*b*) the first spike, were measured by means of a travelling microscope. The latter was also used to measure the time records, and from these data a time of conduction over a certain distance could be assigned to each photograph. A graph was then plotted of time against distance of conduction, and the slope of the straight line giving the best fit to the points found by the methods of linear regression (Fisher, 1944), the regression of time on distance being calculated. The velocity could then be found as the reciprocal of the regression coefficient. In this way lines were obtained for both inflexion and spike velocities.

*Histology*

At the end of each experiment the nerve was removed from the chamber and cut into 1 cm. lengths. The latter were extended on pieces of library card and fixed in Flemming's osmo-chrome-acetic mixture, dehydrated, infiltrated with paraffin, sectioned at 4–5 $\mu$ and stained by the modified Weigert method described by Gutmann & Sanders (1943). The largest fibre in each nerve was then located by means of rough measurement with an ocular micrometer, and photographs were taken of the region of nerve containing it by direct projection on bromide paper at a magnification of $\times 1200$. The axon and total diameters of the fibres were measured by means of the travelling microscope in the way described by Sanders (1946).

*Sources of error*

Excluding the intrinsic variation between animals, the accuracy of any correlation between fibre dimensions and conduction velocity made by the methods described is affected by errors which may be classified as follows:

*Errors of preparation.* The majority of measurements were made upon excised nerve. The magnitude of the effects of this treatment cannot be assessed, and it is possible that the rates recorded are lower than those occurring naturally within the body. However, as all the nerves were treated comparably, all are affected equally by this source of error. A second source of error is that of variation in the temperature of the warm chamber. $Q_{10}$, for mammalian fibres, is about 1·7 (Gasser, 1931), and therefore, since the temperature varied by as much as 1° C., an estimate of velocity based on a single reading might deviate by as much as 6·0%. However, since the conduction rate assigned to each nerve was a statistical estimate based on at least seven readings, and the temperature of the chamber during an experiment, although variable, did not increase or decrease regularly, this source of error is unlikely to be serious, and certainly not systematic.

*Errors in the estimation of conduction rate.* At the beginning and end of each experiment, the distance from the cathode to the proximal recording electrode was measured with callipers to the nearest 0·5 mm. The intervening steps by which the electrodes were moved along the nerve were measured on the rods which projected from the warm chamber. Errors of reading are therefore possible. The regression lines of time on distance of conduction, however, in all cases passed within 1 or 2 mm. of the origin. For a maximal conduction distance of 10 cm., this represents an error of 1–2%. The estimates of conduction time are most susceptible to error. The distances actually measured on each photograph were those between the deflexion of the oscillogram produced by the 'escape' of the stimulating current, and the inflexion and first spike of the action potential. The travelling microscope used in measuring the records was the instrument used by Sanders (1946) who found that its use involved a non-systematic error of $\pm 0{\cdot}07$ mm. (S.D. of 100 observations). The shortest distance measured being of the order of 2 mm., this corresponds to an error of 3·5% at the shortest distances of conduction. This small error of measurement was principally the result of difficulty in judging the exact point of inflexion of the record in the magnified image seen under the microscope. An additional error in the estimation of time lay in the fact that only a 500-cycle oscillator was used to calibrate the time base. This was, however, linear over the range used. The oscillator was checked against time-controlled a.c. mains after every experiment.

*Errors in the estimation of fibre dimensions.* The sources of error involved in measurements of this sort have been discussed by Sanders (1946). The same precautions as those recommended in this author's paper were adopted. Since all the measurements were made on fixed and stained fibres the absolute values recorded may be somewhat less than those for the corresponding fresh fibres, but there is probably no differential change in the axon and total dimensions (Hursh, 1939). Moreover, all the nerves were treated comparably during preparation, so that all would be affected by the errors of microscopical preparation and measurement to relatively the same extent.

*Total effect of errors*

From a consideration of all these sources of error it is clear there are only two serious errors which can affect the results, namely, the unknown effect of removing the nerves from the body, and the alteration in fibre dimensions produced by fixation, embedding and staining. As all nerves were treated in the same way, neither of these affects comparisons between the different nerves, on which the conclusions reached are based. As regards the non-systematic sources of error listed above, an estimate of those which affect conduction rate was made in every case by calculating the standard error of $b$—the regression coefficient of time on distance of conduction. In no case did the error exceed 5% of the value of $b$.

## RESULTS

### *The conduction rate of normal and regenerating nerve fibres*

*Normal peroneal fibres.* Photographs of the action potentials at different distances of conduction were made in the case of each of twelve normal nerves. A sample series of photographs, obtained from a single nerve, is given in Fig. 1. The action potential of the rabbit's peroneal nerve did not show the separation into $\alpha$, $\beta$, $\gamma$, $\delta$, and $C$ elevations which is seen in the saphenous nerve (Gasser & Grundfest, 1939). There were instead two main elevations corresponding to the $\alpha\beta$ and $\gamma\delta$ groups. In addition there was sometimes a small upward inflexion on the rising phase of the main spike, and a small $C$ wave.

The conduction times and distances for the first inflexion of the action potential in these twelve nerves are shown plotted on the same graph in Fig. 2. The conduction velocities obtained for individual nerves are given in Table 1. From these data, the normal peroneal nerve has a mean inflexion velocity of $68{\cdot}6 \pm 1{\cdot}5$ m./sec. (S.E. of mean of twelve observations); similarly, the peak of the $\alpha\beta$ wave has a velocity of $56{\cdot}7 \pm 1{\cdot}4$ m./sec. (12). Therefore, according to the present data, the largest fibre in the peroneal nerve of the rabbit conducts impulses with a velocity of about 69 m./sec. This is somewhat slower than the velocity in the cat's peroneal (108–111 m./sec.; Hursh, 1939), but is within the range found for mammalian alpha fibres (Grundfest, 1940). The interpretation of possible differences in maximal conduction velocity between different mammalian species is further discussed below.

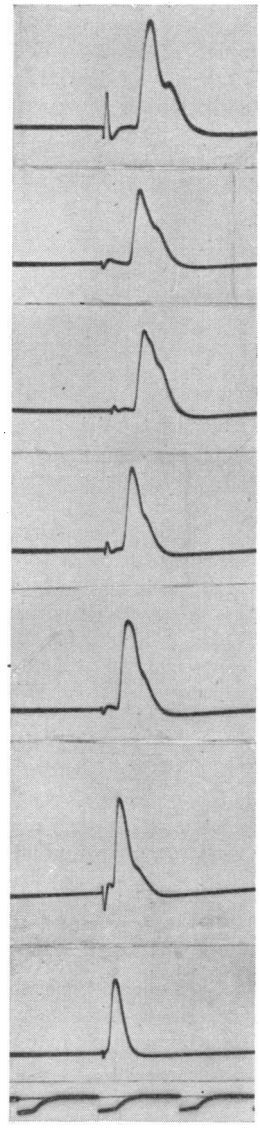

Fig. 1. Action potentials obtained from a normal nerve. Conduction distance 68 mm., decreasing by 10 mm. steps in successive photographs. Time marks = 1/500 sec.

*Conduction rates of regenerating fibres proximal to the point of crushing.* Inflexion and spike velocities proximal to the site of crushing were found for twenty regenerating nerves; in twelve of these the data were sufficiently extensive to be treated statistically. The action potentials obtained from regenerating proximal stumps contained similar elevations to those already described for normal nerve. In all except the late regenerates (456 and

486 days), however, there was a sharp change in velocity at the point of crushing (see Fig. 8). In the latter cases the nerves, within the limits of the experimental error, conducted at the same velocity throughout their length (see Fig. 7) and need not be considered further at present. In Fig. 3 the conduction times and distances for the inflexion of the action potential in the case of the eight proximal stumps between 56 and 123 days are shown plotted on the same graph. The conduction velocities of the individual nerves are given in Table 1. The inflexion has a mean velocity of $76{\cdot}0 \pm 1{\cdot}8$ m./sec. (S.E. of mean of eight observations); the spike, on the other hand, has a mean velocity of $66{\cdot}2 \pm 1{\cdot}8$ m./sec. (8).

TABLE 1. Velocities of the inflexion and spike of the action potential in the peroneal nerves of rabbits: (1) in normal nerves, and (2) in the proximal stumps of nerves which had been crushed 56–123 days previously. All velocities given in m./sec.

| Normal nerves | | | Proximal stumps | | |
|---|---|---|---|---|---|
| Animal | Inflexion velocity (m./sec.) | Spike velocity (m./sec.) | Animal | Inflexion velocity (m./sec.) | Spike velocity (m./sec.) |
| 897 | 71·5 | 59·1 | 897 | 75·6 | 61·2 |
| 921 L | 76·7 | 57·5 | 927 | 79·5 | 62·0 |
| 921 R | 73·7 | 56·3 | 965 | 66·5 | 62·7 |
| 922 | 65·4 | 48·5 | 966 | 79·3 | 74·1 |
| 927 | 61·0 | 49·1 | 967 | 81·7 | 68·5 |
| 961 | 61·3 | 50·5 | 988 L | 72·8 | 66·4 |
| 962 | 75·4 | 58·6 | 988 R | 77·1 | 68·3 |
| 963 | 71·9 | 58·1 | 989 | 75·7 | 66·1 |
| 964 | 65·8 | 60·3 | | | |
| 965 | 69·6 | 66·1 | | | |
| 966 | 64·1 | 54·0 | | | |
| 989 | 66·7 | 62·4 | | | |
| Mean inflexion velocity = 68·6 m./sec. | | | Mean inflexion velocity = 76·0 m./sec. | | |
| Standard error = 1·5. | | | Standard error = 1·8. | | |
| Mean spike velocity = 56·7 m./sec. | | | Mean spike velocity = 66·2 m./sec. | | |
| Standard error = 1·4. | | | Standard error = 1·8. | | |

Comparison of mean velocities:

For inflexion velocity, $t_{18} = 3{\cdot}190$, whence $P = 0{\cdot}01$–$0{\cdot}001$.
For spike velocity, $t_{18} = 4{\cdot}170$, whence $P =$ less than $0{\cdot}001$.

*Comparison of normal and proximal stump velocities.* The broken line in Fig. 3 shows the regression line calculated from the pooled data for the fastest fibres in the twelve normal nerves (see Fig. 2), for comparison with that obtained for the proximal stump data. The line for the proximal stumps has a steeper slope, and it therefore appears that the fibres of the proximal stump of crushed nerves, at times between 56 and 123 days after the lesion, conduct faster than the equivalent fibres in normal nerves. From Table 1 the mean inflexion velocity is 7·4 m./sec. faster, while the mean spike velocity in the crushed nerves exceeds that of its normal counterpart by 9·5 m./sec.

To determine whether these differences in velocity were greater than those to be expected as a result of random sampling, a *t*-test was applied to the data

of Table 1. In the case of the inflexion velocity, $t$, for 18 degrees of freedom, had a value of 3·190, which corresponds to a probability of between 0·01 and 0·001 that the two sets of velocities are different samples taken from the same population. The results of this test therefore indicate, in the case of the first inflexion of the action potential, that the proximal stumps of crushed nerves

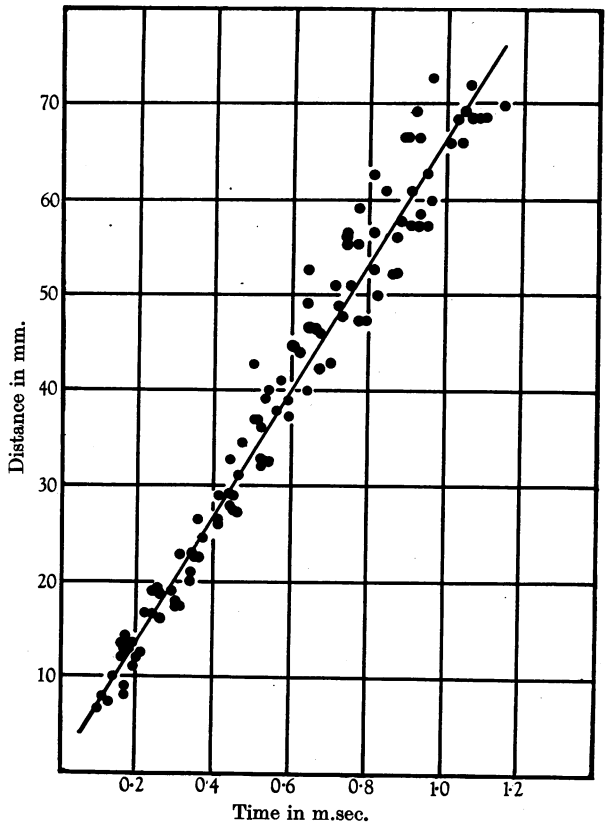


Fig. 2. Graph of conduction distance against time for the inflexion of the action potential in normal nerve. Pooled data from twelve nerves. The line is the calculated regression line for the pooled data.

after the above intervals of regeneration do in fact conduct impulses with significantly greater velocity than normal nerves. A similar test, applied to the data for spike velocity, also showed a significant difference in velocity in favour of the proximal stumps ($t$, for 18 degrees of freedom, $=4{\cdot}17$; $P=$less than 0·001).

*Conduction rates of regenerating fibres distal to the point of crushing.* Action potentials were recorded in twenty-three nerves distal to the point of crushing, and at intervals ranging from 14 to 486 days after operation (Fig. 4). The

inflexion and spike velocities obtained are shown in Table 2 and Fig. 5. In the earliest stages, the action potentials were very small (about 10$\mu$V.) and consisted of a single elevation only (Fig. 4). With increasing times of regeneration, the potentials increased in height, and in addition to the main spike there was sometimes also visible a much later, longer lasting, elevation corresponding

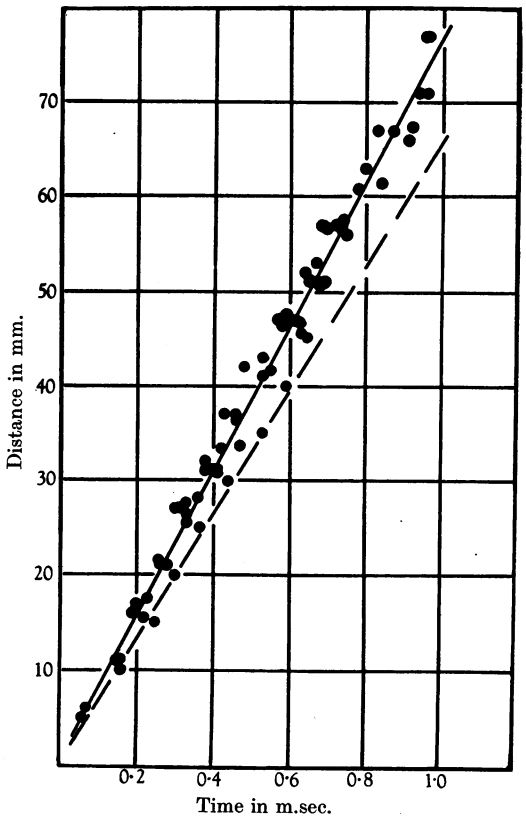


Fig. 3. Graph of conduction distance against time for the inflexion of the action potential in proximal stumps at from 56–123 days after crushing. Pooled data from eight nerves. The solid line is the regression line calculated from these pooled data; the broken line is the regression line for normal nerve given in Fig. 2.

in position to the *C* wave of normal nerve. Only the 456- and 486-day regenerates, however, showed anything corresponding to the $\gamma\delta$ wave of normal nerve.

The results given in Table 2 show that the fibres of nerves regenerating after a crush lesion at first conduct impulses slowly, but that with the passage of time the rate increases, finally approximating to that seen in normal nerve. From Fig. 5 it can be seen that the rate of increase of velocity is at first rapid, and then more gradual, the increment of velocity between 146 and 486 days

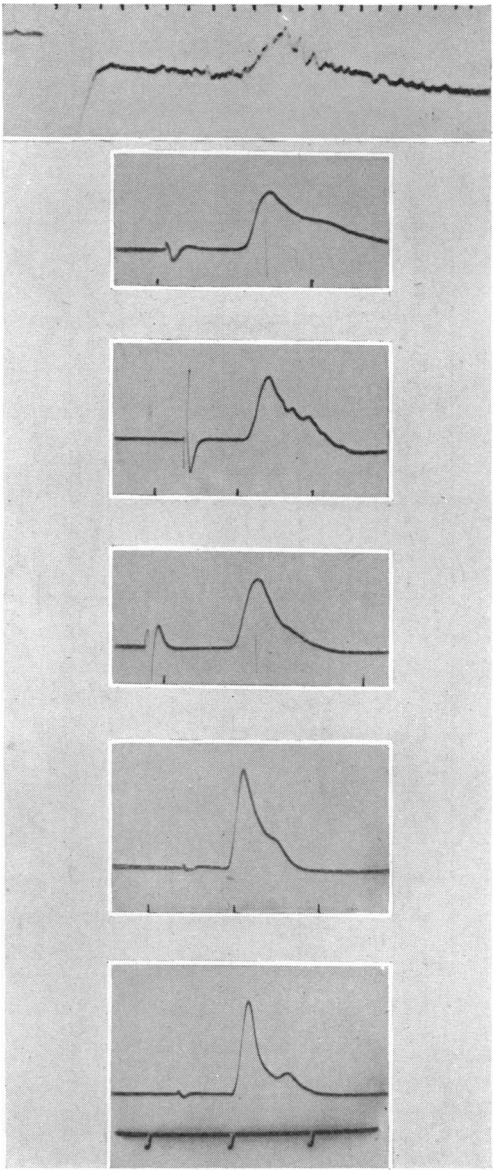

Fig. 4. Action potentials obtained from the distal stumps of regenerating nerves after:

(1) 15 days: conduction distance 69 mm.
(2) 57 „ „ „ 39 „
(3) 72 „ „ „ 75 „
(4) 82 „ „ „ 70 „
(5) 456 „ „ „ 68 „
(6) 486 „ „ „ 86 „

Time marks = 1/500 sec.

being less than that observed during the first 100 days of regeneration. Unfortunately the data are at present incomplete, lacking observations between 146 and 456 days of regeneration, and it is thus impossible to give precise form to the curve of velocity against time of regeneration. However, in a general way the curve resembles, though it does not closely parallel, the curve of diameter increase with time (Fig. 6).

TABLE 2. Conduction velocities distal to the point of crushing in regenerating rabbit peroneal nerves

| Animal | Days of regeneration | Inflexion velocity (m./sec.) | Spike velocity (m./sec.) |
|---|---|---|---|
| 340 | 14 | 12·8 | 10·5 |
| (distally) | | 0·7 | 0·6 |
| 330 | 15 | 9·2 | — |
| (distally) | | 3·7 | 2·3 |
| 329 | 26 | 20·6 | 15·9 |
| (distally) | | 15·1 | 12·0 |
| 922 | 55 | 25·6 | 22·4 |
| 897 | 56 | 27·8 | 25·2 |
| 898 | 57 | 38·3 | 33·0 |
| 899 | 57 | 36·9 | 36·3 |
| 961 | 60 | 35·8 | 32·4 |
| 962 | 63 | 32·8 | 27·4 |
| 963 | 63 | 33·4 | 31·6 |
| 964 | 67 | 35·1 | 33·8 |
| 861 | 72 | 46·1 | 35·6 |
| 872 | 82 | 50·8 | 43·7 |
| 965 | 114 | 50·5 | 44·2 |
| 967 | 114 | 48·7 | 46·2 |
| 966 | 115 | 53·0 | 41·4 |
| 989 | 123 | 45·5 | 43·5 |
| 879R | 146 | 48·7 | 42·6 |
| 879L | 146 | 48·1 | 41·2 |
| 990R | 456 | 66·5 | 54·2 |
| 990L | 456 | 65·9 | 53·5 |
| 948R | 486 | 61·5 | 51·8 |
| 948L | 486 | 59·2 | 51·8 |

Apart from the 14-, 15- and 26-day animals the conduction rates were measured in the way already described. In these three animals, however, the rates were measured on the nerve in situ, with the whole animal enclosed in a warm, humid, chamber. It was thought that the excision, soaking and setting up in the chamber for excised nerve might abolish conduction in the delicate fibres responsible for the slow conducting waves found in these nerves.

A similar curve of conduction velocity against time for regeneration after suture is given by Berry *et al.* (1944). It resembles the present one in that the increase in velocity with time of regeneration is at first rapid, and then later tails off. However, these authors found that even after 466 days of regeneration the conduction rate was still abnormal, falling short of the normal velocity by about 20%. The data from the four nerves at 456 and 486 days are shown plotted on the same graph in Fig. 7. For the four nerves the inflexion had a mean velocity of $63{\cdot}3 \pm 2{\cdot}5$ m./sec. (S.E. of mean of four observations) while the mean spike velocity was $52{\cdot}8 \pm 2{\cdot}4$ m./sec. (4). Comparing these velocities with those obtained for normal nerve by means of a $t$-test it was found that,

taking $P=0{\cdot}05$ as the conventional level of significance, neither the inflexion nor the spike velocity differed significantly from its normal counterpart. For the inflexion velocity, $t$, for 14 degrees of freedom, $=1{\cdot}777$, and therefore

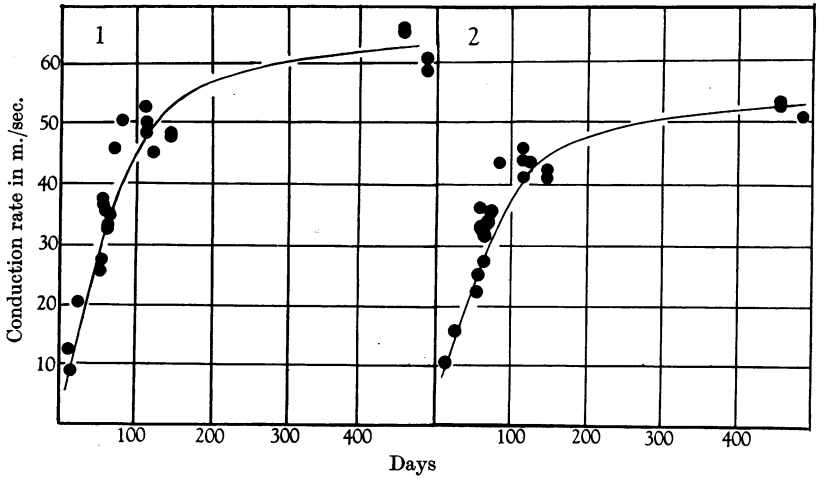


Fig. 5. Graphs of (1) inflexion, and (2) spike velocity in the distal stump against time of regeneration. The curves were drawn by eye.

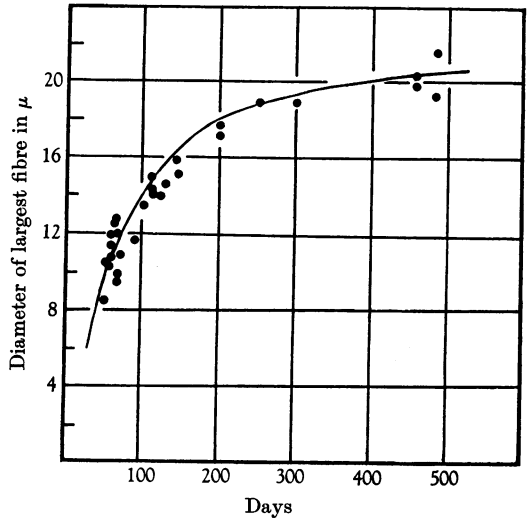


Fig. 6. Graph of the diameters of the largest fibres found in the distal stump against time of regeneration. The extra points in this graph (cf. Fig. 5) were inserted from the data of Gutmann & Sanders (1943). The curve was drawn by eye.

$P \simeq 0{\cdot}1$; while for the spike velocity $t=1{\cdot}404$ and $P=0{\cdot}1$–$0{\cdot}2$. This, combined with the observation that the form of the action potential in these late regenerates is identical with the normal, showing $\alpha\beta$, $\gamma\delta$, and $C$ waves, is strong

evidence that the four nerves measured had indeed returned to the normal state as regards conduction velocity. Yet the differences from normality in the case of both inflexion and spike velocities came close to significance, and had further observations been available at these times, it is possible that the velocities might be found to be still slightly below normal. All our experiments,

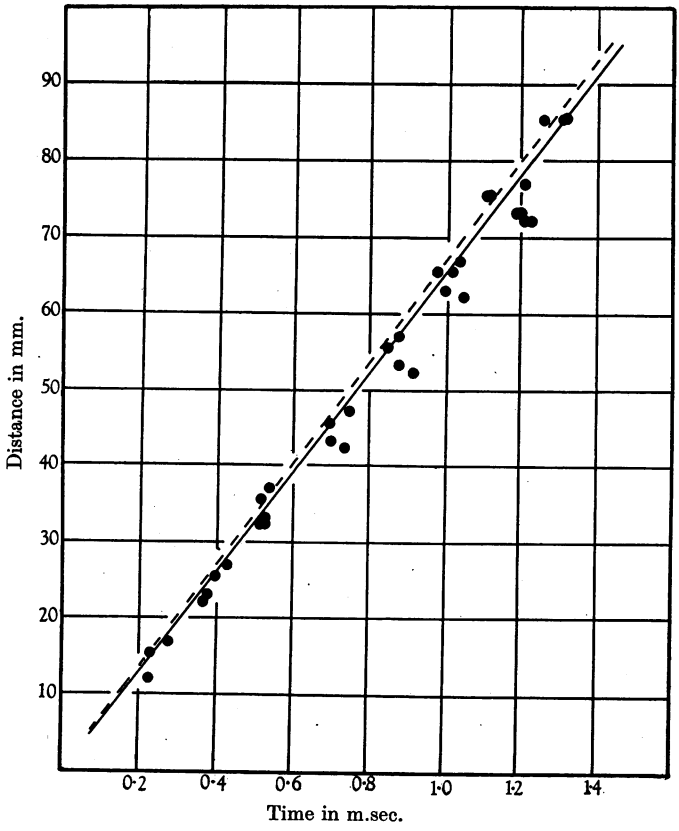


Fig. 7. Graph of conduction distance against time for the inflexion of the action potential in the distal stump after 456 and 486 days of regeneration. Pooled data from four nerves. The solid line is the regression line calculated from these pooled data. The dotted line is the regression line for normal nerve given in Fig. 2.

however, were made upon crushed nerves, while Berry *et al.* (1944) used nerves which had been cut and sutured. It has already been shown (Gutmann & Sanders, 1943) that, after crushing, a nerve becomes completely reconstituted after only 300 days of regeneration, while as late as a year after suture the fibre pattern of the nerve is far from being restored. The results of the present experiments, therefore, emphasize the distinction between regeneration following nerve crushing, and the repair which follows nerve suture. Regeneration after a simple crush injury is the more complete.

In all, except the 456- and 486-day animals, the conduction velocity changed sharply at the point of crushing, the fibres distal to the lesion conducting less fast than those in the proximal stump. Fig. 8 shows a plot of conduction distance against time in the case of a nerve which had been allowed to regenerate for 56 days after it had been crushed (animal 897). Proximal to the lesion the fastest fibre had a velocity of 75·6 m./sec.; distal to the site of crushing the velocity of propagation of the inflexion of the action potential fell to only 27·8 m./sec. The graph of conduction distance against time for the fastest fibre in the normal peroneal nerve of the opposite side of the same animal ($V = 71{\cdot}5$ m./sec.) is also given for comparison. In this regenerating nerve,

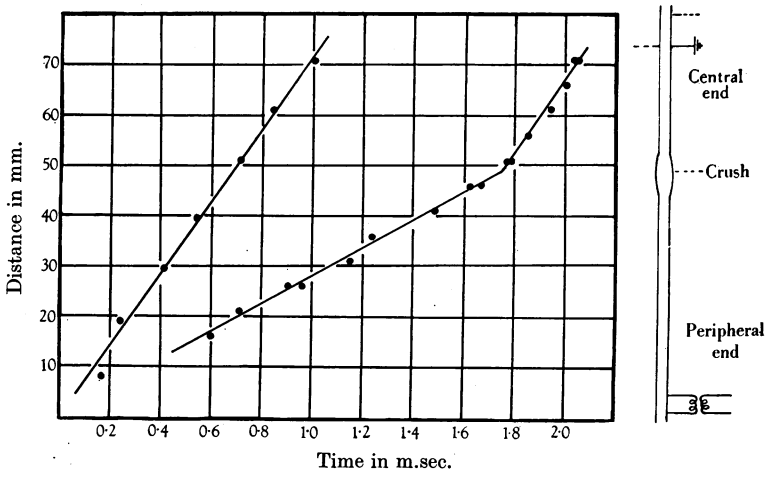


Fig. 8. Graph of conduction distance against time for the inflexion of the action potential in the case of a single animal (897), 56 days after the peroneal nerve of one side had been crushed. The left-hand line is that from the normal, the right-hand that from the crushed nerve. Arrangement of electrodes as in the diagram. The lines are the calculated regression lines.

however, it will be seen that, apart from the change in velocity at the site of crushing, no change in velocity could be detected on passing distally within the distal stump itself. The distal stump was also found to conduct at a constant rate throughout its length in all the other regenerating nerves except those measured at 14, 15 and 26 days after operation. In animal 344 (14 days regeneration time) the conduction rate just distal to the crush was 12·8 m./sec., while lower down the stump it had fallen to 0·7 m./sec., a velocity in the range found for *C* fibres in normal nerve. *C* fibres are unmyelinated, and it was probable that in this region of the stump the impulse was travelling in non-myelinated extensions of the regenerating fibres. Indeed, histological examination of the region of the nerve from which this low rate was recorded, showed that it contained only very fine unmyelinated fibres. In animal 330, measured at 15 days after crushing, the inflexion velocity just below the lesion was

9·2 m./sec., while further distally it was only 3·7 m./sec. This velocity is at the extreme lower limit of the range of normal myelinated fibres (Grundfest, 1940). The third animal (26 days: animal 329) had fibres which conducted at 20·6 m./sec. just below the lesion. Lower down the stump the velocity was 15·1 m./sec. The action potentials in all three cases were obtained when the stimulating electrodes were placed at the upper end of the proximal stump, and the recording electrodes moved to various points distal to the site of crushing. We can therefore be certain that we are here concerned with impulses that have been propagated from fast-conducting fibres in the proximal stump into slow-conducting extensions of the same fibres. In the case of animal 344 the impulse travelled from a region in which the fibres were of large diameter and less thickly myelinated, and finally into a stretch of fibre which did not possess a stainable myelin sheath. In the second case (animal 330) the fibres were probably myelinated throughout, but the results show that the impulse could be propagated into a tapering extension of the presumably large fibres above the lesion.

In the case of most of the nerves recorded in Table 2 whose conduction velocities were recorded after 56–146 days of regeneration, the rising limb of the action potential appeared to have a steeper slope at all distances of conduction than those seen in normal nerve at corresponding conduction distances. The effect of this was to make the conduction time to the spike of the action potential shorter relative to the time of the inflexion, and to bring the inflexion and spike rates closer together. For nine of the nerves in Table 2 there is a corresponding normal nerve from the same animal in Table 1. Comparison of the inflexion-spike velocity differences in these nine pairs of nerves shows that the set of fibres between those responsible for the inflexion and the peak of the $\alpha\beta$ wave in the normal nerve cover a greater range of velocities than a similar set of fibres in distal stumps, at any rate for the first 67 days of regeneration. The fibres in these regenerates are thus more homogeneous than the normal as regards conduction rate which is to be expected since the fibres in such nerves cover a smaller range of diameters than in normal nerves. Since neither the inflexion nor the spike velocities of the normal and late regenerates (456 and 486 days) differ significantly from one another, it is obvious that at the end of regeneration the normal range of velocities is restored.

### *Functional recovery and return of conduction velocity*

Apart from animals which were killed at 14, 15 and 26 days after crushing, all the animals showed complete functional recovery, as estimated by the return of toe-spreading of normal amplitude (Gutmann, 1942), at the time when the nerves were excised for conduction rate measurement. From Table 2 it can be seen that, except for the late regenerates in which the normal conduction velocity had been completely restored, none of these nerves had a

normal conduction rate. Animal 922, for example, in which the distal stump conducted impulses at a rate of only 25·6 m./sec. as compared with 68·6 m./sec. in the normal, nevertheless showed a spreading reflex of normal amplitude. From these results we can conclude that a nerve whose constituent fibres conduct at an abnormally slow rate can nevertheless produce at the end-plates a pattern of impulses sufficiently like the normal to elicit the contractions of the muscles seen in a normal spreading reflex. However, the reflex spreading of the toes is produced by the co-ordinated contraction of a small group of muscles situated close together at about the same distance from the spinal cord. Thus, although the conduction rate of the distal stump of the nerve is abnormally low, impulses can presumably arrive at the end-plates of the different muscles of the group without their time relations being greatly disturbed. Provided sufficient muscle fibres have been re-innervated toe-spreading of normal amplitude will be possible in spite of a slow conduction rate. The total reaction time will probably be increased, but this is not detectable under the conditions of testing used.

*Relation of conduction velocity to myelin sheath thickness*

Measurements of the total diameter, the axon diameter, and the myelin thickness were made on both normal and regenerating nerves in the way already described. The largest fibres in the normal nerves had a mean total diameter of $20{\cdot}4 \pm 0{\cdot}35\,\mu$ (S.E. of mean of 11 observations), an axon diameter of $15{\cdot}4 \pm 0{\cdot}34\,\mu$ (S.E. of mean of 11 observations), and a mean double thickness of the myelin of $5{\cdot}0 \pm 0{\cdot}08\,\mu$ (11). In the eight proximal stumps whose conduction rates were measured after 56–146 days of regeneration, the largest fibre had a mean total diameter of $19{\cdot}2 \pm 0{\cdot}34\,\mu$ (8). The mean axon diameter of these fibres was $13{\cdot}3 \pm 0{\cdot}47\,\mu$ (8), while their myelin sheaths had a mean double thickness of $5{\cdot}8 \pm 0{\cdot}13\,\mu$ (8). The dimensions of the largest fibres in these nerves were compared with those of normal nerves by means of a $t$-test. For total diameter, axon diameter, and sheath thickness $t$ had a value corresponding to a probability of $<0{\cdot}05$, indicating a significant difference between the two types of nerve. Therefore the largest fibres in the proximal stumps of nerves which had been regenerating for 56–146 days had smaller axon and total diameters, but thicker myelin sheaths, than normal nerves (Sanders, 1946). This difference is also shown by the photomicrographs of normal and proximal stump fibres given in Fig. 9.

Were the conduction velocity related solely to either the axon or the total diameter (Gasser & Grundfest, 1939; Hursh, 1939) these proximal stump fibres should conduct more slowly than normal nerves, since they have smaller axon and total diameters. Actually, as has been shown on p. 158, they conduct significantly faster than the corresponding normal fibres. The only positive change with which this increase in velocity can be correlated is the increase in

myelin sheath thickness which occurs during the transformation of normal fibres into the type seen in these proximal stumps. These results therefore suggest that conduction velocity depends, in the first instance, upon myelin sheath thickness, fibres with a thick sheath conducting faster than fibres of similar, or even greater, diameter but with thinner sheaths.

Further evidence of the dependence of conduction velocity upon sheath thickness was obtained from the data relating to distal stumps and normal nerves. Sanders (1946) has shown that the myelin sheaths of fibres in the distal stumps of regenerating nerves are at first thicker than the sheaths of fibres of corresponding diameter in normal nerve. On the assumption that myelin thickness controls conduction velocity, these distal stump fibres should conduct faster than fibres of the same axon diameter in normal nerves. Although a group of normal fibres with which they may be directly compared

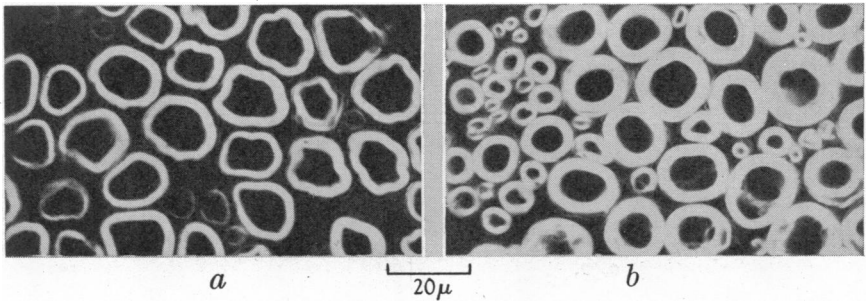


Fig. 9. Photomicrographs, made by direct projection on to bromide paper, of (*a*) fibres from a normal nerve, and (*b*) fibres of corresponding diameter from a proximal stump after 114 days of regeneration. Note thickened myelin sheaths in proximal stump fibres.

cannot be isolated at present, it has been possible to obtain further information in a different way. In Fig. 10 the inflexion velocity of each nerve used, whether normal, proximal, or distal stump, is shown plotted against (1) the total diameter, (2) the axon diameter, and (3) twice the myelin thickness (i.e. total diameter minus axon diameter) of the largest fibre present. The experimental points on inspection show less scatter about the calculated regression line in the case of sheath thickness than in either of the other cases. However, this apparent reduction of scatter may be misleading, since the regression line of velocity on myelin thickness has a somewhat steeper slope than the regression on total or axon diameter. Partial regression analysis of the data was therefore undertaken, and the details are given in Table 3. The total sum of squares of the velocity as found in these data was 10,162 m./sec.$^2$. The multiple regression accounted for 8879 m./sec.$^2$ of this, leaving an error sum of squares of 1283 with 35 degrees of freedom and therefore an error mean square of 36·66. The regression on axon diameter alone gave a sum of squares of 6197; the difference between it and the multiple regression had a mean square of

2682, which was highly significant compared with the mean square for error. Similarly, the regression on total diameter accounted for a sum of squares of 8359, leaving a residue when subtracted from the multiple regression sum of squares of 520 m./sec.$^2$, with one degree of freedom; this difference was again significant when compared with the mean square for error. By contrast the

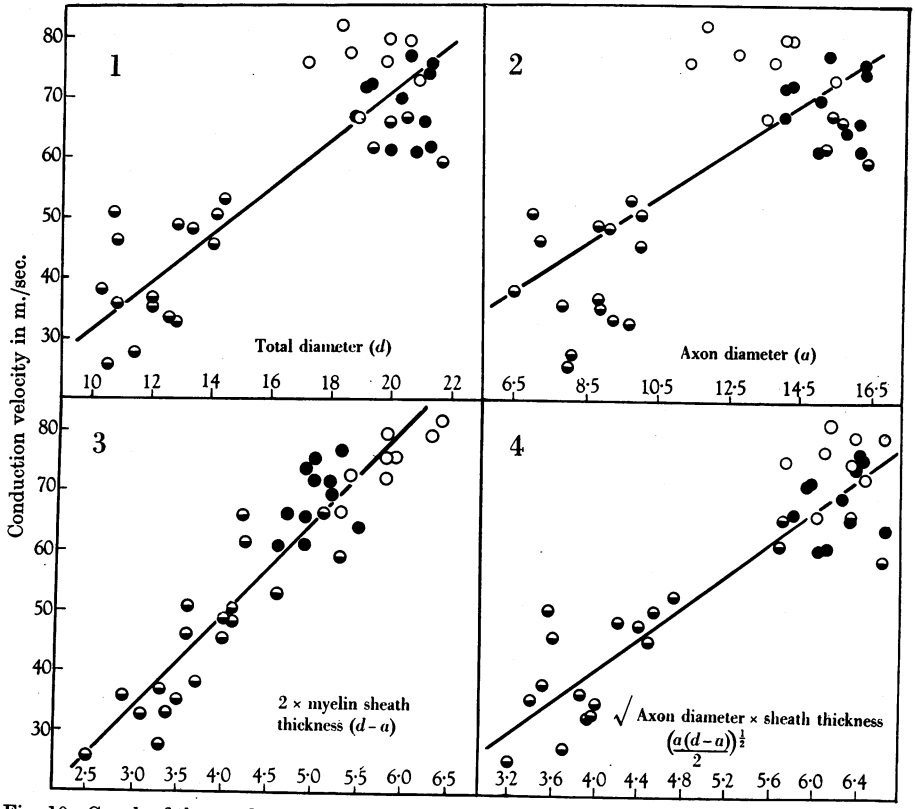


Fig. 10. Graph of the conduction velocity of the inflexion of the action potential against (1) total diameter $d$, (2) axon diameter $a$, (3) 2 × myelin sheath thickness, $d-a$, and (4) $(a(d-a)/2)^{\frac{1}{2}}$, for the largest fibre in each nerve. The lines are the calculated regression lines. ● = normal nerves; ○ = proximal stumps; ◒ = distal stumps. The data on which this figure is based are given in an appendix to this paper.

difference between the sum of squares for the regression on myelin thickness alone and that for the multiple regression was only 55 m./sec.$^2$, a value which was not significantly different from the mean square for error. The analysis therefore indicates that whereas the multiple regression accounts for significantly more of the total variance than the regressions on total diameter or axon diameter, practically the whole of the sum of squares of the multiple regression can be accounted for by considering the regression on myelin thickness alone. The present data from normal and regenerating nerves therefore

indicate that there is a closer correlation between conduction velocity and sheath thickness than between conduction velocity and axon or total diameter. A further point of interest is that the residual sum of squares used as a measure of error (1283; Table 3) is about 12·6% of the total, which is what would be expected on account of the known sources of non-systematic error listed on p. 155.

TABLE 3. Partial regression analysis of the data of Fig. 10

| Item | Degrees of freedom | Sum of squares (m./sec.²) | Mean square | Variance ratio | Probability |
|---|---|---|---|---|---|
| Regression on axon diam. only | 1 | 6197 | | | |
| Difference from multiple regression | 1 | 2682 | 2682 | 73·16 | <0·001 |
| Regression on total diam. only | 1 | 8359 | | | |
| Difference from multiple regression | 1 | 520 | 520 | 14·18 | 0·001 |
| Regression on myelin thickness only | 1 | 8824 | | | |
| Difference from multiple regression | 1 | 55 | 55 | 1·50 | >0·20 |
| Multiple regression | 2 | 8879 | | | |
| Error | 35 | 1283 | 36·66 | | |
| Total | 37 | 10162 | | | |

*Conduction velocity and internodal distance*

In the case of the four distal stumps which had been allowed to regenerate for 456 and 486 days (Table 2), portions were fixed and teased by our colleague Mr A. D. Vizoso to demonstrate the nodes of Ranvier. Whereas in normal nerve large fibres have longer internodes than fibres of smaller diameter, in these regenerated nerves all fibres had internodes of the same length, the actual length being in the case of large fibres much shorter than that found normally. Fig. 11 shows a large fibre taken from one of the 486-day regenerates compared with a fibre of similar diameter taken from a normal peroneal nerve. The regenerated fibre has four internodes in the length occupied by two internodes in the normal fibre. Such differences were a constant feature of all the fibres examined.

Were conduction velocity primarily a function of internodal distance, we should expect such fibres with short internodes to conduct impulses more slowly than normal fibres. In fact, as already stated (p. 162), neither the inflexion nor the spike velocities differed significantly from normal. This must indicate that conduction rate can vary independently of internodal distance. Moreover, the myelin sheath thicknesses of the late regenerated fibres resembled those of normal nerves.

## DISCUSSION

From the results of these experiments we may conclude that within the homogeneous group of fibres provided by a mixed nerve of a single mammalian species, there is a direct dependence of conduction velocity upon myelin-sheath thickness. This conclusion does not invalidate the relation between velocity and total diameter previously found by many workers (Hursh, 1939),

because, as already stated, total diameter and myelin thickness are themselves interrelated. The relation between myelin thickness and the total fibre diameter is however only approximately a linear one (Sanders, 1946), and this may account for the inadequacy of reconstructions of the compound action potential based upon the relation $v = kd$ (Gasser & Grundfest, 1939). These authors, however, found that adequate reconstructions could be made on the assumption that velocity varies linearly with axon diameter. Now it has been found that over the range of fibre diameters in which the observations of Gasser & Grundfest (1939) were made (1–13 $\mu$) there is an approximately linear relationship between myelin thickness and axon diameter, the graph connecting the two quantities having only a slight upward curvature. A similar slight upward convexity is seen in the empirical axon diameter-velocity curve derived by Gasser & Grundfest (1939) from their reconstructions (their Fig. 13*b*). Thus there is reason to believe that the assumption $v = k \times$ *myelin sheath thickness* would also give adequate reconstructions of the action potential.

It is not suggested, however, that sheath thickness is the only variable determining conduction rate. Pumphrey & Young (1938) found that in cephalopod fibres, where there is no 'myelin' sheath in the accepted sense, the axons being surrounded by a thin sheath containing a relatively small proportion of lipids, the velocity varies approximately as the square root of the axon diameter. A similar relationship for this type of fibre has been obtained by Offner, Weinberg & Young (1940) from a mathematical treatment of the core-conductor theory of nerve conduction. Hence it is probable that other dimensions of the fibre in addition to myelin thickness may also play a part in controlling conduction velocity in myelinated fibres. Moreover, while myelin sheath thickness may be the main factor determining conduction rate in a homogeneous group of myelinated fibres, such as those provided by a single peripheral nerve of one mammalian species, it is

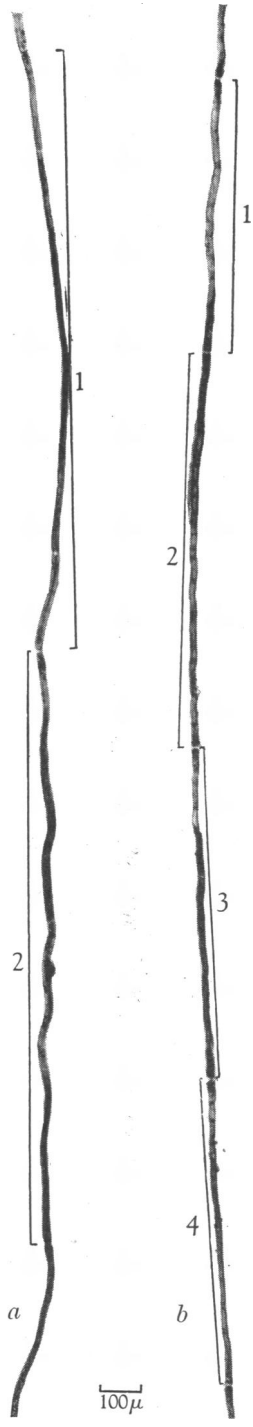


Fig. 11. Photomicrographs of comparable fibres teased from (*a*) normal nerve, and (*b*) a distal stump after 486 days of regeneration. Successive internodes are marked by brackets and the figures 1, 2, etc.

probably necessary to take other factors, such as variations in sheath composition, into account, when interspecific comparisons are made. A reliable estimate of sheath composition in terms of the relative proportions of lipid and protein present is provided by the sheath birefringence (Schmitt & Bear, 1939). The importance of sheath composition with regard to conduction velocity is shown by the work of Taylor (1942) and Taylor & Werndle (1943). These authors compared the fibre diameters and sheath birefringence of a structurally very diverse set of fibres (squid giant: earthworm giant: shrimp giant: frog sciatic: cat saphenous and catfish Muller fibres), all of which conducted impulses at a speed of approximately 25 m./sec. When the fibres were arranged in descending order of diameter, it was found that they were then in ascending order of sheath birefringence, the fibres whose sheaths had the strongest positive birefringence, and accordingly contained the most lipid, being those with the smallest diameters, and vice versa. Whether similar differences in sheath composition cause the nerves of different mammals to conduct at different rates is not known, since data on both conduction rate and birefringence are not available for a sufficiently diverse range of species. That such is the case, however, is suggested by the fact that while the largest fibres in the peroneal nerves of both cat and rabbit have the same diameter ($20\mu$) and the same myelin thickness ($2{\cdot}5\mu$), the cat's nerve conducts at 108–111 m./sec. (Hursh, 1939), whereas the maximum velocity attained in the rabbit's nerve is only in the region of 69 m./sec.

The precise mechanism whereby a change in myelin thickness alters the speed at which the nerve impulse travels is at present largely a matter for speculation. Recent work on large 'unmyelinated' invertebrate fibres has established that in such fibres nervous transmission is brought about by local electric circuits, and it is improbable that the mechanism in myelinated fibres should be fundamentally different. On local circuit theory conduction velocity will be directly proportional to the rate at which the local circuits spread along the fibre. This latter rate is determined by the expression $t = (\text{axon diameter} \times \text{sheath thickness})^{-\frac{1}{2}}$, where $t$ is the time taken by the local circuits to spread a given distance. Hence velocity should vary as (axon diameter × myelin thickness)$^{\frac{1}{2}}$. To test whether the present data are in accord with such a hypothesis, Fig. 10, 4 was constructed. In this figure the velocities for all the different nerves used are shown each plotted against the appropriate value of (axon diameter × myelin thickness)$^{\frac{1}{2}}$, with the calculated regression line drawn to fit the data. On inspection Fig. 10, 4 does not show a markedly closer correlation between conduction velocity and (axon diameter × myelin thickness)$^{\frac{1}{2}}$ than that already seen between velocity and myelin thickness by itself (Fig. 10, 3). Moreover, the data are insufficient to show whether a linear regression or one of higher order should be used to express the relationship plotted in Fig. 10, 4. All that can be said is that the above hypothesis is not excluded by the present data.

On the other hand, the theoretical treatment developed by Offner *et al.* (1940) has led these authors to conclude that velocity is related to some function of the nodal widths and internodal distances, which is contrary to the results reported in the present paper. This theory assumes that the myelin forms an insulating layer over the surface of the fibre, and that the impulse is forced to jump from node to node without causing excitation of the intervening portions of the fibre. Yet in the central nervous system the fibres have no nodes, and conduction velocity is just as high. The whole question of the part played by variations in the dimensions and composition of the myelin sheath is in need of further investigation before an adequate theory of conduction in myelinated fibres can be developed.

SUMMARY

1. Measurements have been made of conduction velocities in rabbits' peroneal nerves regenerating after a crush lesion: (*a*) in the proximal stump after 56–486 days of regeneration; (*b*) in the distal stump after 14–486 days of regeneration.

2. Proximal to the lesion, after 56–146 days of regeneration, the largest fibres conduct at about 7·5 m./sec. (11 %) faster than the corresponding fibres in normal nerves. Such fibres have thicker myelin sheaths than the corresponding normal fibres.

3. In the earliest days of regeneration, fibres distal to the lesion conduct at relatively low velocities ( < 10 m./sec.), and show a diminution in velocity on passing distally within the stump. With increasing time of regeneration, the conduction rate increases, at first rapidly, and thereafter more slowly, until after 456 and 486 days of regeneration the rates recorded do not differ significantly from those of normal nerves.

4. In nerves which have been allowed to regenerate for 456 and 486 days the nodes of Ranvier on the largest fibres are more closely spaced than on fibres of corresponding diameter in normal nerve, although the two types of fibre conduct at similar rates.

5. As a result of these experiments it is concluded that, in the peroneal nerve of the rabbit: (*a*) conduction velocity has a relation to myelin sheath thickness, and is (*b*) independent of internodal distance.

## APPENDIX

In the accompanying table the data for inflexion velocity ($V$), the total diameter ($d$), the axon diameter ($a$), and double myelin thickness ($d-a$) of the largest fibre present are summarized for each of the nerves in which it was possible to measure them all. The first column gives the number of the animal together with the limb, right ($R$), or left ($L$), from which the particular nerve was taken: the second column indicates whether the particular nerve measured was normal ($N$), proximal ($P$), or distal ($D$) stump; subsequent columns give the values found for $V$, $d$, $a$, and $d-a$ in each case.

| Animal | Type of nerve | $V$ | $d$ | $a$ | $(d-a)$ |
|---|---|---|---|---|---|
| 922 *R* | *D* | 25·6 | 10·5 | 8·0 | 2·5 |
| 897 *L* | *D* | 27·8 | 11·4 | 8·1 | 3·3 |
| 962 *L* | *D* | 32·8 | 12·8 | 9·7 | 3·1 |
| 963 *R* | *D* | 33·4 | 12·6 | 9·3 | 3·4 |
| 964 *R* | *D* | 35·1 | 12·4 | 8·9 | 3·5 |
| 961 *R* | *D* | 35·8 | 10·7 | 7·8 | 2·9 |
| 899 *L* | *D* | 36·9 | 12·1 | 8·8 | 3·3 |
| 898 *L* | *D* | 38·3 | 10·2 | 6·5 | 3·7 |
| 989 *R* | *D* | 45·5 | 14·0 | 10·0 | 4.0 |
| 861 *R* | *D* | 46·1 | 10·8 | 7·2 | 3·6 |
| 879 *L* | *D* | 48·1 | 13·3 | 9·2 | 4·1 |
| 879 *R* | *D* | 48·7 | 12·8 | 8·8 | 4·0 |
| 965 *R* | *D* | 50·5 | 14·1 | 10·0 | 4·1 |
| 872 *L* | *D* | 50·8 | 10·6 | 7·0 | 3·6 |
| 966 *L* | *D* | 53·0 | 14·3 | 9·7 | 4·6 |
| 948 *L* | *D+P* | 59·2 | 21·6 | 16·3 | 5·3 |
| 927 *L* | *N* | 61·0 | 20·7 | 16·1 | 4·6 |
| 961 *L* | *N* | 61·3 | 19·9 | 14·9 | 5·0 |
| 948 *R* | *D+P* | 61·5 | 19·3 | 15·1 | 4·2 |
| 966 *L* | *N* | 64·1 | 21·2 | 15·7 | 5·5 |
| 922 *L* | *N* | 65·4 | 22·7 | 17·7 | 5·0 |
| 990 *L* | *D+P* | 65·9 | 19·8 | 15·6 | 4·2 |
| 990 *R* | *D+P* | 66·5 | 20·4 | 15·3 | 5·1 |
| 965 *R* | *P* | 66·5 | 18·8 | 13·5 | 5·3 |
| 989 *L* | *N* | 66·7 | 18·7 | 14·0 | 4·7 |
| 965 *L* | *N* | 69·6 | 20·2 | 15·0 | 5·2 |
| 897 *R* | *N* | 71·5 | 19·0 | 14·0 | 5·0 |
| 963 *L* | *N* | 71·9 | 19·2 | 14·2 | 5·0 |
| 988 *L* | *P* | 72·8 | 20·8 | 15·4 | 5·4 |
| 921 *R* | *N* | 73·7 | 21·1 | 16·2 | 4·9 |
| 962 *R* | *N* | 75·4 | 21·2 | 16·2 | 5·0 |
| 897 *L* | *P* | 75·6 | 17·1 | 11·3 | 5·8 |
| 989 *R* | *P* | 75·7 | 19·7 | 13·8 | 5·9 |
| 921 *L* | *N* | 76·7 | 20·5 | 15·2 | 5·3 |
| 988 *R* | *P* | 77·1 | 18·5 | 12·7 | 5·8 |
| 966 *R* | *P* | 79·3 | 20·5 | 14·2 | 6·3 |
| 927 *R* | *P* | 79·5 | 19·8 | 14·0 | 5·8 |
| 967 *R* | *P* | 81·7 | 18·2 | 11·8 | 6·4 |

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