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Impairment of Growth and Myelinization in Regenerating Nerve Fibers Subject to Constriction.*

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A nerve scar is known to impede the passage of regenerating nerve fibers. However, whether or not fibers that succeed in getting through a constriction remain handicapped in their further development has never been determined. The experiments to be described in the following prove that they do.

Material and Method. In 11 white rats (120-230 g) the sciatic nerve was crushed *cca.* 5 mm above its bifurcation. The tibial and peroneal nerves were cut at the knee, and a segment of artery, small enough to produce local nerve constriction, was slipped over one of the proximal stumps, as described previously.[1,2] Fig. 1 illustrates the operation (C, crush; S, constriction sleeve). Nerve regeneration is thus started simultaneously in both nerves at level C. Fibers of the constricted nerve must grow through the bottleneck, while fibers growing through the unconstricted stump serve as controls. After regeneration periods of from 4 to 15 weeks, the nerves were fixed and stained in osmic acid or in Protargol (Bodian) and Mallory Azan. Fiber diameters were measured and compared at levels both proximal (P) and distal (D) to the constriction sleeve.

Results. In 3 cases the sleeves had failed to constrict. No difference between test and control nerves was noticed; hence, mere presence of a sleeve does not affect regeneration. In the remaining 8 cases, constrictions of varying degrees had persisted, associated as usual with edema and with damming of axoplasm.[2,3] Numerically, fibers have regenerated as extensively through the constricted as through the unconstricted nerves. They contrasted sharply, however, with regard to caliber and myelinization. The fibers that had passed through the constriction were markedly thinner and delayed in myelinization as compared with the regenerated fibers of the control nerve at the same level.

Photographs of representative samples from cross-section D of nerve pairs regenerated with (A) and without (B) constriction, taken at identical magnifications 8 weeks (Fig. 2) and 10 weeks (Fig. 3) after the operation, illustrate the differences. A lower power view (Fig. 4) of both the test (tibial; bottom) and control (peroneal; top) nerves shows the contrast in myelinization (osmic acid stain).

In one case (R 188; 6 weeks regeneration time), all regenerated fibers of the constricted tibial and the unconstricted peroneal (total: 5997 fibers) were measured. Their size distribution is given in Table I. While 50%

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1 Weiss, P., *Arch. Surg.*, 1943, **46**, 525.

2 Weiss, P., and Davis, H., *J. Neurophysiol.*, 1943, **6**, 269.

3 Weiss, P., *Anat. Rec.*, 1943, **86**, 491.

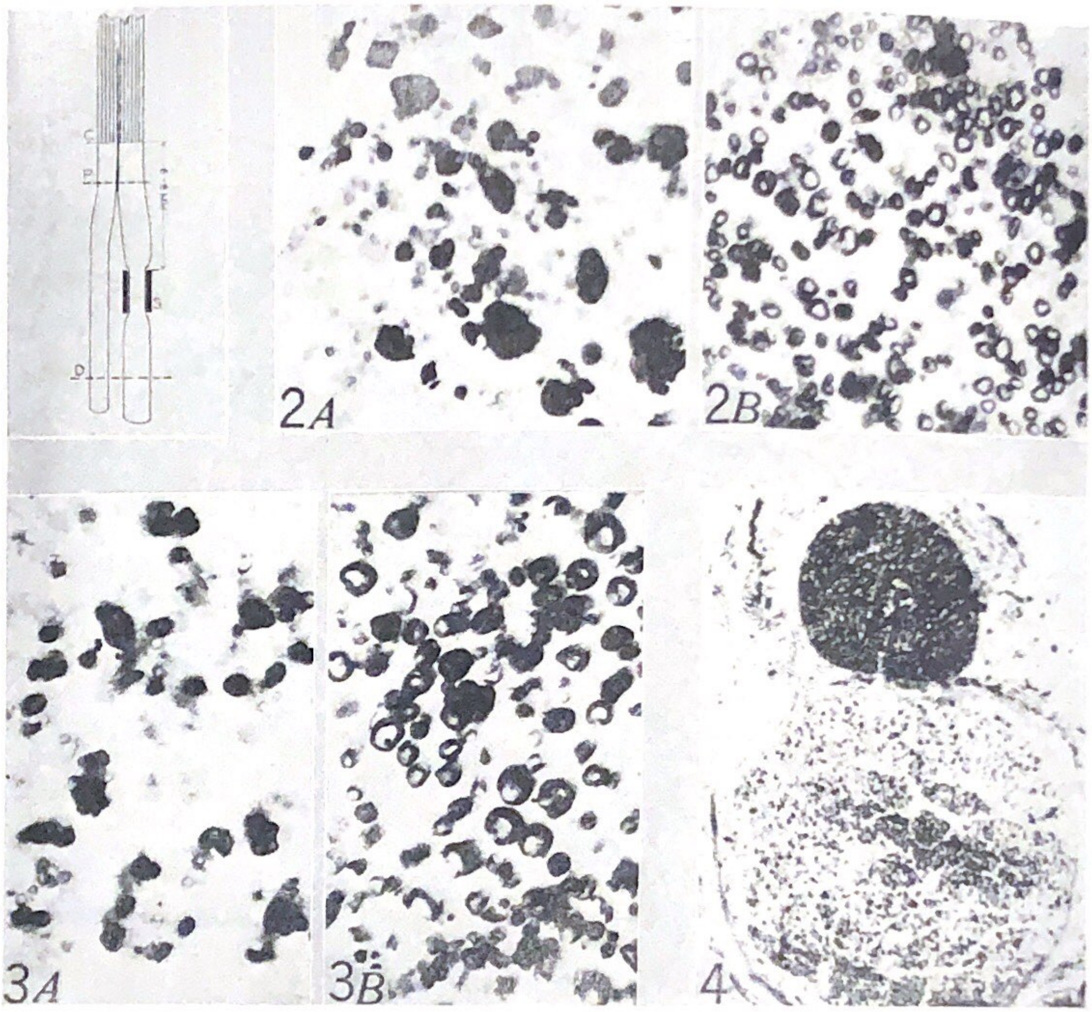

FIG. 1. (Top left) Diagram of operation.

FIG. 2. Cross-sections at level D through the regenerated branches of a sciatic nerve crushed at C, the tibial branch (A) having regenerated through a constriction S, the unconstricted peroneal (B) serving as control. Case R 191; regeneration time 8 weeks. Osmic acid. 450 ×.

FIG. 3. Same as Fig. 2 of a case (R 138) of 10 weeks' regeneration time. Osmic acid. 450 ×.

FIG. 4. Total view of the nerves of Fig. 3 (R 138) at level D, showing the difference in myelinization between the constricted (bottom) and unconstricted (top) nerves. Osmic acid. × 48.

TABLE I.

Size Distribution of the Total Population of Regenerated Fibers in the Constricted and Control Nerves of R188 (6 weeks p.op.), Measured at Level D (Fig. 1) Distal to Constriction.

Nerve	Total No. of fibers	% distribution by equidistant size classes*					%	
		I	II	III	IV	V	$<3\ \mu$	$>3\ \mu$
Control (Peron.)	2350	2.0	47.8	37.9	10.2	2.1	49.8	50.2
Constr. (Tibial)	3647	10.2	80.3	9.5			90.5	9.5

*Class I contains fibers below 1.5 μ, class V those over 6.7 μ, with classes II-IV intermediate at equal intervals.

TABLE II.
Size Distribution and Average Diameter of the 150 Largest Regenerated Fibers in Each Nerve Proximal and Distal to Level of Constriction in R188 (6 weeks p.op.) and R138 (10 weeks p.op.).

Case	Nerve	Level (Fig 1)	Size classes* 4	5	6	7	8	9	10	11	12	13	14	15	16	Diameter Range μ	Diameter Avg μ
R188 (6 wk)	Control (Peron.)	P				69	58	23								5.2- 7.8	6.7
		D			14	88	38	10								4.3- 7.8	6.3
	Constr. (Tib.)	P						66	58	22	4					6.5-10.4	8.5
		D	50	85	15											2.6- 5.2	4.1
R138 (10 wk)	Control (Peron.)	P						86	48	12	4					7.0-10.4	8.3
		D					57	46	37	10						6.1- 9.6	7.8
	Constr. (Tib.)	P									25	67	43	12	3	9.6-13.9	11.6
		D		113	37											3.5- 5.2	4.5

* Class 4 ranges from 2.6 to 3.5 μ, subsequent classes following in steps of 0.87 μ.

of the control fibers had a diameter of more than 3 μ, fewer than 10% of the constricted fibers had attained that size. In this and another case (R 138; 10 weeks) the 150 largest fibers of both the constricted (tibial) and the control (peroneal) nerve were measured and are recorded in Table II.

The table reveals: (1) Proximal to the constriction (level P), the regenerated tibial contains larger fibers than the regenerated peroneal. (2) Distal to the constriction (level D), the tibial fibers are greatly reduced in size, both relative to the peroneal fibers of the same level, and even more so, relative to their own proximal parts. (3) A consistent shift in the fiber spectrum of the control nerves between levels P and D proves that regenerated fibers taper proximo-distally. (4) Fibers in the control nerve and the unconstricted portion of the test nerve are larger in R 138 than in R 188, reflecting the additional 4 weeks regeneration period of the former. Significantly, the test fibers beyond the constriction do not show a corresponding gain. This indicates that constricted fibers are permanently retarded, a view supported by 2 cases studied after 15 weeks, in which the differential between the 2 nerves was found undiminished. Terminal results after still longer regeneration periods will be reported on a later occasion.

Discussion. Discussions of nerve regeneration have in the past centered on the outgrowth phase of the regenerating axon. The further elaboration of the fiber, fittingly referred to as "maturation,"[4] has received less attention. Our present experiments bear on this latter phase. They prove that a localized constriction deprives that part of a regenerating fiber lying beyond it of some factor essential for its further growth in width and myelinization. Evidently, a growing fiber requires continuous contributions from its central cell body, the throttling of which entails a corresponding reduction of growth and myelinization. The damming of axoplasm in front of constrictions, to be detailed in a forthcoming article, suggests a translatory movement of axoplasm as the mechanism involved. This process differs from the substance convection within axons advocated by Gerard[5] and Parker,[6] without excluding it. It would explain the "vis a tergo" of Held and the "formative turgor" of Cajal.

According to our results, the size of any cross-section of a fiber will have a bearing on the size of the same fiber at farther distal levels. Consequently, a small nerve fiber regenerating into a large stump cannot be expected to fill the latter to full size.[7] The condensation of scar tissue in and around suture lines will act much as did our experimental constrictions. Whether the rate of

4 Young, J. Z., *Physiol. Rev.*, 1942, **22**, 318.

5 Gerard, R. W., *Physiol. Rev.*, 1932, **12**, 469.

6 Parker, G. H., and Paine, V. L., *Am. J. Anat.*, 1934, **54**, 1.

7 Nageotte, J., and Guyon, L., *C. R. Soc. Biol.*, 1918, **81**, 571.

lengthwise extension of growing fibers is also affected by constriction, remains to be determined; if it is, the puzzling fact that the rate of advance of nerve fibers inside the distal stump is faster after crushing (precluding scars) than after suture (with some scar formation),[8] would find a ready explanation.

In practical regards, the demonstration of the additional hazard of scar contraction only underlines the value of measures designed to eliminate scar formation. In saying this, we take the desirability of complete caliber recovery and myelinization for granted, although their relevance for functional recovery has never been accurately determined.

Summary. Nerve fibers regenerating in a nerve with localized constriction are greatly reduced in caliber and delayed in myelinization at all levels distal to the constriction. Scar tissue, therefore, presents not only a local impediment to the outgrowth of regenerating fibers, but a continued hazard to their maturation.

8 Gutmann, E. J., *Neurol. and Psych.*, 1942, **5**, 81.

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[8] Gutmann, E. J., *Neurol. and Psych.*, 1942, **5**, 81.