

## THE RATE OF REGENERATION OF NERVE

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### INTRODUCTION

VERY varied estimates of the rate of nervous regeneration have been given by clinical and experimental investigators, but no one has applied the various possible methods in such a way as to enable comparison of their results. We have therefore made estimates by five different methods in the rabbit. The results showed consistent differences between the rate as estimated in various ways, and consideration of these differences has shown that the techniques do not all measure the same quantity, so that it is not surprising that they appear to reveal different rates of regeneration.

For purposes of analysis we may consider that before recovery takes place after suture of the cut ends of a divided nerve the regenerating fibres must pass through three phases. (1) They undergo retrograde degeneration, branching, and the relatively slow process of outgrowth across the suture scar. (2) The tips of the axons advance down the peripheral stump and make connexion with the end-organ. (3) The newly formed fibres increase from their first tiny diameter and acquire myelin sheaths. Since most of the important sensory and motor functions depend on the conduction of suitably timed volleys of nerve impulses it is only after the processes under the third heading have reached a certain stage that function returns.

The factors which determine the time which elapses between suture and recovery are therefore (1) the scar delay or latent period, including the time taken by the processes under the first of the above headings and that which elapses before the third process begins; (2) the rate of progress down the nerve of the processes of maturation or functional completion of the axons. The rate of progress of the tips of the axons is thus not usually a limiting factor in the time necessary for regeneration, nor is the time taken for the establishment of a connexion between the axon tip and the end-organ. Nothing is known about the duration of this latter period, and throughout this paper it has been assumed that it is shorter than the period necessary for completion of maturation of the regenerated nerve to a level at which function is possible (see p. 41).

It will be seen at once that the concept 'rate of regeneration' is far from simple. We have already to distinguish between the apparently simple but clinically less important process of advance of the axon tips down the nerve, and the more obscure, but important, process of advance of capacity to function. If the first alone is considered as the 'rate of regeneration' then there will appear to be a delay after the arrival of fibres at a muscle or sense organ but before the return of reflex functioning. If the spread along the nerve of the process by which the axons develop to a state at which they can function takes place at the same rate as the growth of the axon tips, then the apparent period of delay after arrival of fibres but before functioning will be constant, whatever the distance of the lesion from the end-organ. But there is no a priori reason to suppose that either the delay at the upper end or the spread of the maturation should take place at the same rates, and the experiments detailed below indicate that the delays and rates are in fact different, the advance of the axon tips being the faster.

A further very serious complication is that the degree of maturation of nerve fibres which is necessary to ensure functioning may differ, not only for different muscles and sensory functions but also, perhaps, with the distance of the lesion from any particular end-organ. A relatively little developed stretch of nerve may suffice for conduction over short distances, where the time scatter of impulses cannot become large. We are faced therefore with the possibility that the entity whose rate of advance down the nerve we wish to measure may vary with the distance of the lesion from the end-organ. If this is so no fixed 'rate of regeneration' will appear, the apparent rate being constant only for the recovery of a particular function after lesions at given levels.

Consideration of these complications makes it apparent that when measuring the rates of progress of the processes of regeneration down the nerve it is necessary to define most carefully the function by whose return the advancing front of regeneration is recorded. And this study of the rate of regeneration as estimated by various methods has shown in fact that the apparent rate varies according to the function chosen as an index for recovery.

In any case it is clear that in order to be able to forecast the date at which recovery will occur after a nerve lesion in an animal or man it is necessary to know separately the rates at which the various processes proceed. For instance, the delay in the scar is probably constant whatever the level of the lesion, whereas the time taken by fibre outgrowth and maturation of course increases with the distance to be covered. The 'rate of growth' of nerve fibres cannot be determined simply by dividing the distance from lesion to end-organ by the time taken for recovery.

The experiments described in this paper were designed to estimate the rates of the various stages of the processes of regeneration. The distinction between the rate of advance of axon tip and the rate of advance of functional completion was not appreciated when the experiments were planned, but became apparent when it was seen that there were conspicuous differences in the rates recorded by the use of different methods.

The nerves of the leg of the rabbit have been used for nearly all the observations.

They are very suitable for such experiments because the tibial, peroneal and sural (saphenous minor) divisions of the sciatic can be separated almost to the top of the thigh, allowing any of them to be divided at this level. After interruption of all three divisions rabbits always develop severe sores on the heel and foot, often leading to serious infection. These developments, which have troubled many investigators, can be almost completely avoided if the small sural division is left intact, since this nerve carries sensory fibres from the region of the heel.

All operations were performed aseptically after anaesthesia, usually with intravenous nembutal, followed if necessary by ether. The nerves in the thigh can be very fully exposed by an incision along the line of the femur, followed by retraction of the biceps group of muscles. If necessary the biceps tendon and fascia can be cut away from the knee and the whole muscle reflected. The rabbits used were adults (except for experiments with newborns) and were of various races. As far as possible animals in good health only were taken, but no special attention was applied to uniformity in weight.

#### I. RATE OF GROWTH OF SENSORY FIBRES

Methods of three types have been used to estimate the rate of growth of sensory (pain) fibres. (1) Since reflex responses can be obtained by stimulating the young fibres in the nerve itself, the animal may be opened under anaesthesia at a given time after nerve suture and the nerve stimulated by pinching or electrical means to discover the farthest point to which fibres have reached (Young & Medawar, 1940). (2) Sutures of a given nerve may be made at various distances from a certain skin area and comparison made between the times which elapse before appearance of pain sensibility in that area. (3) After sensory recovery has begun in an analgesic area, the rate at which that area shrinks gives a measure of the rate of growth of the sensory fibres.

It must not be assumed that these three methods all measure exactly the same quantity. Whereas the first type of experiment measures approximately the growth rate of the fastest growing axon tips, the second and third measure the advance down the nerve of the process of maturation of such numbers of the new fibres as are necessary to give reflex functioning (see p. 37).

Estimates have been made by all the three methods, but the present paper is concerned mainly with those made by the first. The results obtained by L. Guttmann and E. Guttmann with the other two methods will be reported in detail later, the main results only being given here for comparison.

##### A. *Methods of following the advance of reflex excitability down the recovering nerve*

Estimation of the distance reached by new fibres by means of mechanical stimulation of the nerve can give very consistent results if suitable precautions are observed. An animal on which, say, the tibial nerve was cut and sutured 25 days earlier is anaesthetized, and the nerve exposed from the level of the lesion down-

wards to the heel. With a fine forceps it is then firmly pinched at its most peripheral point. If no fibres are present here there will of course be no response to this stimulus, and the pinch is then repeated a few millimetres higher, and so on at intervals up the nerve. When the point at which fibres are present is reached an unambiguous reflex response is obtained, usually in several muscle groups. Of course if the anaesthesia is very deep no visible reflexes are seen, and it is therefore important in all cases to make a control by exposing some nerve known to be intact and pinching this, before investigating the nerve which has been sutured.

It might be expected that responses of gradually increasing intensity would be obtained at the tip of the regenerated region. In practice such a zone, though detectable, is short, the threshold between the responsive and unresponsive parts of the nerve being very sharp. Measurement of the distance which fibres have grown from the lesion can therefore be made to the nearest one or two millimetres, if the pinches are made sufficiently close together on the nerve. As an example of the accuracy of the method: in rabbit 191 the peroneal nerve was cut and sutured on 22 May 1940 and on 6 June 1940 the nerve was explored under nembutal anaesthesia. Pinching upwards from the heel gave no response at 41.0 or 39.0 mm. from the suture point, but at 38.0 mm. there was a slight but definite head movement. Two still higher pinches for confirmation gave movements slightly greater than the first. Three separate checks of the distance, measured with dividers, gave 38.0, 37.5 and 38.5 mm.

Errors sometimes arise from the fact that pinching of arteries or veins may give rise to reflex movements of the animal. Moreover, the sensory fibres in question follow the vessels in their distribution, so that it may happen that the *nervi vasorum* in a nerve remain intact even after the fibres of the nerve itself have all been severed. Reflexes obtained in this way from stimulation of severed nerves are especially marked where large arteries enter the nerve, for instance in the sciatic above the popliteal space or in the anterior or posterior tibial nerves above the heel. It is sometimes very striking to see an animal give reflex responses in this way when a pinch is given to a nerve known to be severed from the central nervous system, and it seems possible that such reflexes have been responsible for some of the reports of autogenous regeneration in isolated peripheral stumps.

Errors from this source can be excluded by making it a routine that all collateral connexions of the nerve be severed before the pinching experiment is begun. In fact the nerve should be quite free from the lesion downwards, and cut peripherally.

Histological examination, by Bodian's method, of the most distal regions of the regenerating nerves from which responses were obtained, showed good agreement with the results of pinching experiments. It is not easy to be certain histologically of the farthest point to which regenerating fibres have reached. The youngest fibres are very fine, and growth cones are seen only rarely. However, in a piece of regenerating nerve 10 mm. long with the lowest excitable point half-way along it, there were usually found to be numerous fibres at the upper end, none at the lower. Sometimes even more exact correspondences were obtained, the first fibres being

visible at, or just above, the 'first positive pinch'. This histological examination certainly shows that the reflex response to pinching the nerve gives an estimate of the farthest point to which a few of the fastest growing fibres have reached. The fibres which give the response are unmyelinated and can hardly have been laid down for more than a few hours. Such very young fibres appear to be particularly easy to stimulate mechanically; witness the great sensitivity of some human neuromas when exposed under local anaesthesia.

On the other hand, such small fibres are rather difficult to stimulate electrically. It is not easy to discover exactly the farthest point to which fibres have reached by stimulation with faradic shocks or condenser discharges. Even using fine silver wire electrodes and discharges of relatively large condensers, responses could usually only be obtained from points higher in the nerve than those shown by the pinching experiments.

#### B. *Rate of advance of the axon tips*

With these precautions experiments by different observers were found to give reasonably consistent estimates of the distances reached by new fibres after a given number of days. There is of course a considerable scatter; for instance, among the rabbits examined 15 days after plasma suture of the tibial or peroneal nerves, distances of outgrowth ranging from 23 to 38 mm. were recorded, with a mean of 30.1 and standard deviation of 5.3. This scatter is greater than would be expected from phenotypic and genotypic differences alone, but perhaps not more than might be expected from differences in the making of the suture. There is no reason to suppose that a great part of the scatter is due to observational error.

Fig. 1 shows that beyond all question the distance from the suture point at which responses can be obtained increases with the time between the initial operation and final experiment. This gives the clearest demonstration, on the lines of that given by Howell & Huber (1892), that regeneration consists of a process spreading down the nerve. It appears that after an initial delay in the scar the fibres advance down the nerve at a constant rate. In fact the points of Fig. 1 may reasonably be fitted by a straight line. However, since, in order to facilitate statistical comparisons, nearly all of the observations were made after either 15 or 25 days, it is not possible, from these data, to test the hypothesis that the rate of growth is constant; in all the following calculations it has been assumed to be so.

#### C. *The delay in the scar*

The data indicate clearly that during the first few days after suturing no fibres appear in the peripheral stump. Presumably during this period they are undergoing retrograde degeneration for a few millimetres from the injured surface, splitting up into branches and advancing across the scar between the stumps. Cajal (1928) estimated that the first part of this period, before fibres reach the upper margin of the scar, was about 2-3 days, and that fibres then grow at a rate of 0.25 mm./day across the scar. He therefore found that axons only appeared in the peripheral stump '7 or more days' after suture in rabbits.

In the present experiments the length of this period has not been carefully determined by independent experiments, though the points marked on the base-line of Fig. 1 show that it could be so determined. If the assumption that the rate of growth in the peripheral stump is constant be correct then the latent period can readily be calculated as the point at which the regression line of distance travelled by the new fibres on time cuts the base-line. The data of Fig. 1 thus give as an

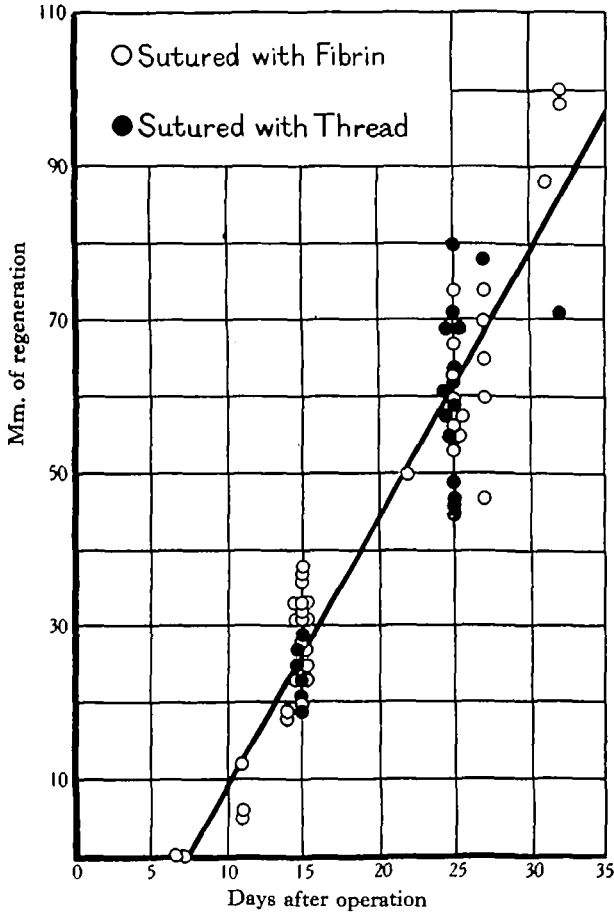


Fig. 1. Plot of the distances reached by new fibres as revealed by pinching the exposed nerve at various times after suture by stitching or with concentrated fowl plasma. The line is the calculated regression line for all the points considered together.

estimate of this delay 7.27 days after plasma suture. Presumably the period will vary considerably with the closeness of apposition of the stumps when the suture is made, and a large part of the variation in the distances of outgrowth recorded must be due to this cause. Indeed, it is possible to some extent to forecast from observation of the closeness of apposition at operation, whether the distance reached will be large or small. For instance, in rabbit 191 it was recorded at operation that whereas the apposition on the right side was 'excellent', that on the left was 'rather

less successful'. After 15 days it proved that fibres had reached to 38 mm. on the right but only 33 mm. on the left. Histological examinations confirmed that the union was closer on the right.

Nevertheless, it is probable that a great part of this period of delay is occupied by retrograde degeneration and other preliminary processes. For in experiments in which the nerve was heavily crushed at the original operation (p. 22), rather than being cut and sutured, the figure for the delay in the scar is 5.23 days, that is to say only slightly shorter than after suture, although the scar separating the central axons from the intact peripheral stump is certainly shorter and simpler after a crush than after a cut and suture.

This close correspondence between the latent period after crushing and after complete division suggests that a definite time is needed for the processes preparatory to axon regeneration, irrespective of the rate at which this regeneration takes place. That the bulk of the scattering in the estimates for distances is due to differences in regeneration rates between different animals rather than to differences in latent period can be shown in another way. If the differences were due chiefly to differences in the latent periods, then the scatter (expressed arithmetically as the variance) should be no greater after 25 days than after 15 days, always assuming that the rate of regeneration is constant in each individual. On the other hand, differences between rates of regeneration would produce a scatter which became more marked at a greater distance from the point of the lesion, and which ideally would be proportional to this distance. By analysis of the variance at 15 and 25 days it is in fact found that the variance is roughly proportional to the mean distance which the fibres have travelled, so that dividing the variance of the distances by the mean distance gives a 'specific factor of variance' which is approximately the same after 25 days as it is after 15 days. This leads us to suppose that it is individual differences in the rate of regeneration rather than in the latent period that is responsible for the scatter of the estimates.

These figures for delay would agree with Cajal's estimate of 2-3 days as the period before fibres leave the central stump and enter the scar. It must be remembered that these estimates give the delay before the tips of the fibres begin to advance down the peripheral stump. There are strong indications that there is a further delay before the process of medullation also begins to advance (see p. 40).

#### D. *Rate of growth after suturing*

Having crossed the scar the fibres grow down the peripheral stump at a greatly increased rate. The revised estimates of rate of growth of sensory fibres now obtained, and including new data, differ a little, though not significantly, from the provisional ones recorded by Young & Medawar (1940).

The regression coefficients obtained from the data of Fig. 1 show that the rates in twenty-two experiments in which either the tibial or the peroneal was cut and sutured with white or black silk was 3.42 mm./day, whereas in the forty experiments in which the suture was made with concentrated fowl plasma it was 3.53 mm./day.

Table 1. *The results of estimation of the rate of nervous regeneration by various methods in the rabbit. Where alternative estimates, obtained from the pooled data of a number of animals and the paired data from opposite sides of the same animal, are given, the figure which is to be preferred is italicized*

Quantity measured	No. of readings	Rate of progress $\pm$ standard error mm./day	Latent period
1 Rate of advance of tips of pain fibres in nerve			
11 After nerve suture	62	3.45 $\pm$ 0.16	7.27
111 Stitch sutures	22	3.42 $\pm$ 0.41	7.68
112 Fibrin sutures	40	3.53 $\pm$ 0.17	7.27
1121 in tibial	18	3.44 $\pm$ 0.28	7.29
1122 in peroneal	12	3.82 $\pm$ 0.25	7.62
1123 peroneal into tibial	10	3.37 $\pm$ 0.40	6.58
12 After crushing nerve			
121 Adults	34	4.36 $\pm$ 0.24	5.23
1211 High crushes	20	4.48 $\pm$ 0.28	5.19
1212 Low crushes	14	4.11 $\pm$ 0.41	5.08
122 Babies	11	4.27 $\pm$ 0.36	2.35
2 Rate of advance of functional completion			
21 Sensory function			
211 After crushing peroneal nerve at various levels	13	3.04 $\pm$ 0.35	9.76
Paired data	4 $\times$ 2	3.4	19.0
212 From shrinkage of anaesthetic area			
2121 After crushing	6	2.05 $\pm$ 0.14	—
2122 After suture	6	1.57 $\pm$ 0.15	—
22 Motor function			
221 From recovery after lesions at different levels			
2211 After crushing			
22111 Adults	38	3.05 $\pm$ 0.14	20.77
Paired data	4 $\times$ 2	3.1	21.6
22112 Babies: paired data	2 $\times$ 2	5.4	10.2
2212 After suture			
22121 Adults	19	2.02 $\pm$ 0.32	32.35
Paired data	4 $\times$ 2	2.6	36.5
22122 Babies: paired data	2 $\times$ 2	2.5	16.2
222 From times of recovery of muscles at different levels			
2221 After crushing	6	2.2	—
2222 After suture	1	1.2	—

Table 1 shows that neither the rates, nor the periods of delay in the scar, differ significantly in the two cases. Table 1 also shows that in eighteen of these experiments in which fibrin junctions were made in the tibial nerve neither the rate nor the latent period differ significantly from the twelve junctions made in the peroneal nerve. Evidence from interruption of all three parts of the sciatic by a single crush (p. 22) also indicates that there are no large differences in the rate of advance of the tips of the axons in different nerves. Similarly in ten experiments in which a peroneal central stump was sutured into a tibial distal one the rate is similar, and the latent period only possibly a little shorter. In this type of experiment the apposition between the cut ends should be particularly satisfactory because any desired length of central stump is available and perfect apposition can be achieved, without any tension. The similarity of the latent periods therefore further supports the view that



this time is mainly occupied with processes of reorganization which are independent of the nature of the lesion and scar between the stumps. Since there are no significant differences after the different types of suture, we may pool all the results and say that after suture the rate of growth is  $3.45 \pm 0.16$  mm./day and the latent period 7.27 days.

#### E. *Rate of growth after suture of nerves in the dog*

With the co-operation of Miss E. Bülbring of the Department of Pharmacology similar experiments were made on three dogs. The sciatic nerves were cut, sutured with plasma and explored after 11, 24 and 26 days. In so few experiments it is difficult to gain sufficient experience of the level of anaesthesia required to give decisive results, but the reflexes were obtained at distances from the suture points which indicate growth rates of the same order as those found in the rabbit. For instance, in dog 3 the exploration made after 26 days showed a clear response at 41 mm. from the junction. Histological examination showed that fibres were present at this point and for some distance beyond it. The delay in the scar cannot have been less than 8 days, and may have been more, since the junction was not a very satisfactory one, so the rate of growth of the fibres must have been at least 2.3 mm./day. Similarly dog 4 gave 40 mm. after 24 days, giving a rate of at least 2.5 mm./day.

#### F. *Rate of growth after interruption of a nerve by crushing*

Since even with the most careful technique it is impossible to make unions of cut stumps in an absolutely uniform manner in different experiments, it was hoped that results showing smaller variability might be obtained by examining the distances reached by new fibres after a nerve had been crushed at one point. Such crushing, if properly performed, interrupts all the axons, which therefore undergo Wallerian degeneration. However, the connective tissues maintain the continuity of the nerve, so that new outgrowth of the severed axons can take place under optimal conditions. In small nerves, such as the peroneal and tibial of the rabbit, suitable crushes can be made very effectively by using fine, sharp, smooth-faced watchmakers' forceps. Crushing several times at one spot reduces the nerve to a transparent thread over a length of 1 or 2 mm. During the following minutes opaque matter flows back into the crushed region, so that the site of the crush can hardly be recognized. Subsequently only a very slight swelling develops at the injured point, and sections suggest that in many cases the new growing fibres remain within their original connective tissue tubes, which were apparently not destroyed by the crushing. Such a lesion must be distinguished from one in which all the internal structure of the nerve is distorted by the crush so that new fibres have to cross a scar and become criss-crossed much as they would do after suture. Crushing with broad instruments, such as toothed artery forceps, may well produce such disturbances, leaving the ends in a position which may be quite unfavourable for regeneration.

That simple crushes of the first type do completely interrupt all the fibres is shown by the fact that the distance from the crush at which reflexes can be elicited

increases with time, as after simple suture. Fig. 2 shows the results of thirty-four experiments in which the nerve was explored at times varying from 13 to 25 days after the initial crush injury. The distances reached are considerably greater than those which would have been found after complete severance and suture of the nerve, although, as already mentioned, the latent period is hardly different in the

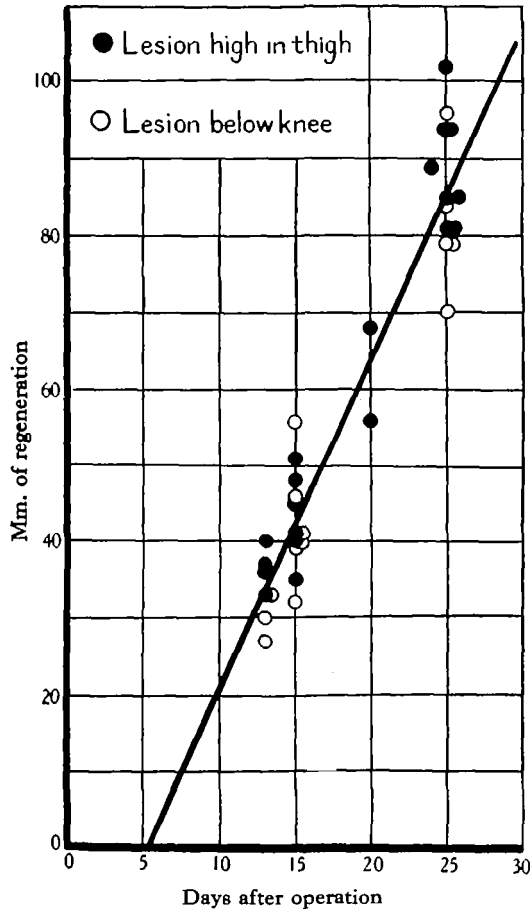


Fig. 2. Plot of the distances reached by new fibres as recorded by pinching the exposed nerve at various times after interruption of the axons by crushing. The lesions were made either high up or low down, but the regression line is that for all the points considered together.

two cases. In fact, the greater distances reached after the operation of crushing are due to the faster growth of fibres in the peripheral stump (Table 1), the estimate being  $4.36 \pm 0.24$  mm./day.

It is somewhat surprising to find that the rate of growth in the peripheral stump varies with the conditions at the point of injury. Presumably the scar at a suture line presents a region at which the fibres are much attenuated, without endoneural support, and accompanied only by Schwann cells. Being therefore thin and ill-nourished they are less well able to grow in the peripheral

stump than when they remain throughout in a partially intact nerve, as they do after a crush. It is possible that the crushing interferes less than does cutting with the blood supply of the stumps, or at least that a good circulation is more rapidly re-established after a crush.

If the rate of outgrowth really depends on conditions between the growing point and the nerve cell body it is a striking confirmation of the doctrine that regeneration of nerves consists of an outgrowth from the central stump, in the full sense. Some relatively recent workers (e.g. Spielmeyer, 1929), although admitting that continuity with the nerve cell body is necessary for regeneration, have yet held that the nerve fibre is secreted, as it were, by the activities of the tissues of the peripheral stump, and that in this sense the doctrines of Bethe and others of autogenous regeneration are correct.

In any case the fact that the rate of growth in a particular nerve varies with the nature of the interruption is a warning against assuming that such growth rates can be considered as fixed and unvarying quantities. The high rate of growth after crushing is itself of interest for the prognosis of recovery in nerves which have been accidentally crushed at one point in man. It has frequently been observed that recovery after such lesions is more rapid than after suture, even when the latter is performed under optimal conditions.

#### *G. Rate of growth after injury at various levels*

It is important to discover whether the rate of outgrowth of new fibres varies with the distance of the lesion from the nerve cell body. Stopford (1920), from study of the times of recovery after lesions at different levels, has suggested that the rate of growth of new fibres may be higher when the lesion is closer to the cord.

The data analysed above include experiments in which nerves, mostly the peroneals, were crushed at various levels, but so far they have all been considered together. We may now consider separately those crushes made high up and low down. The highest point which can be satisfactorily reached on the peroneal nerve exposed in the leg is 5-10 mm. below the obturator foramen, and this is the level described as 'high'. The sensory fibres at this point are about 25 mm. from their cells in the dorsal root ganglia. The 'low' crushes were made in the peroneal nerve reached by a small incision just below the knee. This arrangement gives the maximum difference of level which is experimentally possible with this method in the rabbit, namely, up to 120 mm.

Twenty 'high' and fourteen 'low' experiments were made, and as Table 1 shows the rates of growth and latent periods are not significantly different at the two levels. In fourteen cases the nerve was crushed high on one and low on the other side of the same animal. In eleven of these the outgrowth had proceeded slightly farther on the side crushed high, but the greatest difference was only 11 mm. This proportion (11-3) would be expected to occur in about 2% of an infinite set of trials, and the difference is therefore suggestive of a slightly greater rate of outgrowth in the nerves crushed high.

It must be remembered that what is here measured is the rate of growth of the axon tips. It may be that the rate of advance of the process of maturation of the nerve fibres appear to be lower after high lesions than low ones, because the degree of completion necessary to allow functioning increases with the distance of the lesion from the end-organ.

## II. RATE OF ADVANCE OF FUNCTIONAL COMPLETION OF FIBRES

### (I) *Recovery in skin after lesions at different levels*

In all the experiments so far described the rate of regeneration has been determined by study of the distance from the point of injury at which reflex responses could be obtained when the nerve itself was exposed and pinched. A completely separate estimate has been obtained by the use of the criterion of the first appearance of recovery in a given area of skin rendered insensitive by injury. The method has been to interrupt the peroneal nerve by crushing at various levels, and then to watch for the return of reflex responses to pin prick in the denervated area on the leg and foot (Fig. 4). Recovery in such an area of sensory loss is a very complex

Table 2. *Times taken for complete sensory recovery after crushing the peroneal nerve at various levels. The distances given are from the lesion to the lowest point of the analgesic area on the foot, and the number of days is that necessary for recovery to reach this point*

Animal	Distance mm.	Days
562	160	67
560	161	70
494	166	68
493	171	68
485	198	67
486	198	90
579	201	70
304	212	94
562	240	90
560	244	91
546	250	96
390	252	85
494	266	101
493	273	101
509	282	95

process, including overlap as well as regeneration, therefore it is not possible to use as a datum the moment at which recovery begins at the upper margin. Instead we have used the criterion of recovery at a fixed level, namely, the reappearance of pain sensibility in the most distal part of the affected area. The animals were examined weekly until recovery had taken place, and the distance from the lesion of this most distal part, that is, the dorsum of the second and third toes, was then carefully determined. The details of this investigation will be later described by two of us (E. G. and L. G.).

Table 2 and Fig. 3 show the results in the fifteen animals studied. The lesions were made at three levels: 160–170, 195–210 and 240–280 mm. from the most distal point in the area of sensory loss. There is undoubtedly a progression, in that the recoveries took longer after the more proximal lesions, but the data are not so consistent as those obtained by the pinching of exposed nerves. This is only to be expected since more variables may intervene between injury to the nerve and

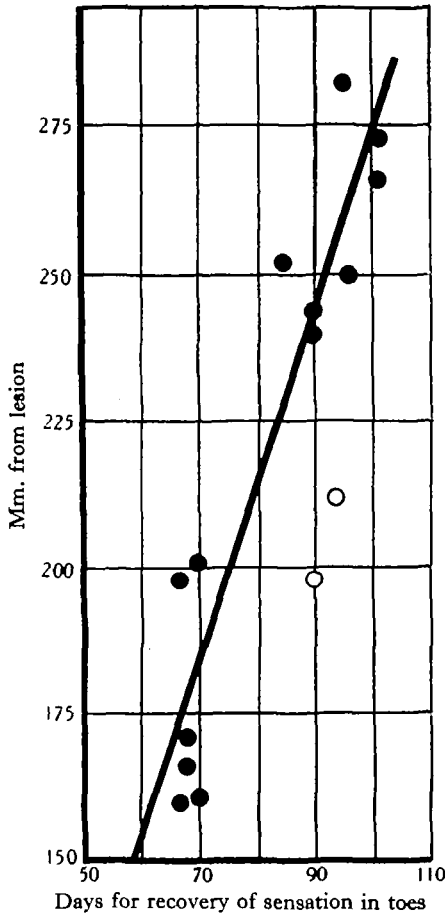


Fig. 3. Plot to show number of days needed for recovery of sensibility at the tips of the toes after crushing the peroneal nerve at various levels. The two points marked with open circles have not been considered in calculating the regression.

recovery in the skin than when the nerve itself is exposed. Taking all the data together and calculating the regression coefficient we find a rate of  $2.74 \pm 0.44$  mm./day. That this rate is unduly small is shown by the fact that the corresponding calculated latent period is no more than 3.8 days. The points which seem to fit least well into the data are those for the middle distance, and if we eliminate the two very delayed recoveries marked with open circles we obtain a rate of growth of  $3.04 \pm 0.35$  mm./day with a latent period of 9.8 days, which is still a shorter delay than is shown by other and more reliable methods (p. 27).

In four of these experiments the nerves were pinched at different levels on the two sides of the same animal. As shown by Table 3 comparison of the times of recovery on the two sides of these animals gives estimates of the rates of recovery which are slightly higher than those obtained from the above regression line, the

Table 3. *Comparison of times necessary for complete sensory recovery when peroneal nerve was interrupted high on one side and low on the other side of the same animal. Distances and times calculated as in Table 2*

Animal	Distance mm.	Days	Difference of distance	Difference of days	Rate mm./day	Latent period
560 L	161	70	83	21	3.95	29.2
R	244	91				
562 L	160	67	80	23	3.48	21.0
R	240	90				
493 L	273	101	102	33	3.09	12.7
R	171	68				
494 L	266	101	100	33	3.03	13.2
R	166	68				
				Mean	3.39	19.0

average of the four experiments giving a rate of 3.39 mm./day. By subtracting the time taken for functional completion in the nerve from the total we can then obtain estimates of the latent periods from these data (last column of Table 3) and the mean estimate is 19.0 days. This is the time which elapses before maturation reaches the level of functional completion close to the lesion, and this completion begins to advance down the nerve.

None of these estimates approaches the value of 4.36 given by the method of exposing and stimulating the nerve (see p. 23), and it is not necessarily to be expected that they would do so. For the lower figure is an estimate of the advance down the nerve of that process of recovery which makes the nerve able to mediate reflex functions, and there is no special reason to suppose that the process advances at the same rate as the growth of the axon tips.

### (2) *Progress of recovery within an insensitive area*

The second method which has been used for estimates of the rate of advance of functional completion of sensory nerve fibres has been to follow the progress of shrinkage of an analgesic area during recovery. A nerve, usually the peroneal, was interrupted, and the area of sensory loss mapped by testing the responses of the animal to pin prick, marks being made on the skin with silver nitrate (30%) and either photographed or carefully measured and plotted on outline drawings of the leg. Examinations were made, usually at weekly intervals, until there began to be a shrinkage in a zone in which recovery could not be accounted for by overlap from adjacent nerves. Further examination and measurement then showed the rate at which shrinkage proceeded. There is not a simple steadily advancing front to the sensitive region; usually the area shrinks from the sides as well as from above downwards. Sometimes the downward progression stops while the shrinkage from the

sides progresses. But nevertheless, a very definite downward progression can nearly always be detected. This is especially easy to follow on the dorsum of the foot after interruption of the peroneal nerve. As the example in Fig. 4 shows, the margin of recovery has a  $\Lambda$  shape and the advance of the apex of the  $\Lambda$  is very readily followed.

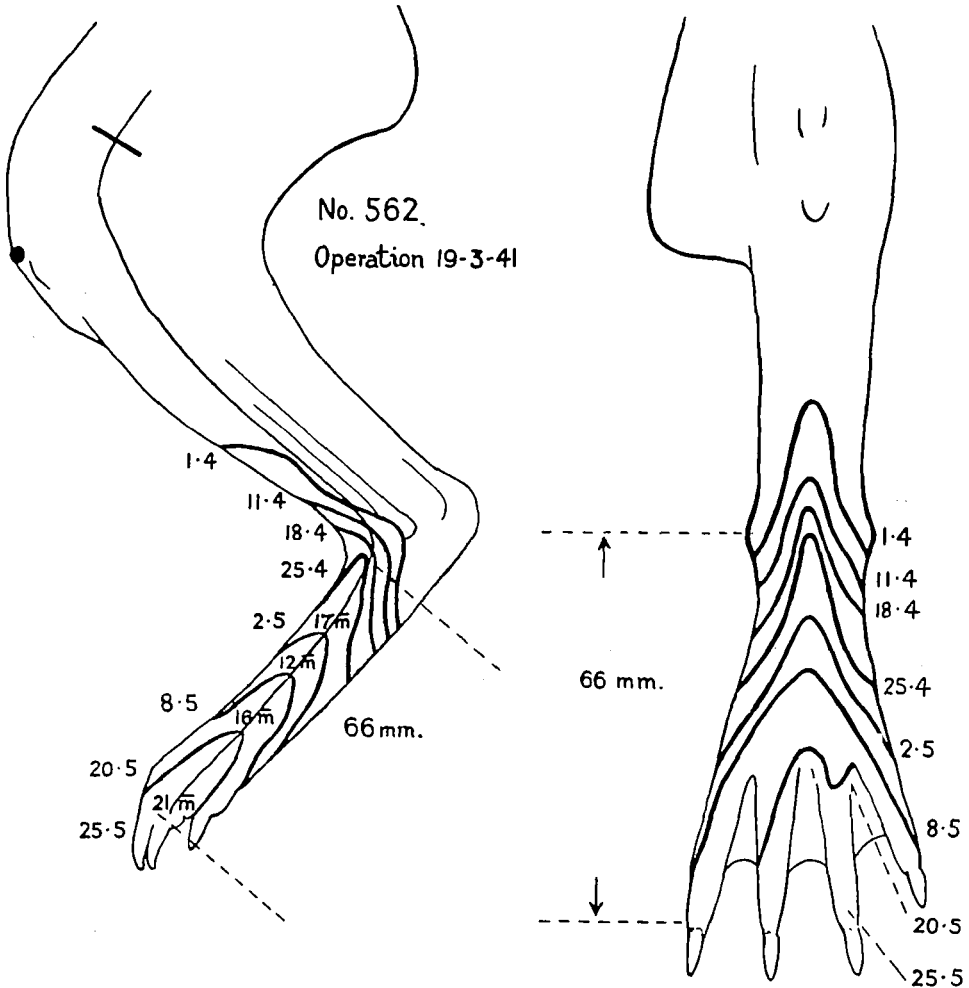


Fig. 4. Anterior and lateral views of the advance of the margin of recovery on the foot of a rabbit after crushing the peroneal nerve at the knee. The course of the nerve and the point of interruption are shown in the lateral view.

Table 4 summarizes the results in six cases in which recovery was followed after crushing the peroneal nerve. The average rate of regeneration is 2.05 mm./day, but there are large variations between the different animals. Moreover, as Fig. 4 shows, the shrinkage does not always take place steadily throughout the area. In fact great care must be taken in deciding which points to choose in making the estimates. In one case the shrinkage in the area supplied by the saphenous nerve proceeded at the high rate of 3.1 mm./day. After suture the advance is still more

Table 4. Rates of growth from progress of recovery on the dorsum of the foot after crushing the peroneal nerve. The distances are those observed after true recovery had begun, and before it had ended (see text)

Animal	Distance on skin mm.	Days	Rate mm./day
560 L	55	22	2.50
494 R	59	31	1.90
562 L	66	30	2.20
579 L	69	36	1.92
546 R	74	49	1.51
509 R	80	35	2.29
Mean rate			2.05 ± 0.14

irregular and the individual results will be reported later. The average rate given by 6 experiments was  $1.57 \pm 0.15$  mm./day.

Before accepting figures obtained in this way as an estimate of the rate of progress of new nerve fibres it must be remembered that the distance between two points on the surface of the skin does not represent the whole of the distance that nerve fibres must travel before the advancing edge of sensitivity moves from the upper to the lower point. The distance is greatly increased by the ramifications of the cutaneous nerve plexus. It is also possible that regenerative processes proceed more slowly in these ultimate plexuses than in the main nerve trunks.

### (3) Rate of advance of functional completion of motor nerve fibres

In order to establish the rate of growth of motor nerve fibres it is necessary to compare the times of recovery of motor function after lesions at various distances from the muscle. The function chosen was the spreading of the toes of the rabbit, a movement which can be elicited by holding the animal by the scruff of the neck and lowering it suddenly. The spreading is produced by the action of the small peroneal muscles II-IV, which are innervated by the peroneal division of the sciatic. Abduction of the first toe is also produced by the m. abductor digiti primi,<sup>1</sup> which is innervated by the tibial nerve.

After interruption of the peroneal nerve the absence of impulses tending to spread the toes produces a characteristic narrow appearance of the whole foot, and when the animal is suddenly lowered the only spreading is an abduction of the first toe. The first signs of recovery, usually abduction of the fourth toe, can thus readily be detected. Daily examination of the animals can be made very quickly. Recovery after crushing a nerve occurs unambiguously, with an error of as little as 1 day. After division and suture, however, the earliest signs of recovery remain doubtful for 2-4 days.

#### (1) Motor recovery after crushing a nerve.

At an initial aseptic operation the peroneal nerve was crushed on both sides of each rabbit, but at varying distances from the muscles. Each distance was measured

<sup>1</sup> The muscle called by Krause (1884), m. extensor digiti primi.



approximately at the time of operation and then confirmed to the nearest millimetre after recovery had taken place by direct measurement on the exposed nerve. Thirty-eight nerves were interrupted in this way, at distances varying from 8 to 110 mm. from the muscle, recovery times varying correspondingly from 24 to 58 days. Table 5 and Fig. 5 show the results. It will be seen that the period necessary for recovery after a lesion at any given distance from the muscle is reasonably constant.

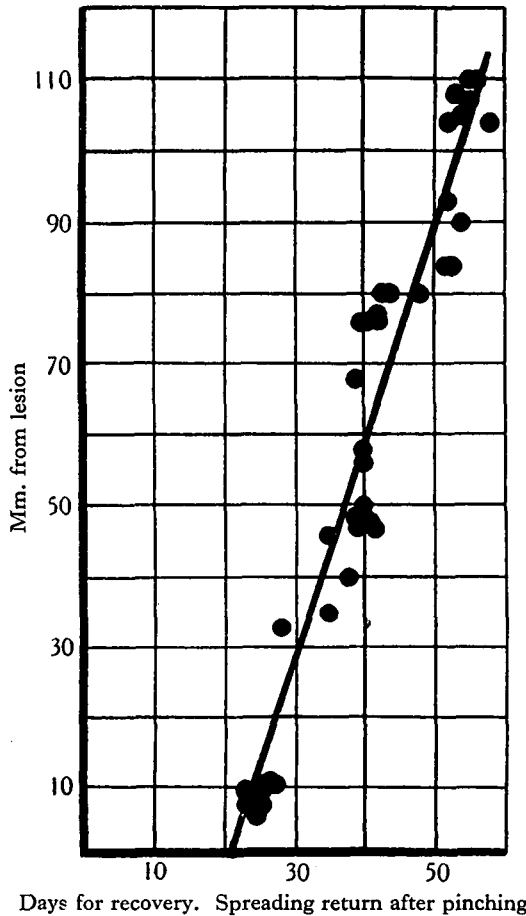


Fig. 5. Plot to show the time necessary for recovery of spreading of the toes after crushing the peroneal nerve at various distances from the muscle.

For instance, for distance 8–10 mm. the times of recoveries vary only from 23 to 26 days. For 40–50 mm. the variation is 35–40 days, and for 100–110 mm. 52–58 days. The regression coefficient of distance from muscle on days necessary for recovery gives us an estimate of the rate of growth of functionally complete motor fibres, and the figure is  $3.05 \pm 0.14$  mm./day, with a latent period of 20.8 days. In four cases, shown in Table 6, the recovery was followed after the peroneal nerve had been crushed high on one side of the animal and low on the other. The average rate calculated for these four animals is 3.1 mm./day, with a latent period of 21.6 days.

This latent period of 21 days is that necessary for the reaching of functional completion at the point of the lesion, and agrees with the 19.0 days found for sensory fibres (see p. 27).

Table 5. *Times necessary for motor recovery (first appearance of spreading of the toes) after crushing the peroneal nerve*

Animal	Distance mm.	Days	Animal	Distance mm.	Days
493	8	24	522	68	39
494	8	23	536	76	42
567	8	25	546	76	40
542	10	25	588	76	40
566	10	23	636	77	42
579	10	24	635	80	43
562	10	26	623	80	43
560	10	25	390	80	48
579	33	28	468	84	52
578	35	35	468	84	52
584	40	38	562	90	54
486	46	35	560	93	52
422	48	40	509	104	58
422	48	40	561	104	52
421	48	40	561	105	54
421	48	40	507	107	55
485	50	40	508	108	53
364	56	40	493	110	55
363	58	40	494	110	56

Rate  $3.05 \pm 0.14$  mm./day. Latent period 20.8 days.

In suggesting that the rates of advance down the nerve are those of a process of maturation which moves less fast than the tips of the axons it has been assumed that there is no considerable extra delay in the muscle which has been denervated for the longer period. This assumption needs to be checked, since it is not unlikely that after a long period of denervation and hence of atrophy this delay

Table 6. *Time necessary for recovery of spreading of toes after peroneal nerve crushed high up on one side and low down on the other side of the same animal*

Animal	Distance mm.	Days	Difference of distance	Difference of time	Rate mm./day	Latent period
493 R	8	24	102	31	3.29	21.57
L	110	55				
494 R	8	23	102	33	3.09	20.41
L	110	56				
562 L	10	26	80	28	2.86	22.52
R	90	54				
560 L	10	25	83	27	3.07	21.72
R	93	52				
Mean					3.1	21.6

would be greater than after denervation for, say, only 25 days. If this proved to be the case then the whole of the above calculation would be upset, the length of nerve traversed not being then the only variable. In other words, since we are using the return of muscular response as the criterion of arrival of fibres that have

started at different distances, we must be sure that this criterion is the same in all experiments.

This possible source of error has been controlled by first interrupting the nerve high on one side of a rabbit and low on the other, and then at about the time when it was calculated that motor fibres have grown from the high lesion down to the level of the low one, interrupting the latter again. The fibres should then start again level and will reach muscles which have been denervated for equal times. To put it another way the first operation on the low operated side serves merely to prepare the muscle on that side, so that it shall be similar, as an index, to its opposite.<sup>1</sup> Table 7 shows the results in the three animals treated in this way. It will be seen that function returned after approximately the same times of total denervation on the two sides, giving rates of growth of the same order as those obtained from the above regression line. The times taken for recovery after the double lesions (distances 40-46 mm.), were 36-39 days, and are quite comparable to the times required for recovery in the two experiments in which single lesions were made at this level (35 and 38 days). The average rate of growth given by these three experiments, 3.5 mm./day, is slightly higher than that given by the thirty-eight experiments in which the nerves were crushed once only, and this difference is what would be expected if the period of delay between arrival of fibres at the muscle

Table 7. *Time taken for motor recovery after peroneal nerve crushed high up on one side and on the other crushed twice to ensure the same total period of denervation on both sides*

Animal	Distance mm.	Days	Difference of distance	Difference of time	Rate mm./day	Latent period
507 L	46	36	61	19	3.21	21.7
R	107	55				
508 L	45	37	63	16	3.94	25.6
R	108	53				
509 L	40	39	64	19	3.37	27.1
R	104	58				
				Mean	3.5	25

and the return of function is greater after longer periods of denervation. While the difference is slight, 3.0 against 3.5, and cannot be tested statistically, it is perhaps just suggestive of a prolongation of the period of delay with progressive atrophy of the muscle. For our present purpose we may draw the conclusion that the rate of advance of functionally complete motor fibres after a nerve has been crushed cannot be less than 3 mm./day and is almost certainly rather greater.

There are two possible sources of error in these control experiments themselves, which must be mentioned. (1) After the second interruption the outgrowth of fibres might be less (or conceivably more) vigorous than after an initial lesion. But in other experiments (Holmes & Young) the outgrowth has been found to be at all

<sup>1</sup> Since the distance between the two lesions was greater than that of the lower one from the muscle it is clear that for a few days before the second operation axon tips must have been present in the muscle on the low-operated side, and might possibly have had some effect on the condition of the muscle fibres.

times equally vigorous under such conditions. (2) The scar resulting from the first operation might delay the fibres. Other experiments in which high rates of growth have been observed through much more seriously scarred nerves show that this is unlikely. The effect of any such delay would be to increase the apparent rate of regeneration, but it can be dismissed as the basis of the high rate actually observed since the absolute times needed for recovery were not greater after these double than after single lesions (see above).

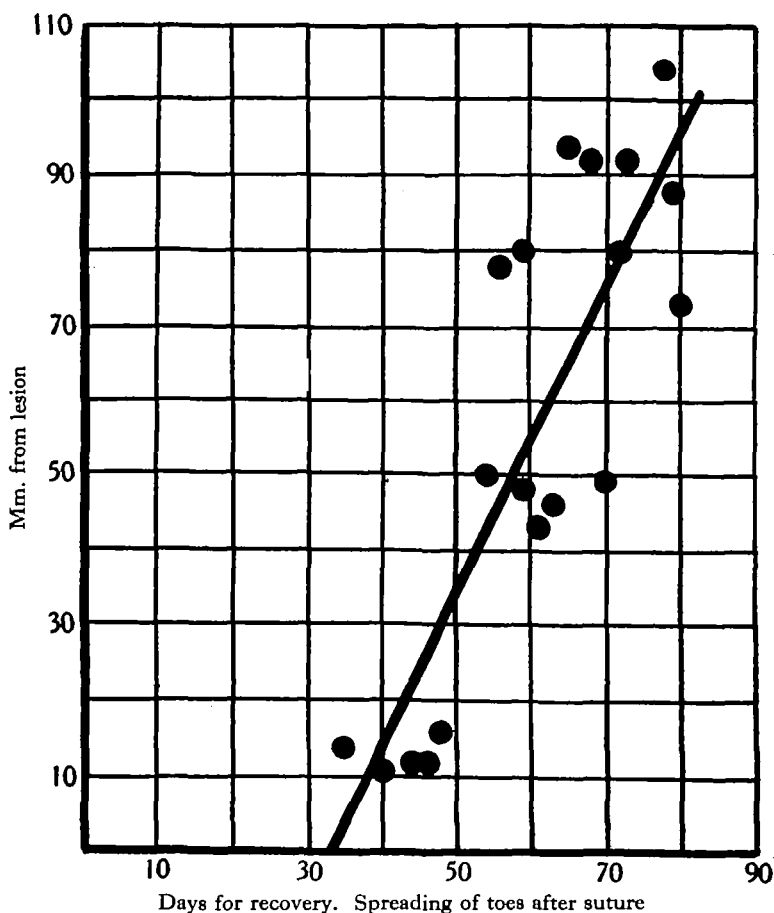


Fig. 6. Plot to show the times taken for recovery of the spreading of the toes after severance and suture of the peroneal nerve at various levels.

(2) *Rate of growth of motor fibres after suture.*

This estimation was made by cutting the peroneal nerve at various distances from the muscle, suturing with plasma, and looking for recovery of the function of spreading the toes. Table 8 and Fig. 6 show the results of the nineteen experiments made. The time between operation and recovery was much longer than after crushing the nerve. For instance, after injury 40-50 mm. from the muscle

Table 8. *Times necessary for motor recovery (first appearance of spreading of the toes) after cutting and suture of the peroneal nerve*

Animal	Distance mm.	Days	Animal	Distance mm.	Days
569	11	40	521	73	80
584	12	46	590	78	56
503	12	44	589	80	59
564	14	35	604	80	72
572	16	48	563	88	79
486	43	61	569	92	73
574	46	63	573	92	68
574	48	59	564	94	65
485	49	70	572	104	78
573	50	54			

Rate  $2.0 \pm 0.3$  mm./day. Latent period 32.3.

the times required for recovery were 38–40 days after crushing, 54–70 days after cutting. Histological examination confirmed the existence of variations at the suture line; for instance in rabbit 485, which recovered very late, there was a separation of nearly 3 mm. between the stumps.

In spite of this variation it has been thought best to treat the data as a single body, and the regression coefficient of time for recovery on distance from the muscle gives a rate of growth of 2.02 mm./day and a total latent period of 32.35 days.

In four animals the nerve was cut and sutured high on one side and low on the other of the same animal. The results of these are recorded in Table 9, and they give a somewhat higher estimate of the rate of growth, namely, 2.6 mm./day and

Table 9. *Times necessary for recovery of spreading of toes after peroneal nerve has been cut and sutured high on one side and low on the other of the same animal*

Animal	Distance mm.	Days	Difference of distance	Difference of time	Rate mm./day	Latent period
564 L	14	35	80	30	2.67	29.8
R	94	65				
569 L	11	40	81	33	2.45	35.5
R	92	73				
572 L	16	48	88	30	2.93	42.5
R	104	78				
563 L	12	44	76	35	2.17	38.5
R	88	79				
Mean					2.6	36.5

a latent period of 36.5 days, which are probably better estimates than those derived from the regression line. In any case we may conclude that, as with sensory fibres, the rate is lower, and the latent period longer, after cutting and suturing the nerve than after interruption by crushing.

### (3) *Rate of growth of motor fibres measured by times of recovery of different muscles.*

By recording the times at which separate muscles lying at different distances from a lesion recover, we obtain an estimate of rate of growth partly comparable

to that obtained by watching the progress of recovery in the skin (p. 27). Of the muscles innervated by the peroneal nerve the peroneus I (longus), which lies highest, produces pronation of the foot, a function not easy to elicit reflexly, and therefore not used for our present purpose. Mm. peronei II–IV all produce spreading of the toes, and all of them lie higher than m. tibialis anterior and m. extensor digitorum, which produce dorsiflexion of the foot and toes respectively, movements easily elicited by pricking the plantar surface of the foot.

Since these movements are produced by several muscles it is somewhat difficult to decide what distances to take for estimation. The m. peroneus II being the highest muscle producing spreading of the fourth toe, and m. tibialis ant. the highest to give dorsiflexion, these alone may be considered. The distance between them was carefully measured on two medium-sized rabbits, as follows. From a fixed point on the peroneal nerve to the entry of its branch into m. peroneus II the average distance was 16 mm., to m. tibialis ant. 23 mm. Since we do not know how much of the intramuscular course of the nerve must be run before function returns we may, as an approximation, add to these figures the distances between the point of entry of the nerve to the muscle and the mid-point of the length of the latter. These additions (6 mm. for m. peroneus II and 10 mm. for m. tibialis ant.) give us distances of 22 and 33 mm. from our fixed point to the centres of the muscles, and our estimate is then that fibres must grow 11 mm. farther to reach m. tibialis ant. than m. peroneus II. In six rabbits careful examination was made of the times at which spreading and dorsiflexion returned after crushing the peroneal nerve. In every case the spreading returned first, the times between the appearance of the two functions being 5, 5, 4, 6, 4 and 6 days. Taking the mean of this delay as 5 days, and the distance as 11 mm. we obtain an estimate of rate of advance of functional ability of 2.2 mm./day. This is at best a very rough approximation; indeed, the small distances between the muscles make the rabbit unsuitable for studies of this sort. Nevertheless there is a general agreement with the figure arrived at for rate of growth of sensory fibres in the skin (p. 27).

In one case the difference between spreading and dorsiflexion was carefully examined after severance and suture of the peroneal nerve and found to be 9 days. This would give a growth rate of 1.2 mm./day, clearly lower than that after crushing the nerve. But no great reliance can be placed on this single case.

### III. RATE OF REGENERATION IN YOUNG ANIMALS

A few experiments comparable to the above were made to determine the rates of sensory and motor regeneration in rabbits about one month old. Twelve nerves were crushed in the middle of the thigh in animals 16–22 days after birth. At intervals of 6–13 days after operation they were examined by the method described on p. 16, to discover how far from the lesion reflex responses could be elicited by pinching the nerve. The results are shown on Fig. 7. It is impossible to fit a single straight line to these points, because the data from the one animal examined after

13 days show a distance of regeneration relatively much greater than any of the others. It is possible that the data from this animal are anomalous, since it becomes very difficult to make the experiment as the fibres approach the end of the nerve, as they do after 13 days in these small animals. For purpose of discussion the results up to 12 days are considered, and Fig. 7 shows the regression line calculated for the first 12 days only. The latent period is obviously very short, the estimate

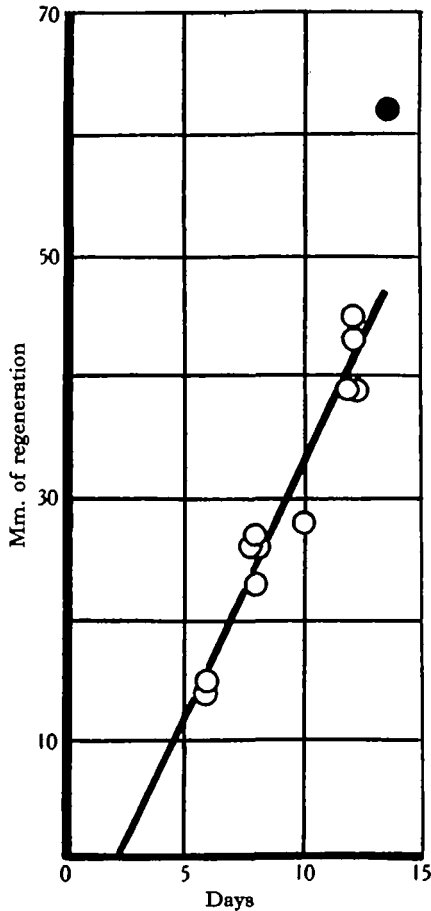


Fig. 7. Plot to show the distances reached by new fibres in animals one month old, as revealed by pinching the exposed nerve at various times after it had been interrupted by crushing.

being 2.4 days, and this is confirmed directly by the nerves examined after 6 and 8 days, in which fibres have grown for considerable distances. On the other hand the rate of advance down the nerve, estimated at  $4.3 \pm 0.4$  mm./day, is of the same order as that found in adults. This is, perhaps, what would be expected, since the processes in the scar, which are essentially those of healing, may well be more rapid in young animals, while the progress of fibres down the nerve trunk, which is as much a movement as a growth process, might be less affected by the age.

Motor recovery was tested in four animals, of the same age as the above, by interrupting the nerve high on one side and close to the muscle on the other. The results are shown in Table 10. In the two animals in which the nerves were crushed the average estimate is 5.4 mm./day, and when the nerves were cut and sutured with plasma 2.5 mm./day. The first of these figures is considerably higher than that of 3.1 mm./day estimated for adults, whereas after suturing the nerve the rate in adults (2.0 mm./day, or more, see p. 34) was little less than in the babies.

Taken with the results for the rate of growth of sensory fibres in young animals these results indicate that the dramatically rapid recoveries made by young animals,

Table 10. *Motor recovery (spreading of toes) after interruption of peroneal nerve in rabbits less than one month old*

Animal	Distance mm.	Days	Difference of distance	Difference of time	Rate mm./day	Latent period
Nerves crushed						
547 L	40	17	35	7	5.00	9.0
R	5	10				
548 R	44	19	40	7	5.71	11.1
L	4	12				
				Mean	5.4	10
Nerves cut and sutured						
655 R	38	31	31	12	2.58	16.4
L	7	19				
656 R	38	32	31	13	2.38	16.2
L	7	19				
				Mean	2.5	16

and children, are not due to very high rates of growth of axon tips in the nerve trunks, but to great shortening of the delay in the suture scar, and probably acceleration of functional completion, combined, of course, with the fact that the distances to be covered are small.

#### DISCUSSION

It must be remembered that in no case have the measurements been of the growth rate of single fibres; indeed, this would only be possible if the growing tips were watched under the microscope as in the experiments of Speidel (1933). In the experiments in which the nerve was pinched to discover how far the new fibres had reached, the critical point is that at which a sufficient number,  $n_1$ , of sufficiently completed fibres is present. Presumably  $n_1$  is greater than 1, but since histological studies have shown that such reflexes are obtained from stretches of nerve in which only a few very fine fibres are present (p. 17) it is clear that it represents only a small proportion of the number of pain fibres originally present in the nerve. During regeneration fibres cross the scar and grow down the stump at varying rates, and studies of the distances at which responses to pinching can be obtained give us an estimate of the rates for a group close to the fastest.



When a nerve is interrupted by a crush the fibres are all damaged alike and probably regenerate at more uniform rates than after severance and suture of a nerve. Therefore the distance from a lesion at which the number  $n_1$  of fibres necessary to give a 'pinch reflex' is present is probably less after cutting than after crushing. If  $n_1$  was large this statistical fact might account for the greater distances of regeneration recorded after crushing, but since  $n_1$  is known to be small, and since the estimates of growth rate are independent of such factors, we must conclude that the actual rate of advance of axon tips in the peripheral stump is slower after suture than after crushing.

On the other hand, in motor recovery it is probable that the number ( $n_2$ ) of fibres that must grow down into the muscle before reflex contraction can occur is perhaps half of the original number of motor fibres in the nerve, since many muscle fibres must be innervated before visible reflex movement is produced. Certainly  $n_2$  is greater than  $n_1$  and therefore in the experiment on motor recovery we are investigating not the fibres that cross the scar first and grow fastest, but the rate of some fibres about the middle of the distribution. This will have the effect of making all estimates of motor fibre growth rate appear lower than those for sensory fibres as determined by pinching the nerve. But since we do not know the extent of the scatter of the rates it is impossible to say whether this factor can account for the greater part of the observed difference.

Probably a more important point is that the estimate of sensory fibre growth rate by pinching is based on the point in the nerve to which a small number of very thin and scantily medullated fibres have grown. But the estimate for motor fibres is of the rate, not of the almost unmedullated tips, but of the progress of the process of growth in diameter and of medullation. The fact that studies of recovery, both sensory and motor, give estimates of rates of regeneration close to 3 mm./day (after crushing), compared with the 4.4 mm./day found by pinching the exposed regenerating nerve, suggests that the lower rate is that at which this process of maturation to functional completion proceeds. The control experiments described on p. 32 have shown that the relatively slow rate of growth recorded in studies of motor recovery is not the result of any significant delay in the re-establishment of the function of muscles due to greater atrophy in those which have been denervated for long periods. There remains, however, the possibility mentioned on p. 15 that the degree of maturation of nerve fibres necessary for functioning is less when a lesion is close to a muscle than when it is far away. If such close lesions recover relatively quickly then the estimates of rate of advance of functional completion along the nerve will be too low.

Since both of these two factors which will tend to produce low estimates of growth rate are of unknown magnitude the conclusion that functional completion spreads down the nerve more slowly than does growth of the axon tips can at present be only provisional. The important point however is to recognize that there is such a spread along the nerve, and that what is being measured when sensory or motor recovery is recorded is the rate of spread of this full nervous regeneration, not that of the axon tips alone. Our estimates give about 2.0 mm./day after suture,

and 3.0 mm./day after crushing as the rate of this advance for both sensory and motor functions.

The average figure for the rate of shrinkage of an analgesic area, 2.1 mm./day, after crushing the peroneal nerve, is still lower than any of the above estimates of growth rate. This would seem to indicate either that the nervous pathways between two points on the skin are much longer than the measured distance, or that a slower growth process occurs at the periphery. Indeed both may be true.

On this evidence the conclusion is that the actual growth rate of the axon tips does not vary between the different functional fibre types, in all of which it probably advances, in the fastest fibres, at about 3.5 mm./day after suture and 4.5 mm./day after crushing of a nerve. In all cases advance of recovery down the nerve has been faster after crushing than after severance and suture. The data indicate that the greater rate is due not only to less interference with the pathways but to a greater rate of advance of fibre tips, and probably of maturation. Though no critical data on this point exist in the literature, quick recoveries after crushing have frequently been recorded (e.g. in 19 days after crushing the peroneal in the cat (Langley, 1918)) and are fortunately a commonplace of surgical practice.

Whatever may be the explanation for the apparent differences in rates of growth the point of practical importance is that when using different methods in the same animal such differences have appeared with great regularity. Estimates of regeneration made clinically with similar methods are likely to yield similar differences of apparent rate. A surgeon stimulating exposed nerves under local anaesthesia may find rates of growth after suture approaching the 3.5 mm./day recorded in the above pinching experiments. Studies of the progress of Tinel's sign, since they depend on the stimulation of relatively few and little-medullated fibres, are likely to give high rates.

On the other hand the neurologist, studying recovery of sensory and motor functions, will estimate that the rate of growth in the nerve is lower, since his criteria depend on the presence of greater numbers of fibres, and on their medullation. If he studies the times of recovery of function in a certain muscle or part of the skin after lesions at various levels he will obtain some estimate of the rate of advance of functional completion down the nerve trunk, and perhaps find it to be, as in the rabbit, some 2 mm./day after suture, or 3 mm./day after crushing the nerve. On the other hand if the rate of progress of sensory recovery in the skin, or the times of recovery of muscles at various distances from the lesions are followed, the rates may appear still lower. However, in man, with the greater distances between muscles, the last-mentioned method should give good estimates of the rate of growth in the nerve trunks.

In clinical studies there is likely to be the further complication that the apparent rates of growth become lower as the functions studied become more complex. Thus it may be that only in those motor fibres responsible for 'simple' movements will functional completion advance down the nerve at the 2.0 mm./day found after the suture for the simple functions of spreading of the toes in the rabbit. It would be interesting to know whether responses to, say, light touch, and particularly tem-

perature, which recover later than pain, also advance more slowly. The work of Head & Sherren (1905) and Trotter & Davies (1909) clearly shows that there is a regular advance in recovery of all modalities of sensation.

With these qualifications in mind we may, finally, compare the above estimates of rates of regeneration with those found experimentally and clinically by previous workers. Few of these have taken account of the need to divide the process of recovery into its various periods. Vanlair (1894) did so in his pioneer paper on the subject, in which he speaks of the time ( $d$ ) taken by proliferation and exodus of new fibres,  $d'$  the time in the scar, and  $\delta$  that in the peripheral stump. He estimated  $d$  (by making, in dogs, two sutures in the sciatic on one side and one on the other!) at 40 days.  $d'$  was estimated, by removing 1 or 2 cm. of nerve altogether, as 0.25 mm./day, and  $\delta$ , by a method very similar to that here used for the study of sensory recovery after high and low lesions, was found to be 1 mm./day. The latter estimate by what is at best a very difficult and ambiguous experiment, on two dogs, is apparently the foundation for many of the text-book statements that regeneration proceeds at this rate.

The most complete of later works is that of Cajal (1928). His method was the direct one of histological study. He recognized clearly that there is a delay before the fibres enter the scar, and that they then cross it slowly. Moreover, 'the sprouts are not equally vigorous and... the invasion of the foreign tissues occurs progressively as if by shifts'. He gives no details about the experiments he has done, but under conditions apparently comparable to our sutures the fibres take 'seven or more days' to reach the peripheral stump. Later however he says that 'usually one sees with certainty the axons in the peripheral stump... only from the 12th to the 15th day'. Our estimate of the scar delay for axon tips seems to lie within the limits of his observations.

Dustin (1917) used Tinel's sign to discover the length of the scar delay in man, and found that the sign began to be elicitable in the peripheral stump between 30 and 60 days after a good suture. Since this sign is usually held to depend on pressure on unmyelinated fibres this seems a rather long delay. Huber (1920) considers that the regeneration is 'well under way by the end of the first week', and Lewis (1920) that 'neurofibrillae... grow down in the old sheaths by the fourteenth day'. Perroncito (1907) found new fibres in the peripheral stump after 6 days, even sooner in young animals.

It is clear from such results, and from our own, that axon tips begin to pass down the peripheral stump not more than about 10 days after a good suture. It is much more difficult to get any data from the literature about the time at which maturation to the level which we have called that of functional completion begins to advance. This delay would only be revealed by studies after lesions at various levels. Our results give 18 and 22 days for the degree of completion necessary to give return of response to pin prick in the foot and spreading of the toes respectively after crushing the nerve. After suture the data of p. 34 indicate that about 36 days elapse before the degree of functional completion necessary to give spreading of the toes begins to advance down the nerve. This is the figure which may be taken when

considering the recovery of simple functions in man. Rates of advance of functional completion could then be calculated by subtraction of this number of days from the time taken for recovery, and dividing into the distance of the end-organ from the lesion

It has been assumed throughout this paper that the latent period recorded in experiments on recovery of sensory and motor function is the time necessary before the portion of the nerve nearest to the lesion has matured to the state of functional completion. But there is the possibility, in the case of the experiments on motor recovery (Fig. 5), that the delay before the earliest recoveries is partly taken up by the process of making a functional contact with the muscle. If this were so then all the lower points on Fig. 5 should be relatively far to the right, because they include an extra delay, not present in the later recoveries. There are indications that this factor operates to a slight extent, and it is perhaps significant that the estimate of latent period from these motor recoveries (22 days) is somewhat greater than that from sensory recoveries (18 days), which, being based on distant lesions only, are not affected by this factor.

Very few estimates of rates of growth in the peripheral stump make sufficient distinction between the various factors to allow strict comparisons. Cajal, again, has an unambiguous estimate since he used histological methods. But he is aware of the obscurities of the problem. 'Speed of growth of the nerve sprouts... is a problem to which, owing to many disturbing causes difficult to measure, only a roughly approximate solution can be given.' He assumes that the 'vigour of growth, the *vis a tergo* of Held, declines progressively with time, until it becomes nil or almost nil', and 'It would be very desirable to know the formula of this retardation'. The data of our Fig. 1 show no clear sign of such decline, though, as explained on p. 18, they are not sufficient to show for certain that the growth rate remains constant. After this very proper reservation Cajal then gives conclusions 'from numerous measurements in rabbits, cats and dogs whose sciatic nerve was completely cut across'. The individual data are not given, but the conclusion is that 'The velocity of the cones of growth in the peripheral stump is between 2 and 3 mm. in 24 hours... In some really exceptional cases the velocity of growth was 4 mm. per day'.

Huber (1920) states that 'the area of neurotisation progresses towards the periphery at the rate of 1-2 mm. per 24 hours', but he does not give the data on which this figure is based, and it is certainly much too low.

Cajal's estimate agrees remarkably well with our estimate obtained from pinching the exposed nerves, namely 3.5 mm./day after suture, and we may safely conclude that this is close to the rate of advance of axon tips in the rabbit and probably in the dog and cat. The few estimates available indicate that the rate of advance of axon tips is also similar in Man. Studying the advance of Tinel's sign several workers have recorded figures much higher than the 1 mm./day of the text-books. Tinel himself (1917) gives 1-2 mm./day, but with no details, but Dustin (1917) finds rates in the median nerve of 2.0-4.5 mm./day, and in the radial as high as 4-5 mm./day.

Very few studies are available from which the rate of advance of functional completion of nerve fibres can be estimated. Osborne & Kilvington (1908), by the ingenious method of eliciting axon reflexes, found advances of motor fibres by as much as 140 mm. in 120 days (1.2 mm./day) and as little as 30 mm. in 91 days (0.3 mm./day). We have tried to use this method in the rabbit but though elegant in conception it does not give an estimate of rate of advance of recovery to a biologically significant level, and it proved very difficult to apply accurately in practice. That the recovery is quick if the lesion is nearer to the muscle is a commonplace of clinical studies but none have recorded the times or distances accurately enough to allow latent periods or rates of advance of functional completion to be studied accurately. Yet the data of Stopford (see M.R.C. Report, 1920) show that this process must advance much faster than 1 mm./day. For instance, m. pronator teres is said to recover in 4 months when the nerve is damaged at the elbow, 6 months when in the upper arm. The difference in average distance is not recorded but can hardly be less than 150 mm., which in 60 days give 2.5 mm./day.

The published data of clinical cases in man do not afford an accurate means of calculating the rate of advance of functional completion. They are mostly complex lesions (e.g. gunshot wounds) in which even after suture there may be fibrous scar tissue in the nerve and infection may further interfere with the process of regeneration. Moreover, secondary suture is performed after varying periods, and we do not know the extent to which this delay affects recovery. Also accurate measurements of the distance from the lesion to the end-organ have rarely been made clinically, and examinations are not made at strict and frequent intervals, so that an estimate of rate is extremely difficult.

Even studies of the rate of advance of recovery in the skin, which our studies show to give the lowest figures, usually show rates higher than 1 mm./day. Thus from the carefully recorded results of Trotter & Davies (1907) it can be calculated that in the skin the rates in mm./day are approximately 1.3 for touch (3480 mg.), 2.1 for cold sensation, 2.1 for pain and 1.2 for pilomotor function. These calculations assume that the distances travelled by the fibres are the same as those between points on the skin. Actually the distances are certainly greater and the rates therefore higher. The first return of sensation to the area shown in Fig. 14 of their paper occurred after 4 months (say 120 days) and the distance of the point of this recovery from the lesion was 200 mm. Subtracting 36 days for the delay before the process of functional completion advances this gives an estimate of growth rate of 2.4 mm./day, which is close to the rate which we have found in the rabbit.

We must conclude then, not only from our own results, but also from the published data, that the process of functional completion proceeds down the nerve faster than the usually accepted 1 mm./day, and that the axon tips grow faster still.

SUMMARY

1. Five different methods have been used to estimate the rate of advance of regeneration along nerves in the rabbit. Not all give the same result. When the advance of the axon tips is studied a higher rate is recorded than when the times necessary for functional recovery are considered. In the latter case the experiment gives a measure of the rate of advance not of the tips of the axons but of completed nerve fibres, able to function.

2. The 'scar delay', or period before arrival of new axon tips in the peripheral stump, is nearly constant in all well-made sutures (7.3 days). After interruption of a nerve by complete crushing at one spot this delay is only slightly shorter (5.2 days).

3. There is a further delay before maturation to the level of functional completion begins to advance down the nerve, so that the total latent period before advance of recovery begins is about 36 days after suture and 20 days after crushing a nerve.

4. The rate of advance of the fastest axon tips was determined by finding the furthest point from the lesion at which reflexes could be elicited by pinching the nerve. After suture of the n. peroneus in the rabbit this rate was 3.5 and after crushing 4.4 mm./day.

5. This rate of the axon tips is not significantly different after crushing the nerve at different distances from the nerve cell body, namely high in the thigh or below the knee.

6. This rate is similar in the tibial, peroneal and sural divisions of the sciatic in the rabbit.

7. The rate of advance of functional completion of nerve fibres was measured by making lesions at various levels and studying (*a*) the time necessary for return of response to nociceptive stimuli to a given point on the skin and (*b*) the time for recovery of a given motor function, namely, spreading of the toes. Both methods give rates of 2.0 mm./day after suture, and 3.0 mm./day after crushing.

8. Analgesic areas on the foot shrink at the rate of 2.1 mm./day during recovery after crushing the nerve, 1.6 mm./day after suture.

9. Comparison of the times of recovery of muscles at varying distances from a lesion gives an estimate of rate of advance of functional completion of 2.2 mm./day after crushing a nerve, but this method is only approximate in the rabbit.

10. In rabbits of 1 month old the rate of advance of the axon tips is not greatly different from that in adults, but the delay in the scar is less, and maturation of the nerve fibres proceeds more rapidly.

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