THE EFFECT OF PERIPHERAL CONNEXIONS ON THE MATURATION OF REGENERATING NERVE FIBRES

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INTRODUCTION

Previous work (Sanders & Young, 1944, 1946; Weiss & Taylor, 1944; Weiss, Edds & Cavanaugh, 1945; Aitken, Sharman & Young, 1947) has shown the importance of the effect which the peripheral connexions have on regenerating nerve fibres. The present work was planned to examine in detail some of the factors which might contribute to this effect.

Answers were sought to the following questions: (*a*) Does the distance travelled by regenerating fibres modify the process of regeneration? (*b*) Do fibres turn at the cut end of a nerve and grow back in a central direction? (*c*) To what extent does close contact of regenerating fibres with muscle tissue influence maturation?

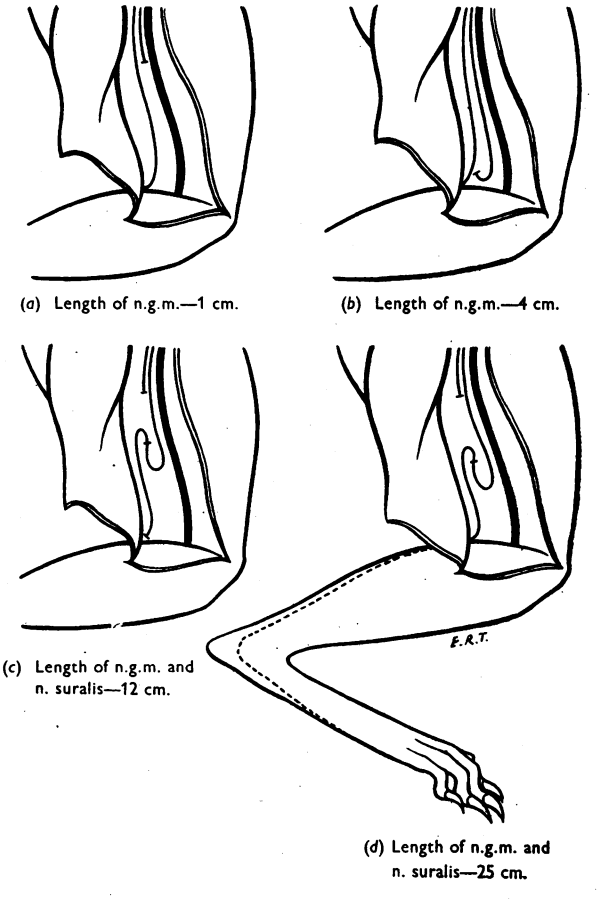
MATERIALS AND METHODS

Adult rabbits of different breeds were used, and the nerve to the medial head of the gastrocnemius muscle (n.g.m.) was selected, as in the previous work (Aitken *et al.* 1947). In all cases the n.g.m. was crushed as high as possible, with fine smooth watch-maker's forceps. In order to study the behaviour of new fibres growing down various lengths of nerve but not reaching the end organ, the nerve was also cut distally and the peripheral stump avulsed from the muscle. In this way a neuroma was caused to form at distances 1–4 cm. from the crushed point. In order to study regeneration over still longer lengths the proximal cut end of the n.g.m. was joined to the distal end of the severed sural nerve, which lies near by, and in this way the length of the regenerating pathway could be increased up to about 25 cm. in large rabbits. The union was maintained by means of coagulated fibrin. In some animals the sural nerve was again cut below the knee (see Text-fig. 1).

In this series, regeneration was allowed to proceed for 100 days, and the nerves were then fixed in order to determine the diameter of the new fibres at a level 1·5 cm. below the crushed point.

In another series of animals, to determine the effect of the muscle on regeneration, the n.g.m., after being crushed as high as possible and cut distally, was implanted into a nearby muscle. Biceps femoris was used and the implantation was made near the nutrient artery, by passing the end of n.g.m. along the track in the muscle made by carefully inserting a round-bodied needle between the fasciculi. This manipulation was carried out with the minimum amount of trauma to avoid damaging the muscle fibres. The implant was held in position with coagulated fibrin. The functional state of biceps femoris was varied by removal of the entering nerves from the sciatic trunk on one side, using the opposite side as a control with a normally innervated biceps muscle. In an attempt to prevent reinnervation, the cut ends of the nerve to biceps femoris were formalinized in some animals, but the method was abandoned owing to the danger of injury to the main sciatic trunk.

All animals were examined after a 100-day period and the n.g.m. was identified. In most cases the nerve was stimulated with an induction coil and any muscle response noted. Occasionally, the implantation was not maintained in the muscle and in these cases the nerve formed a neuroma on the surface of biceps or in the fascia.



Text-fig. 1. Diagram showing the four types of experiment to determine the effect of the distance travelled by regenerating fibres on the maturation of the nerves. (*a*) and (*b*), lengths of 1 and 4 cm. from n.g.m.; (*c*) and (*d*), lengths of 12 and 25 cm. from n.g.m. and n. suralis.

Where the implant was undisturbed, the minimum stimulus needed to evoke a muscle response was noted with the electrodes placed about 2 cm. from the site of the implantation. As far as possible, the stimulation of the nerves was standardized and in each case, after the threshold with the nerve intact had been noted, the nerve was then cut centrally, isolating it from the central nervous system and finally crushed close to the muscle to show whether the contraction was due to spread of the stimulus along the nerve.

In all the different experiments the nerves were fixed in the following modification of Flemming's solution (2 % osmic acid in distilled water, 4 c.c.; 1 % chromic acid

in distilled water, 15 c.c.; glacial acetic acid, 1 drop) for not more than 24 hr., dehydrated without preliminary washing, cleared in cedar-wood oil and embedded in paraffin wax after an intermediate stage in cedar-wood oil-benzol-paraffin wax mixture. Sections, 5μ thick, were cut at comparable levels in all nerves. In the shortest lengths of n.g.m. the sections were cut as near as possible to the neuroma, but in all others at a point about 1·5 cm. below the crush. The site of the crush was determined by a slight swelling in the nerve, by the presence of a marker (indian ink or black silk) or by the central cut end of the sural nerve which was found usually close to the crushed point. The sections were stained by Weigert's method and photographed at 750 × magnification on to bromide paper. Differential counts were made of the fibre diameters which were classified into 2μ groups.

RESULTS

A. *Effect of length of regeneration path on the degree of maturation*

The experiments provided data for the following lengths of the regenerating path: (*a*) regeneration along 1 cm. of peripheral path, n.g.m.; (*b*) regeneration along 4 cm. of peripheral path, n.g.m.; (*c*) regeneration along 12 cm. of peripheral path, n.g.m. and n. suralis; (*d*) regeneration along 25 cm. of peripheral path, n.g.m. and n.suralis.

The differential counts of the fibre sizes just below the crush obtained from the nerves in these experiments are given in Table 1 along with the total number of fibres in the nerve, the mean diameter for the fibres over 6μ in diameter and their number.

Table 1 (*a*) shows that in the short peripheral path the total number of fibres is large and usually over 2000. The great majority of these fibres are small and poorly myelinated, and the mean diameter of the groups over 6μ is 7·33μ. As the neuroma in these experiments is very near to the crush there will be a large number of fibres which turn at the neuroma and pass back along the nerve. They will have been counted twice (see later).

Table 1 (*b*) contains the results obtained from those nerves where the distance travelled by the regenerating fibres was 4 cm. of n.g.m. There is a diminution in the total number of fibres, with an increase in the number of those over 6μ in diameter, the mean of the latter being 7·68μ.

Table 1 (*c*) and (*d*) show a continuation of this trend, the total number falls with an increase in the over 6μ groups and an increase in the mean diameter to 7·98μ in the 12 cm. pathway and 8·74μ in the 25 cm. pathway.

After an examination of Table 1, it was decided to consider only the larger fibre (over 6μ) groups and the mean fibre diameter of those fibres was taken as an index of maturation in the nerve. This was done in an attempt to prevent the number of small fibres (some of which would be counted twice) masking the effect of any change in the number and distribution of the larger fibres. From each set of comparable experiments a series of mean diameters was obtained and considered as a sample of nerves regenerating for the given length. Is there any simple relationship between the degree of maturation and the length of the regeneration path? Inspection of the figures in Table 1 suggests a high degree of correlation between maturation and length regenerated, but the estimation of the correlation coefficient would only give

the extent of the connexion between the two variables. It would not give any information as to the degree in which change in length affects maturation.

If, however, the mean maturation estimations for all the nerves are plotted against the length regenerated, it appears that there is some reason to anticipate a linear relationship between the two variables. In more statistical terminology we may reasonably expect a significant linear regression of maturation on length of the regeneration pathway. This line can be computed and is shown in the diagram (Text-fig. 2), indicating that over the range of the experiments there is an increase

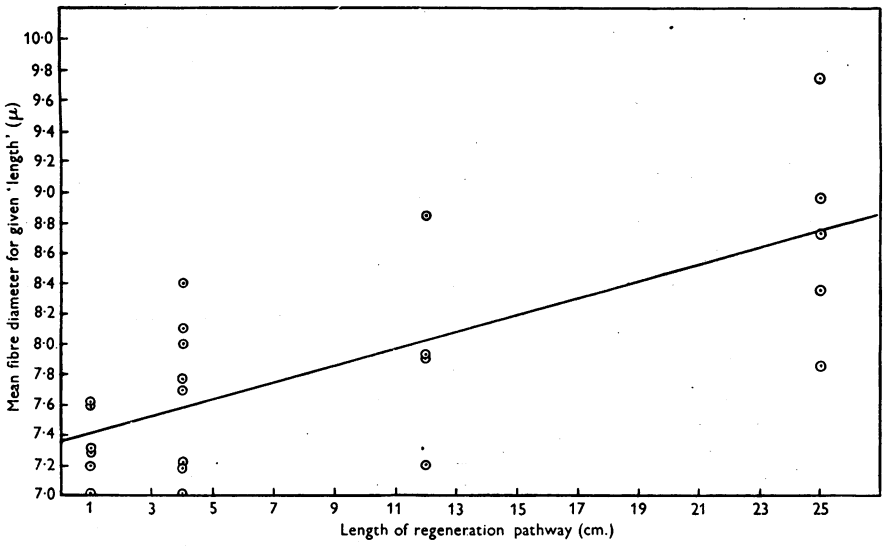
Table 1. *Distribution of fibres in regenerating nerves of different lengths*

	Diameter (μ)										
Specimen	0–2	2–4	4–6	6–8	8–10	10–12	12–14	14–16	Total	No. $>6\mu$	Mean of $>6\mu$ fibres
					(*a*) N.g.m. with 1 cm. peripheral path						
48 (*a*)	326	1254	381	91	8	—	—	—	2060	99	7·32
51 (*f*)	96	1528	558	207	66	8	—	—	2463	281	7·58
56 (*f*)	311	2513	521	198	81	2	—	—	3625	281	7·60
57 (*f*)	378	1576	409	199	22	—	—	—	2584	221	7·20
58 (*c*)	216	1115	363	150	27	—	—	—	1871	177	7·31
59 (*a*)	315	1750	375	23	—	—	—	—	2463	23	7·00
									Mean of group		7·33
					(*b*) N.g.m. with 4 cm. peripheral path						
29 (*b*)	132	724	318	124	41	9	3	—	1351	177	7·77
51 (*a*)	95	764	349	102	10	—	—	—	1320	112	7·18
55 (*a*)	41	540	193	125	115	41	—	—	1055	281	8·40
56 (*a*)	35	560	166	162	107	18	—	—	1048	287	8·00
57 (*c*)	85	711	311	122	3	—	—	—	1222	125	7·05
58 (*a*)	186	1058	368	128	16	—	—	—	1756	144	7·22
59 (*b*)	45	583	194	124	117	11	—	—	1074	252	8·10
185 (*b*)	36	518	263	138	67	3	—	—	1025	208	7·70
									Mean of group		7·68
					(*c*) N.g.m. joined to sural nerve—total length 12 cm.						
83 (*a*)	35	414	172	112	160	73	6	—	972	351	8·85
89 (*a*)	53	589	279	119	68	11	—	—	1199	198	7·91
95 (*a*)	61	503	117	143	110	5	—	—	939	258	7·93
105 (*b*)	124	322	270	233	5	9	1	—	964	248	7·21
									Mean of group		7·98
					(*d*) N.g.m. joined to sural nerve—total length 25 cm.						
89 (*c*)	20	589	277	144	114	46	—	—	1190	304	8·36
539 (*d*)	55	274	108	94	120	58	—	—	709	272	8·74
539 (*a*)	14	160	110	119	129	104	5	—	641	357	8·97
541 (*a*)	56	444	225	152	93	8	—	—	978	253	7·86
546 (*a*)	23	192	83	86	106	94	53	5	642	344	9·75
									Mean of group		8·74

of 0·5 μ in the mean diameter of the larger regenerating fibres in each 10 cm. travelled. That the effect is a real one and not due to chance is shown by the regression coefficient which is significant on the 0·001 level ($t=4·7$, $f=21$).

These experiments therefore definitely indicate that regenerating fibres which grow for a greater distance become larger. Nothing very definite can be said about the form of the relationship, the diameter more nearly follows the length than its logarithm, but other possibilities are not excluded. It must also be remembered that the very longest fibres make endings different from the others (in the skin rather than in a neuroma).

As the length of the peripheral path and the rate of progress of the nerve fibres down the path can be estimated, it is possible to calculate the time taken to reach the end of the Schwann tubes, and by deducting this figure from 100 to determine the time allowed for maturation. Allowing 5 days for the fibres to cross the crush, 5 days to cross the union and a growth rate of 5 mm. per day above the union but of only 3 mm. a day below the union (Gutmann, Guttman, Medawar & Young, 1942) we obtain the results summarized in Table 2.



Text-fig. 2. Linear regression of degree of maturation on the length travelled by the regenerating nerves, maturation being measured as the mean diameter of the larger ($> 6\mu$ in diameter) fibres.

Table 2. *The estimated times allowed for maturation of the regenerating fibres of n.g.m. after travelling different distances*

Distance travelled (mm.)	Estimated time to reach end of nerve (days)	Time allowed for maturation (days)
10	7	93
40	13	87
120	45	55
250	88	12

In the longest nerves many of the fibres would be actively growing until 12 days before the biopsy. The specimens with neuromas at the cut end of the n. suralis below the knee (12 cm.) would have 55 days in which to mature, yet the mean diameter of the fibres is less than that found in the longest nerves (25 cm.) (see Text-fig. 1).

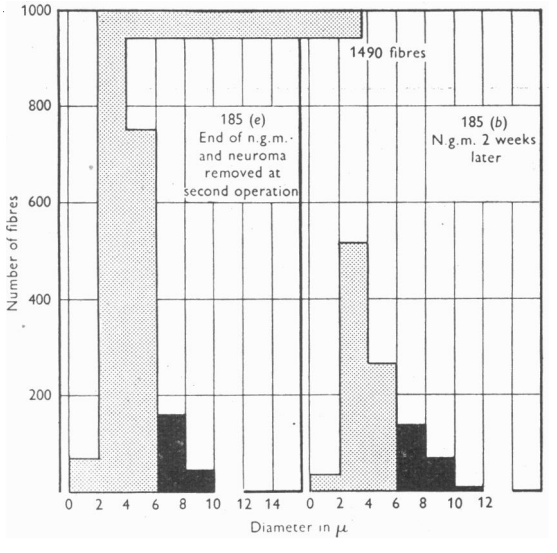
B. *Turning back of regenerating fibres at a neuroma*

When regenerating nerve fibres reach the end of the Schwann tubes, without making contact with a peripheral end organ, a neuroma is formed. It has been suggested by Weiss *et al.* (1945) that some of the fibres turn and re-enter the Schwann tubes and travel in a central direction. This phenomenon was clearly demonstrated

in one of the experiments where the nerve was crushed, cut lower down, allowed to regenerate and form a neuroma, and was then operated on again, the neuroma being cut off 2 weeks before the terminal biopsy. Comparison of the two specimens 185 (*e*) and 185 (*b*) in Table 3 shows that the total number of fibres had been reduced to one-half.

Table 3 (*a*) and (*b*). *Counts of regenerating fibres in the n.g.m. immediately proximal to a neuroma and in the same nerve 2 weeks after the neuroma had been removed*

Specimen	Diameter (μ) 0–2	2–4	4–6	6–8	8–10	10–12	Total
	(*a*) Portion of n.g.m. with neuroma attached						
185 (*e*)	68	1490	750	158	42	—	2508
	(*b*) N.g.m. 2 weeks after removal of neuroma						
185 (*b*)	36	518	263	138	67	3	1025



Text-fig. 3. Histograms of the distributions of fibre sizes in n.g.m. immediately proximal to a neuroma and in the same nerve two weeks after the neuroma had been removed.

Unfortunately, owing to the nearness to the neuroma, it was impossible to obtain a section of which all parts could be counted. However, a reliable estimate was made by determining the total area of the photograph with a planimeter (727 sq.cm.) and calculating the number of fibres in the size groups from the area which was capable of being counted (426 sq.cm.).

Examination of the figures in Table 3 shows that the greater differences are to be found in the groups of smaller fibres. From these findings it does seem that a great number of small regenerating fibres returns along the nerve trunk and this suggests that the larger fibres are the better index of maturation as the number of larger fibres was almost the same in the two specimens.

Text-fig. 3 shows the histograms of these two specimens, and Pl. 1, figs. 1 and 2 the photographs of the cross-sections which were counted. The difference in size of the sections is due to the proximity to the neuroma and the number of fibres.

C. *Effect of muscle state on maturation of regenerating fibres*

On examination of the animals after 100 days, it was found that the biceps muscle was not greatly wasted on the paralysed side, though stimulation of the sciatic nerve high in the thigh produced no contraction of the muscle. Stimulation of n.g.m. gave rise to a contraction of a bundle of muscle fibres. By increasing the strength of the stimulus it was possible to make more muscle fibres respond, though never did the muscle react as when the nerve to the normal biceps on the other side was stimulated. The paralysed muscles responded at a higher threshold of stimulation of the nerve than did the normal muscles. The differential counts of the fibre populations of these nerves are given in Table 4.

Table 4. *Distribution of fibres in regenerating n.g.m. when implanted into normal and paralysed biceps femoris muscles*

Specimen	Diameter (μ) 0–2	2–4	4–6	6–8	8–10	10–12	12–14	Total	No. $>6\mu$	Root mean sq. diameter of fibres $>6\mu$ (D)
				(*a*) Implantation into normal biceps						
417 (*r*)	52	1035	256	177	23	—	—	1790	200	7·2
428 (*r*)	115	716	287	165	67	8	—	1358	240	7·9
443 (*c*)	175	586	194	163	81	—	—	1199	244	7·7
483 (*a*)	316	973	419	77	216	2	—	2003	295	6·3
642 (*c*)	12	588	375	228	66	10	—	1279	304	7·4
643 (*b*)	55	426	193	189	78	1	2	944	270	7·7
				(*b*) Implantation into paralysed biceps						
417 (*l*)	6	817	394	326	160	97	11	1811	594	8·5
428 (*l*)	85	730	285	150	29	13	—	1292	192	7·7
443 (*a*)	151	612	183	134	66	9	—	1155	209	7·9
483 (*d*)	138	968	185	85	111	69	49	1605	314	9·7
642 (*a*)	13	347	133	110	105	65	30	803	310	9·3
643 (*c*)	44	416	216	136	98	36	5	951	275	8·5

The larger fibres ($>6\mu$) were again used as an indication of the degree of maturation. The hypothesis that the distributions of the larger-sized fibres are the same whether the nerve terminates in a normal or paralysed muscle was made and calculations gave the following results:

Animal	X^2	f	$P(X)^2$
417	50·84	1	0·001
428	11·757	2	0·01
443	0·36	1	0·6
483	13·91	2	0·001
642	118·86	2	0·0001
643	21·8	2	0·001

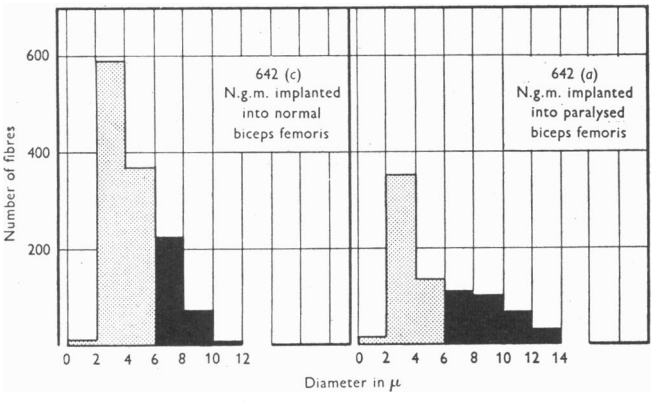
Except in animal 443, the hypothesis is rejected in every case. When the total X^2 is considered, $X^2=217{\cdot}53$, $f=10$ and $P(X)^2=0{\cdot}001$.

The possibility of these samples belonging to the same population is therefore exceedingly remote.

When the root mean square diameters (D) of the fibres more than 6μ in diameter

are considered it is evident that the nerves which regenerate into the paralysed muscles are larger in all experiments except one (428).

Pl. 1, figs. 3 and 4 show photographs of sections of nerves (642*a* and 642*c*) which have been implanted into normal and paralysed muscles. It will be noticed that the former produces a condition in which the Schwann tubes are filled with many small fibres whereas, in the latter, the tubes contain fewer fibres and usually one large fibre. Histograms of the same two nerves (Text-fig. 4) demonstrate the reduction in the total number of fibres—especially the smaller groups—and an increase in the larger fibre groups.



Text-fig. 4. Histograms of the distributions of fibre sizes in regenerating n.g.m. after implantation into normal or paralysed biceps femoris.

DISCUSSION

From the preceding results, it is evident that the distance travelled by regenerating nerve fibres and the possibility of making a functional connexion with muscle both influence in a marked degree the process of maturation.

A. *Effect of length*

The diameters of normal nerve fibres are usually taken to vary according to the type of functional activity of the fibres (Erlanger & Gasser, 1937). In these experiments, the regenerating fibres rarely attain a size more than 12–14 μ in diameter, they are non-functional and therefore the peripheral connexion can have no stimulating effect on the maturation processes. Where the n.g.m. was united with the n. suralis, certain new factors were introduced which must be considered. The union of the nerves, the smaller Schwann tubes of the sensory nerve and the presence of peripheral end-organs in the skin are the most important factors. The unions were made with no tension between the two nerves and the positions were maintained with coagulated plasma. At biopsy, the unions were examined and found to be intact and sections of the n. suralis proved that many fibres had passed down into this nerve. Pl. 1, fig. 5 is a photograph of a longitudinal section of one of these unions (95*d*). There is always the possibility, however, that a few fibres escaped at the site of the union into the surrounding fascia or even turned back up the n.g.m.

The smaller Schwann tubes might tend to impede the flow of axoplasm and so increase the diameter of the fibres above the union.

In the normal n.g.m. there are a number of sensory fibres which might find a pathway towards an end organ in the skin. These fibres were presumably non-functional, as stimulation of the skin supplied by n. suralis evoked no response, and when the n. suralis was electrically stimulated no withdrawal of the leg occurred.

The effect of the length of nerve regenerated was investigated by Sanders & Young (1946), who considered that the process of maturation was independent of it. They employed varying lengths of the large peroneal nerve and joined it to the still larger tibial nerve. Both of these nerves have considerable mixed muscle and sensory (skin) components and the regenerating fibres would eventually make contact with end organs, many of them on the muscle fibres. Though the two sides would be partly comparable, no indication is given of the possibility of the motor end-plate connexion masking the length effect. The difference in length was 55 mm., which according to the present findings would account for a shift upwards of $0{\cdot}25\,\mu$ in the mean diameter of the fibres, a figure which could almost certainly be accounted for by the varying peripheral connexions of the fibres.

Time for maturation. It has been suggested by Weiss *et al.* (1945) that the nerve fibre increases in diameter after the growing end comes to rest. In the present series of experiments, those nerves which could regenerate along the terminal branches of n. suralis into the foot would have only 10–20 days in which to mature, whereas those nerves which ended in neuromas in the thigh or leg would have 70 or 50 days. The degree of maturation, however, bore an inverse relationship to the time. In spite of the long time which could be used for maturation, the shorter lengths had nerves of a smaller mean diameter than the longer nerves. Previous work (Aitken *et al.* 1947) has shown that when nerves are allowed to regenerate and form neuromas for periods up to 200 days, though the number of fibres is reduced in the longer periods, there is no increase in the diameter of the fibres. It is therefore probable that in considering the degree of maturation the distance travelled is a more important factor than the time.

B. *Effect of overcrowding*

The effect of overcrowding the Schwann tubes by a large number of small fibres is most pronounced in the shorter lengths of nerve. Their numbers over the total series of nerves vary considerably and for no very obvious reason, even some of the implants into paralysed muscle (Table 4 (*b*)) having a total of over 1000 fibres in the groups under $6\,\mu$ in diameter. The larger-sized fibres seem to form a more constant and stable series, and in the nerve which had the neuroma removed 2 weeks before biopsy there was a difference of only 8 between the number of large fibres in the specimen of nerve with the neuroma and that found in the nerve itself. It is realized that there is a difficulty in making a definite statement concerning this matter as sections cut near the neuroma are very difficult to count accurately owing to the obliquity of many of the fibres.

When Pl. 1, figs. 1 and 2 are compared, it is seen that many of the Schwann tubes in fig. 2 (section of n.g.m. after removal of the neuroma) contain only one fibre and often this fibre is small. During the 14 days since operation, many of the small fibres have degenerated, but the remainder do not seem to have increased in size.

C. *Effect of muscle state*

It has long been maintained that the nerve supply of mammalian muscle is such that each muscle fibre has one motor end-plate (Wilkinson, 1929; Denny-Brown & Pennybacker, 1938), though some workers (Aghduhr, 1916; Cuajunca, 1932) have reported the presence of multiple endings on a fibre. In normally innervated muscle there will therefore be a state of equilibrium between muscle and nerve, and when a 'foreign' regenerating nerve is made to grow into a fully innervated muscle, it will lie in contact with muscle fibres which are already innervated. Yet when the nerve is examined the process of maturation is found to have proceeded farther than in a nerve of comparable length which forms a neuroma in the fascia (compare results in Tables 1 (*b*) and 4 (*a*)). The close proximity to muscle tissues does thus seem to have an effect on the process of maturation. Examination of nerves in normal muscles which is being carried out shows that many fine fibres pass between the muscle fibres with little or no attempt to form motor end-plates.

When a regenerating nerve is implanted into a paralysed muscle the process of maturation is greatly facilitated (Table 4 (*b*)); not only is the number of small fibres usually reduced but the larger fibres are significantly increased. When these results are compared with those previously reported (Aitken *et al.* 1947) for maturation in regenerating nerves following union with a muscle nerve, it is found that the maturation after implantation is poorer than after union. In the latter case, most of the motor end-plates were reinnervated and the whole muscle contracted on stimulation. Stimulation of an implanted nerve in a paralysed muscle gave rise to contraction of a few bundles of muscle fibres. Histological examination of the paralysed muscle revealed no innervated 'native' motor end-plate but a very different neuroma from that which was found in a normal muscle. It would seem that the implant responds to the demand on the part of the paralysed muscle and that the most potent stimulus to maturation is the opportunity to form new motor end-plates. As counts of the new motor end-plates were not made, it is impossible to correlate the degree of maturation with the number of functioning muscle units but the number of endings was never very great and they were mostly close to the implant.

The growing ends of the nerve fibres would reach the muscle in about 13 days after the operation and they would have 87 days in which to ramify amongst the muscle fibres. The results of electrical stimulation showed that the functional spread of the nerves was restricted. This was specially marked in the experiments where the implantation was made into a normal muscle.

Fort (1938), working with Weiss, has studied some of the factors involved in the establishment of neuro-muscular connexions in toads. He suggests that denervated muscle may be more 'permeable', though he admits that his evidence is inconclusive. Attempts to arrest the process of reinnervation in a denervated muscle by use of a Ringer extract of normal muscle were also not effective.

From the present work it would seem that the juxtaposition of regenerating nerve fibres and muscle fibres is sufficient to initiate the process of maturation, but that the opportunity of forming a functional motor end-plate on muscle fibres is a very strong stimulus to continuation of the process.

SUMMARY

1. Experiments in which nerves were allowed to regenerate along pathways of different lengths (1, 4, 12 cm. ending in a neuroma, and 25 cm. ending in the skin) showed that the longest nerves had largest diameters. Over the range investigated there was an increase of $0{\cdot}5\mu$ in the mean diameter of the fibres for each 10 cm. travelled.

2. Almost half the regenerating fibres turn round at a neuroma and travel back along the nerve. This effect is greatest in those cases where the regeneration path is short.

3. Maturation of a regenerating nerve is much more complete when the nerve fibres are allowed to make contact with paralysed (denervated) muscle fibres than when the nerves grow into a normal muscle or into fascia. The possibility of making new functional motor end-plates or reinnervating those which have been denervated produces a marked increase in the degree of maturation.

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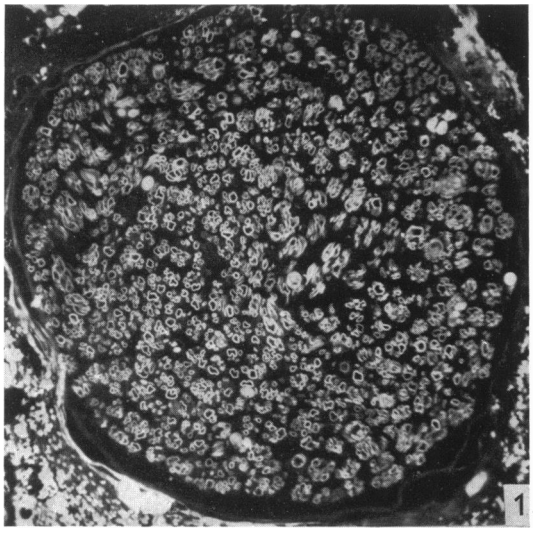
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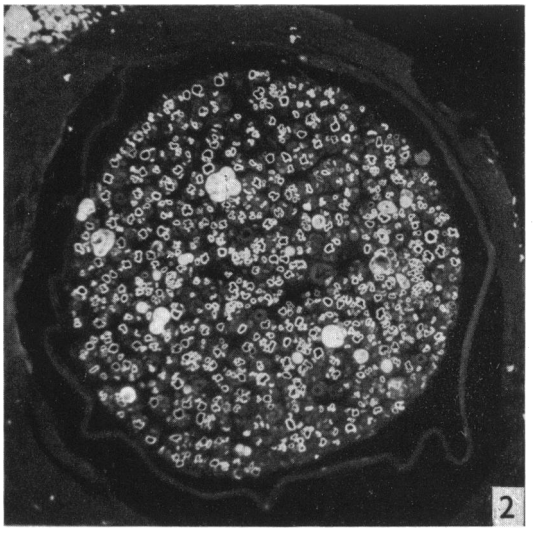
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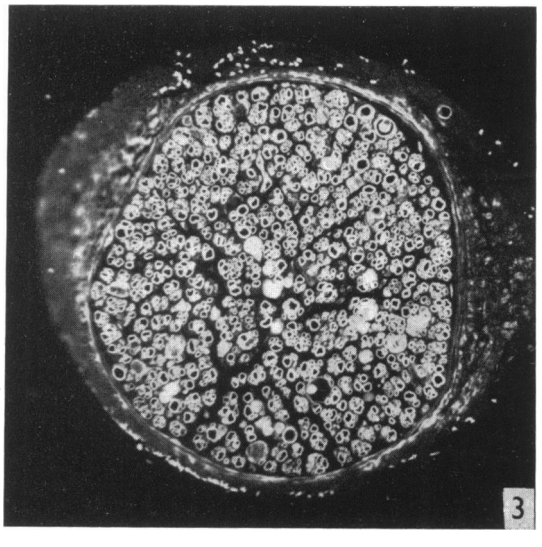
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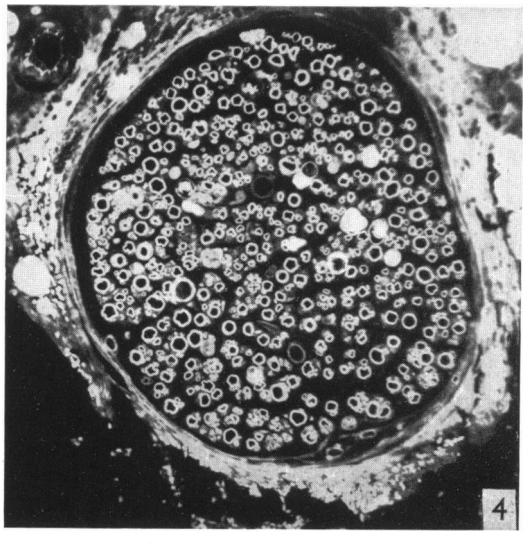
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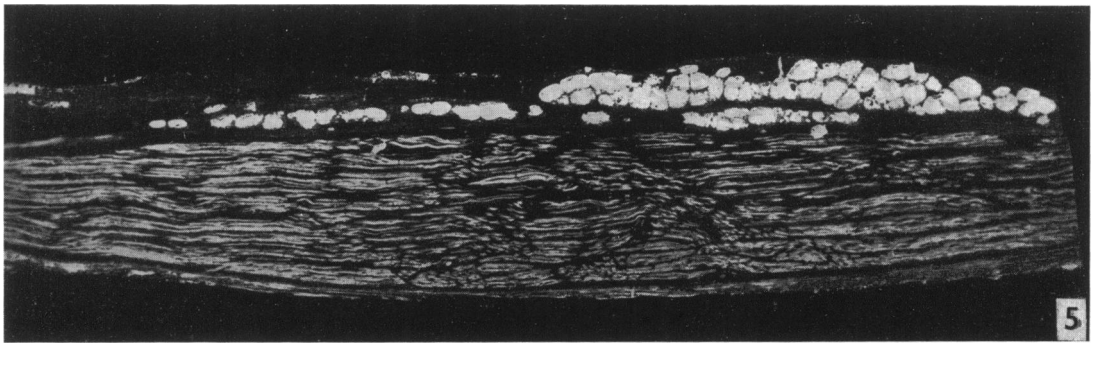
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EXPLANATION OF PLATE

All figures are of specimens fixed in Flemming's solution, Weigert-stained and photographed directly on to bromide paper.

Fig. 1. Regenerating nervus gastrocnemii medialis immediately proximal to the neuroma (185*e*). (×150.)

Fig. 2. Regenerating nervus gastrocnemii medialis 2 weeks after removal of the neuroma (185*b*). (×150.)

Fig. 3. Regenerating nervus gastrocnemii medialis which was implanted into a normally innervated muscle (642*c*). (×150.)

Fig. 4. Regenerating nervus gastrocnemii medialis which was implanted into a paralysed (denervated) muscle (642*a*). (×150.)

Fig. 5. Longitudinal section of union between nervus gastrocnemii medialis and n. suralis 95*d*). (×55.)