THE BEHAVIOR OF RESIDUAL AXONS IN PARTIALLY DENERVATED MUSCLES OF THE MONKEY*

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PLATES 6 AND 7

The "spontaneous" recovery exhibited by partially denervated muscles of several laboratory mammals (rat, cat, dog, rabbit) has been studied frequently (1–6). Indirect evidence derived from analyses of the weight, strength, and fiber size of these paretic muscles has suggested that most of the recovery is due to the extension of residual, intact axons which adopt denervated muscle fibers. The recent histological demonstration that undamaged axons in paretic muscles of the rat develop collateral branches which reinnervate adjacent denervated motor end-plates, has conclusively substantiated this explanation (7).

Several of the authors cited have suggested that the recovery which occurs in some paretic muscles during the convalescent stage of human poliomyelitis, may be attributable in part to a similar enlargement of residual motor units (*cf.* also references 8 and 9). In an effort to provide further evidence bearing on the validity of this suggestion, partially denervated muscles have been studied in a series of monkeys. The major aim of the investigation has been to determine whether or not the intact nerve fibers in paretic muscles of a primate behave similarly to those in other, previously studied mammals. The results obtained indicate that in the monkey, collateral regeneration of residual, intramuscular axons occurs only to a limited degree following slight denervation, and does not take place after marked denervation.

Material and Methods

Six *rhesus* monkeys (*Macaca mulatta*), ranging from 3.5 to 5.0 kg. body weight, constituted the experimental material. All operations were performed under intravenous nembutal anesthesia using aseptic technique. With the animal in the supine position, the right psoas major muscle was exposed retroperitoneally through an inguinal incision. The dissection was then extended along the medial border of the psoas muscle until the origin of the sciatic nerve from the lumbosacral plexus was exposed. From one to three of the lumbar nerves which contribute to the sciatic nerve were tied and interrupted about 1 cm. central to their point of union. The proximal stumps of the sectioned nerves were then capped with methyl

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methacrylate to inhibit regeneration (10), diverted laterally, and sutured to the belly of the psoas muscle.

The specific lumbar nerves eliminated in the different cases are listed in Table I. The sciatic nerve in the monkey (actually, the tibial, common peroneal, and femoral flexor nerves since, in most instances, there is no true sciatic nerve; *cf.* reference 11) receives fibers from the last 4 lumbar (L_4, L_5, L_6, L_7) and the first 2 sacral (S_1, S_2) nerves, L_4, L_5, and S_2 being inconstant. The largest contributors are generally L_6 and L_7, but these two nerves are variable in size (*cf.* case M 66, in which about 50 per cent of the tibial-peroneal fibers come from L_6, with case M 25, in which L_5 and L_6 together contribute only 25 per cent of these fibers). In general, the peroneal nerve obtains more fibers from the more cephalic spinal nerves, the tibial nerve, more from the more caudal levels. In 4 cases, a severe partial deneurotization of the sciatic nerve was effected (Table I, M 68, M 70, M 71, M 69); in the two remaining cases (M 66, M 25), the loss of fibers was moderate.

The animals were followed for periods ranging from 33 to 200 days after operation. The functional status and the degree of atrophy of the leg that was operated upon were observed at frequent intervals, comparisons being made with the left leg which was not operated upon. After the stated periods, the animals were sacrificed with an overdose of nembutal.

The sciatic nerves operated upon (right) and the controls (left) and their main branches were explored to estimate the pattern of deneurotization. From each sciatic nerve a segment was then removed which included the still intact, contributory spinal nerves as well as the distal stumps of those which had been interrupted. Samples of the following were also excised from both legs: nervi flexores femoris, tibialis, and peroneus communis (from popliteal space), and soleus (just proximal to soleus muscle). All nerves were fixed under slight longitudinal tension in 2 per cent osmic acid; sample transverse sections were then prepared for use in counting myelinated nerve fibers.

Selected muscles from each of the legs were carefully excised and weighed. The specific muscles taken, varied from case to case depending on the pattern of paresis which had been produced. Wet weights were always determined, however, for the plantar extensor group (musculi gastrocnemius, plantaris, and soleus) and for two dorsiflexors (musculi tibialis anterior and extensor digitorum longus). Small pieces (roughly $10 \times 5 \times 2$ mm.) were then cut from different regions of each muscle which had been weighed, and impregnated according to the gold chloride method of Carey (12, 13; *cf.* reference 7).

Following impregnation with gold, muscle fragments were teased and mounted *in toto* for histological study. This permitted observations on the relationship between entire intramuscular nerve bundles and large numbers of muscle fibers (*cf.* Fig. 1). As previously explained (7), direct counts can be made on these preparations of the number of end-plates supported by the terminal axon branches in any given terminal bundle of nerve fibers. An estimate of the "terminal innervation ratio" can be derived from these values. The ratio, which may be defined as the average number of end-plates supplied by a single fiber in a terminal bundle, is relatively constant in normal muscle (*vide infra*). In partly denervated muscles in which collateral regeneration has taken place, the terminal innervation ratio is markedly increased.

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<div style="text-align:center">RESULTS</div>

Degree of Partial Denervation.—Sections of the nerves which were experimented upon demonstrated that the procedures employed to inhibit the regeneration of the severed spinal nerves were successful. Although a few

regenerating fibers escaped around the plastic caps (*cf*. reference 14), none penetrated the distal stumps for more than a few millimeters. All the medullated axons in the muscular branches of the affected nerves, therefore, were derived from the intact spinal nerves. The approximate number of residual axons in the peroneal and tibial nerves, expressed in per cent of those present in the control nerves, is given in Table I. These values indicate that the structures supplied by the branches of these nerves suffered an average loss of nerve fibers ranging in the 6 cases from about 25 per cent to 90 per cent. The degree of denervation varied even more widely in the individual muscles of different animals.

Functional Observations.—In evaluating the functional performance of the legs utilized for experiment, it was essential to keep in mind that muscles acting on the hip and knee joints were little impaired by the operation. With

TABLE I

Residual Innervation and Wet Weights of the Affected Leg Muscles

Case	Time after operation	Spinal nerves eliminated	Residual fibers in peroneal-tibial nerves in per cent of controls	Muscle weights in per cent of controls	
				Plantar extensors	Dorsiflexors
	days				
M 68	33	L_5, L_6, L_7	10	32.2	51.3
M 66	63	L_6	50	93.5	89.0
M 70	78	L_5, L_6, L_7	10	28.8	35.8
M 71	117	L_5, L_6, L_7	17	51.6	29.8
M 69	160	L_5, L_6, L_7	10	17.4	19.8
M 25	200	L_5, L_6	75	96.2	91.4

the exception of the moderate paresis produced in some of the gluteal and hamstring muscles (which in these animals received most of their innervation from L_7 and S_1), only the muscles below the knee were affected; all muscles innervated by the femoral and obturator nerves were normal. The following remarks thus refer specifically to movements of the foot and digits (*cf*. reference 15).

The affected legs in the less paretic cases M 66 and M 25 were only slightly disabled, and began to function almost normally as soon as the animals had fully recovered from the operation. Atrophy of the affected muscles was barely detectable throughout the postoperative periods and, at the time of sacrifice, the weights of all the affected muscles were within 11 per cent of those of the contralateral controls (Table I).

In the remaining severely paretic cases, the affected muscles were almost completely paralyzed throughout the respective postoperative periods (from 33 to 160 days). Serviceable toe and foot movements were regularly observed

in M 71, but they were weak and the involved muscles became markedly atrophic, particularly the dorsiflexor group. Cases M 68, M 70, and M 69 usually carried the limb with both the hip and knee joints in flexion; the lower leg and foot were dragged passively. No refined tests were made to detect small changes in the amount of residual muscle power but, so far as their functional utility was concerned, the paretic legs in these cases showed no improvement up to the time of sacrifice. The severe, progressive atrophy of the affected muscles (Table I) accords with these findings.

Histological Observations

Normal Muscles.—The intramuscular nerve bundles in normal leg muscles of the monkey form a more complex pattern of ramifying plexuses than that described in the rat (7). Moreover, the natural branching of the individual nerve fibers is richer in the monkey and the size of the average motor unit is therefore presumably larger. As in the rat, however, the terminal branches of the individual axons are finally collected in terminal bundles containing a few to over 30 fibers. The latter course for a short distance, usually about 0.5 to 1.0 mm., then spray out and pass directly to their respective motor end-plates (Figs. 1 and 2).

Most of the fibers in terminal bundles are actually terminal; that is, each innervates only 1 end-plate. Natural branching at this level, however, does occur. To estimate its frequency, counts were made both of the number of nerve fibers entering 71 terminal bundles in 8 different thigh and leg muscles, and of the number of end-plates innervated by these bundles. A total of 538 end-plates were associated with 430 nerve fibers, giving an average terminal innervation ratio of 1.25:1 (standard error of the ratio is ±0.08). This result indicates that on the average 1 out of every 4 nerve fibers in a terminal bundle will branch naturally and innervate 2 end-plates. Natural branching within these bundles is therefore more frequent in the monkey than in the rat, in which smaller terminal innervation ratios are encountered (7).

Affected Muscles.—In the 4 most severely paretic cases (M 68, M 70, M 71, and M 69), the degree of denervation in most of the muscles studied ranges from 75 to 100 per cent; a few exceptional muscles retain up to roughly half of their original nerve fibers. The intramuscular nerve trunks are markedly depleted, and residual axons are intermingled with many cords of Schwann cells (Büngner's bands). These bands, derived from the non-neural elements of the interrupted axons, can be traced directly to remnants of the denervated motor end-plates (Fig. 6). The latter are still distinguishable even in the oldest cases.

The course of many of the residual, intact axons can be followed easily; frequently only two or three of them remain in a small intramuscular nerve trunk, and their natural side branches can be seen diverging into terminal

bundles (Fig. 3). These side branches, which constitute "terminal" axon branches as defined above, are almost without exception devoid of newly formed collateral twigs (Figs. 4 to 6). They divide naturally about as often as do similar fibers in normal muscles, but there is no evidence, based either on the nature of the connections effected by these fibers or on terminal innervation ratios, that any marked collateral regeneration has occurred. In a few exceptional instances, one or two apparently new collateral branches were detected in a small spray of residual fibers (Fig. 3). They were never abundant enough to increase the number of innervated end-plates significantly, however, and the interpretation to be placed on them is questionable.

Figs. 5 and 6 also demonstrate that the absence of collateral regeneration cannot be attributed to a sharp segregation of intact and denervated muscle fibers. Characteristically, these two elements are closely intermingled, a condition previously recognized as essential for the collateral sprouting of residual axons (4, 7).

Case M 66, and particularly case M 25, which suffered relatively light denervation, provide evidence of moderate collateral regeneration of the residual axons in several of the affected muscles. The terminal bundles from the paretic gastrocnemius muscle of M 25 which appear in Figs. 7 and 8 illustrate our findings. In contrast to the normal and affected bundles previously described, the nerve fibers illustrated each support an average of almost 2 end-plates. The bundle in Fig. 7 contains 7 intact fibers which innervate 13 end-plates. The two adjacent bundles in Fig. 8 together include 16 fibers which enter 32 end-plates.

A comparison of the individual fibers in Fig. 7 with those in the control bundle shown in Fig. 2 (contralateral muscle; same magnification) indicates that many of the former are below normal in size. These small fibers are interpreted as immature collateral branches, the presence of which has increased the terminal innervation ratio to nearly 2:1. Virtually all the new branches in the sprays shown in Fig. 8 have attained normal dimensions and can no longer be distinguished by size alone; only the enhanced terminal innervation ratio reveals that collateral regeneration has occurred.

Counts of "terminal" nerve fibers and their associated end-plates in three paretic and three control muscles of M 25 gave even more conclusive evidence for collateral regeneration. The terminal innervation ratios obtained (Table II) demonstrate that on the average, the experimental nerve fibers supply almost twice as many end-plates as do the control fibers. The statistical significance of the differences between comparable experimental and control ratios was determined by converting the actual figures on which the proportions are based to percentages, and calculating the standard error of a percentage. The probability values obtained were all 0.01 or less, indicating that the differences are significant.

Fig. 7 also demonstrates that the process of repair is incomplete, since several still "empty" strands of Schwann cells can be seen passing to denervated end-plates. This was a common finding in these muscles and it contrasts sharply with earlier observations on paretic muscles of the rat. In the latter, collateral branches regularly take over all but an occasional end-plate; consequently, much larger terminal innervation ratios are encountered, ranging up to more than 30:1 (7).

The results which have been described lead to the conclusion that although a moderate degree of collateral regeneration of the residual axons can be demonstrated conclusively in lightly denervated muscles of the monkey, the process fails to go to completion, and many muscle fibers are permanently deprived of their innervation. The percentage of muscle fibers which fail to regain innervation must increase sharply as the degree of denervation is increased,

TABLE II

Terminal Innervation Ratios in Case M 25

Muscle	Control muscles				Affected muscles			
	Sprays counted	End-plates	Nerve fibers	Average terminal innervation ratio	Sprays counted	End-plates	Nerve fibers	Average terminal innervation ratio
Medial gastrocnemius	11	125	94	1.3:1	8	146	76	1.9:1
Lateral gastrocnemius	7	77	62	1.2:1	5	81	48	1.7:1
Anterior tibial	7	64	57	1.1:1	7	54	20	2.7:1

for in muscles which have been rendered severely paretic, collateral regeneration either fails altogether or is so sporadic as to be of no functional consequence.

COMMENT

The results of this investigation demonstrate that the behavior of intact axons in partially denervated muscles of the monkey differs makedly from that of comparable nerve fibers in the rat. The rapid and profuse collateral regeneration of residual axons in the latter occurs only to a moderate degree, or not at all, in the former. The reasons for this puzzling difference are obscure, and until a more complete understanding of the mechanism of collateral regeneration is obtained, little can be gained by speculation.

As noted in the introduction, several authors have suggested that part of the recovery of paretic muscles in human patients convalescing from poliomyelitis may be due to an enlargement of residual motor units. This suggestion, which derived considerable support from the earlier investigations on other mammals, must now be reappraised. Assuming that the present results are more nearly representative of what occurs in partially denervated human

muscles, it would appear that collateral regeneration cannot play a major role in the improvement of paretic muscles following partial poliomyelitic lesions. In severely affected muscles, extensive recovery due to the expansion of intact motor units would be most unlikely, a conclusion which accords with clinical experience (16). The precise share of regenerative processes in the gradual improvement of lightly paralyzed muscles remains to be determined. Indeed, there is a continuing and urgent need for a complete morphological survey of the effects of poliomyelitis on peripheral nerves and their end-organs, comparable to the excellent analysis by Bodian (17, 18) of the progressive changes in the central nervous system.

The attention currently devoted to "closed neurotripsy," a method which is designed to enhance the recovery of paretic muscles by crushing them (19), might appear to gain some support from our results. It has been argued that the trauma associated with this treatment injures the residual intramuscular axons which then regenerate an increased number of branches and adopt new motor end-plates. Neurotripsy might thus be a means of circumventing the low tendency of primate motor axons to regenerate collaterally which is demonstrated in the present study.

Actually, neurotripsy does not have this effect on the nerve fibers of either normal or partially denervated muscles of the rat (Edds, unpublished data). Crushing such muscles with a vibrating hammer produces little change in the intramuscular axons or their endings, unless the damage is sufficient to cause necrosis and destruction of the muscle fibers. In the latter instance, there is extensive local degeneration of the nerve fibers. But even if the interrupted axons should subsequently regenerate and branch profusely, little improvement could be anticipated in view of the limited regenerative capacity of mammalian muscle (20).

SUMMARY

Leg muscles of the monkey have been studied following partial denervation produced by surgical elimination of from 25 to 90 per cent of the axons entering the sciatic nerve from the lumbosacral plexus. The investigation included observations on function, rate and degree of muscle atrophy, and neurohistological appearance of the affected muscles.

In most of the cases, from 83 to 90 per cent of the residual nerve fibers in the peroneal and tibial nerves were destroyed and a severe paresis of the leg muscles was produced. No functional improvement was noted up to 160 days after operation, and the affected muscles became markedly atrophic. Histological examination of these muscles failed to reveal more than sporadic collateral regeneration of the residual axons.

In two cases 50 and 75 per cent of the peroneal and tibial nerve fibers remained intact 63 and 200 days, respectively, after operation. The legs operated

upon in these cases functioned almost normally and all muscles weighed within 11 per cent of those of the contralateral, normal leg. Histological study and counts of end-plate:nerve fiber ratios showed that many residual axons had regenerated collateral branches which entered denervated end-plates. Collateral regeneration was incomplete, however, and many end-plates remained without innervation.

These results indicate that residual axons in paretic muscles of a primate do not regenerate collaterally as readily as do those of other previously studied mammals.

BIBLIOGRAPHY

1. Hines, H. M., Wehrmacher, W. H., and Thomson, J. D., *Am. J. Physiol.*, 1945, **145**, 48.
2. van Harreveld, A., *Am. J. Physiol.*, 1945, **144**, 477.
3. van Harreveld, A., *Am. J. Physiol.*, 1947, **150**, 670.
4. Weiss, P., and Edds, M. V., Jr., *Am. J. Physiol.*, 1946, **145**, 587.
5. Frederick, J. N., and Kosman, A. J., *Proc. Soc. Exp. Biol. and Med.*, 1948, **69**, 605.
6. Kosman, A. J., Wood, E. C., and Osborne, S. L., *Am. J. Physiol.*, 1948, **154**, 451.
7. Edds, M. V., Jr., *J. Exp. Zool.*, 1950, **113**, 517.
8. Bodian, D., *J. Am. Med. Assn.*, 1947, **134**, 1148.
9. Guyton, A. C., *Arch. Int. Med.*, 1949, **83**, 27.
10. Edds, M. V., Jr., *J. Neurosurg.*, 1945, **2**, 507.
11. Howell, A. B., and Straus, W. L., Jr., *in* The Anatomy of the *rhesus* Monkey, (C. G. Hartman and W. L. Straus, Jr., editors), Baltimore, Williams and Wilkins Co., 1933, 307.
12. Carey, E. J., *Am. J. Path.*, 1942, **18**, 237.
13. Carey, E. J., *in* Laboratory Technique in Biology and Medicine, (E. V. Cowdry, editor), Baltimore, Williams and Wilkins Co., 2nd edition, 1948, 52.
14. Edds, M. V., Jr., *J. Exp. Zool.*, 1949, **111**, 211.
15. Sperry, R. W., *Quart. Rev. Biol.*, 1945, **20**, 311.
16. Green, W. T., Poliomyelitis, Papers and Discussions Presented at the 1st International Poliomyelitis Congress, Philadelphia, J. B. Lippincott Co., 1949, 165.
17. Bodian, D., *Bull. Johns Hopkins Hosp.*, 1948, **83**, 1.
18. Bodian, D., Poliomyelitis, Papers and Discussions Presented at the 1st International Poliomyelitis Congress, Philadelphia, J. B. Lippincott Co., 1949, 62.
19. Billig, H. E., van Harreveld, A., and Wiersma, C. A. G., *J. Neuropath. and Exp. Neurol.*, 1946, **5**, 1.
20. Clark, W. E. L., *J. Anat.*, 1945, **79**, 15.

EXPLANATION OF PLATES

All figures are unretouched photomicrographs of gold-impregnated, teased preparations of normal (Figs. 1 and 2) and partially denervated (Figs. 3 to 8) muscles.
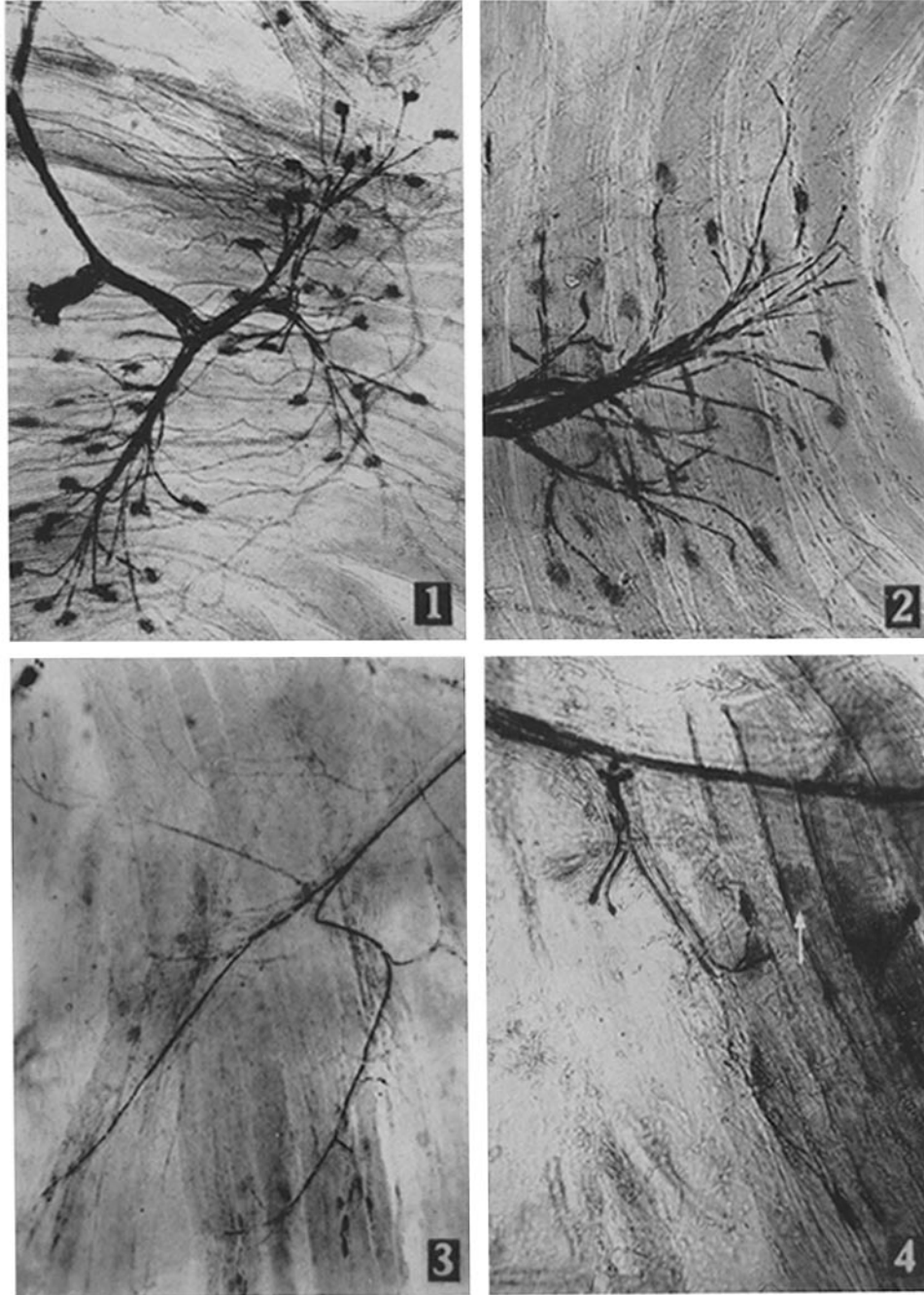
PLATE 6

FIG. 1. A small intramuscular bundle of nerve fibers in a normal musculus extensor digitorum longus. Three terminal bundles and their associated motor end-plates can be distinguished. × 75.

FIG. 2. Three terminal bundles of axons and their endings in a normal gastrocnemius muscle. Case M 25. × 125.

FIG. 3. Severely denervated area of the musculus soleus in case M 69, 160 days after operation. The nerve to this muscle had only 2 per cent of its normal complement of medullated axons. A single axon enters the field at top right, and branches naturally at the first node of Ranvier. The branch coursing diagonally to the left emits one collateral and terminates in the lower left corner. The other branch produces 3 collaterals which divide to innervate 6 end-plates. × 70.

FIG. 4. An almost completely deneurotized terminal bundle runs diagonally downward from left to right. It contains numerous empty Büngner cords and 2 intact fibers. One fiber (right) innervates one end-plate, the other, 2. No collateral regeneration; note denervated end-plate at arrow. Musculus soleus (about 98 per cent denervated), case M 70, 78 days after operation. × 130.
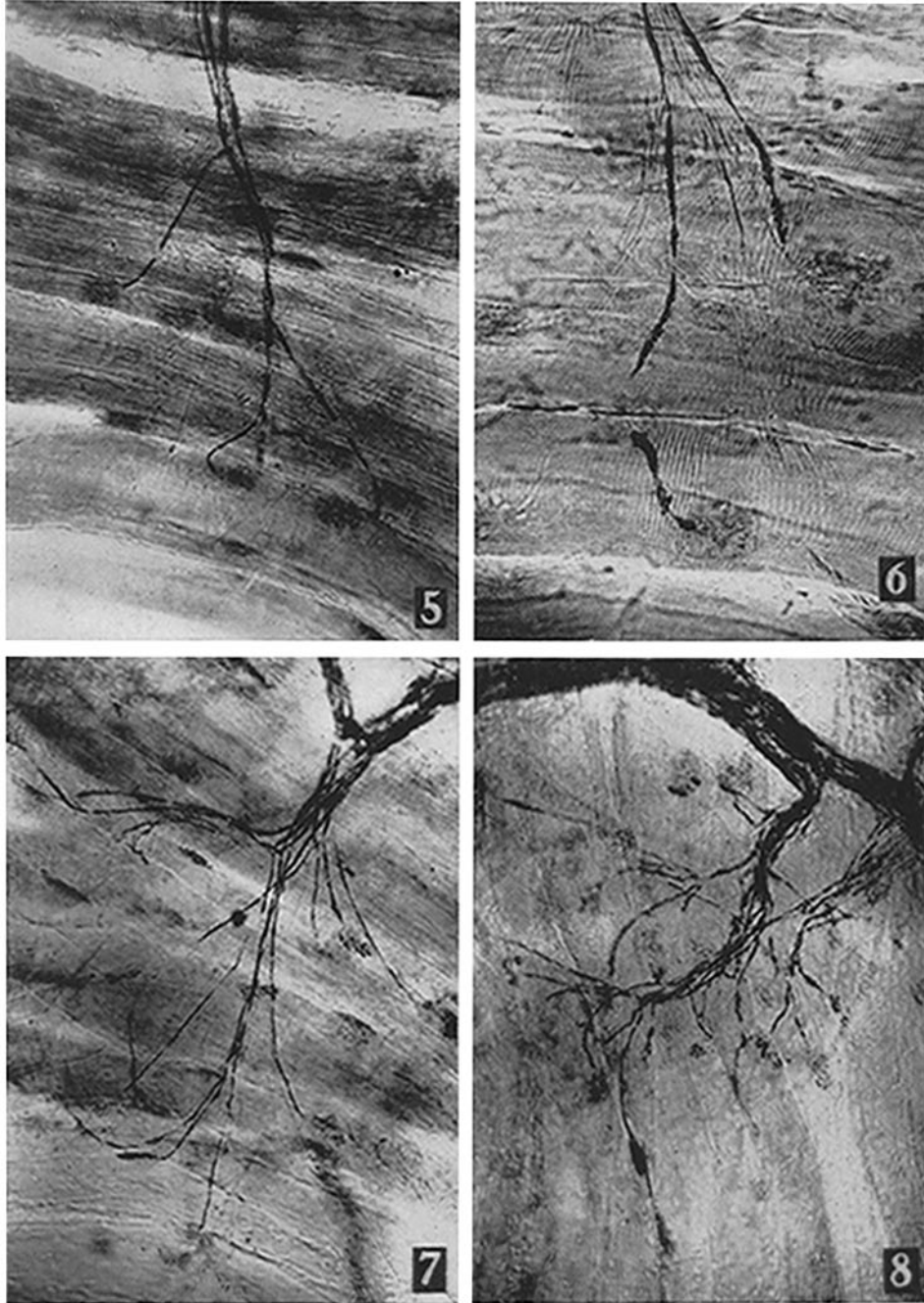
(Edds and Small: Partially denervated muscles)

PLATE 7

FIG. 5. A partially deneurotized terminal bundle containing 4 residual fibers, enters the field at top. One fiber branches; the bundle innervates 5 end-plates. No new branches have formed to supply the denervated end-plates which are scattered through the field. Musculus biceps femoris (about 65 per cent denervated), case M 71, 117 days after operation. × 130.

FIG. 6. Two original terminal fibers running in a bundle of Büngner cords. Each fiber enters a single end-plate and one (left) has an ultraterminal branch to a second end-plate. Note close intermingling of intact and denervated elements. Same muscle as shown in Fig. 5. × 290.

FIG. 7. Seven intact fibers enter the terminal bundle at top right and, with their collateral branches, innervate 13 end-plates. The increased terminal innervation ratio and the small caliber of some of the collaterals indicate that collateral regeneration has occurred. Several still deneurotized strands of Schwann cells are visible. Musculus gastrocnemius (about 10 per cent denervated), case M 25, 200 days after operation. × 125.

FIG. 8. Another area from same muscle as shown in Fig. 7, containing 2 terminal bundles and their associated end-plates. The terminal innervation ratio is 2:1 (32 end-plates:16 fibers). All fibers are of normal dimensions (cf. Fig. 2), indicating that the new branches arose earlier than those in the previous figure. × 125.

(Edds and Small: Partially denervated muscles)