Effect of Nerve Compression on Wallerian Degeneration *in Vitro*.

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Effect of Nerve Compression on Wallerian Degeneration *in Vitro*.*

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Wallerian degeneration is an essential preliminary to successful nerve regeneration in that it prepares the distal stump for reinvasion by regenerating fibers. Physiological insight into its mechanism, however, lags greatly behind our descriptive acquaintance with its morphological features. The following experiments represent part of a broader study of the factors determining the character and rate of Wallerian degeneration.

It was known that when a nerve locally constricted by a ligature,[1] clamp,[2,3] or arterial cuff,[4] undergoes Wallerian degeneration, those parts of the axis cylinders lying within the compressed zone may fail to break down. This effect could be ascribed (1) to injury of the fibers, or (2) to paralysis of the mechanism of degeneration either (a) by the local ischæmia, or (b) by direct pressure action on the nerve fiber. Alternative a and b could be decided by reproducing the compression effect *in vitro*, *i.e.*, with the whole nerve removed from blood supply; alternative 1 and 2 by the demonstration that fibers arrested in their degeneration by compression may degenerate after decompression.

The capacity of nerve fibers to undergo typical Wallerian degeneration in a properly composed medium *in vitro* has been known.[5,6] Our own observations confirm, with certain qualifications, the earlier reports: Degeneration occurs promptly in Ringer's, Tyrode's solution, blood serum, but less regularly or not at all in NaCl solution or blood plasma. Our present experiments were carried out with fragments (*cca.* 1 cm) of rat nerves (sciatic, brachial, intercostal; 100-200 g donors), explanted into Ringer's solution in Petri dishes at 37°C, with or without compression, fixed after varying intervals, sectioned longitudinally, and silver-impregnated according to Bodian.[7] Criteria of degenera-

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1 Cajal, S. Ramôn y, *Degeneration and Regeneration of the Nervous System*, Oxford University Press, London, 1928, Vol. I, p. 292.

2 Stroebe, H., *Beitr. z. path. Anat.*, 1893, **13**, 160.

3 Denny-Brown, D., and Brenner, C., *Arch. Neurol. and Psych.*, 1944, in press.

4 Weiss, Paul, and Davis, Hallowell, *J. Neurophysiol.*, 1943, **6**, 269.

5 Ingebrigtsen, Ragnvald, *J. Exp. Med.*, 1916, **23**, 251.

6 Nageotte, Jean, *L'Organisation de la Matière*, Paris, Félix Alcan, 1922, p. 274.

7 Bodian, David, *Anat. Rec.*, 1937, **69**, 153.

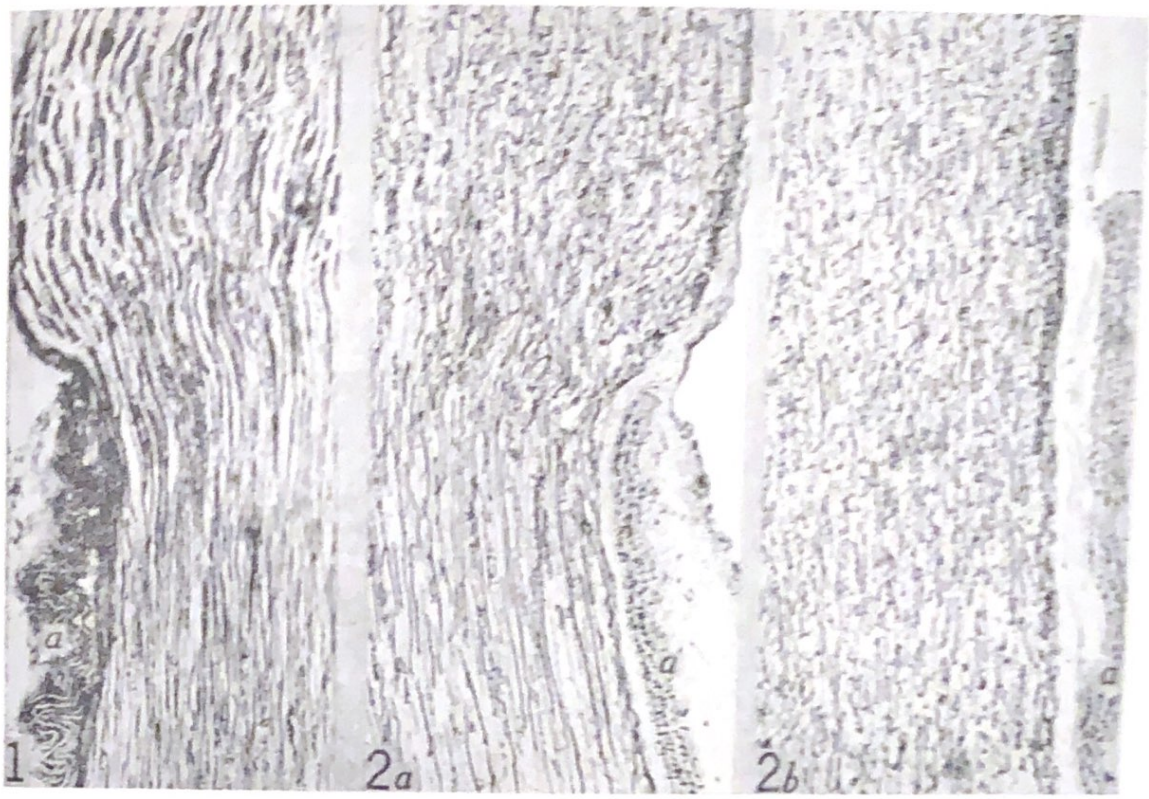

FIG. 1.

Tibial nerve with constricting arterial cuff (*a*) after 12 hours *in vitro*. Most fibers inside sleeve still intact, fibers outside vacuolated and in fragmentation. × 114.

FIG. 2.

Tibial nerve with arterial cuffs (*a*) after 24 hours *in vitro*. × 138.

(a) Constricting cuff. Many intact fibers inside of cuff; practically all fibers outside of cuff degenerated. Note the sharpness of demarcation line.

(b) Non-constricting cuff. Practically all fibers uniformly degenerated both inside and outside of cuff.

tion were the changes of the axis cylinder, progressing as follows: swelling → vacuolization → fragmentation → ovoids.

In a preliminary series, nerves were placed under 25 g glass slides and compared with unweighted nerves in the same dish. Nine nerves thus compressed showed definite delay of degeneration as compared with their controls after 18, 26, and 48 hours. That the delay was due to pressure and no other factors (oxygen lack, accumulation of metabolites, etc.), is evidenced by 3 nerves kept between slides propped on silk threads of corresponding size, which degenerated without delay. In 5 of 8 nerves compressed to only half their lengths, degeneration in the compressed end lagged behind that of the free end.

More uniform and effective application of pressure was achieved by the use of constricting sleeves of live artery (carotid or femoral) slipped over the nerve fragments as described previously.[8,3] By a suitable choice of size combinations of nerves and arteries, various degrees of constriction were obtained. Nerve parts left bare or provided with sleeves of matching size served as controls. In some cases, the sleeves were removed after varying periods of explantation as a test of the results of decompression. Samples for histological study were taken from the same preparation at different stages of its *in vitro* life.

In all cases in which constriction had been adequate, Wallerian degeneration was markedly retarded in the compressed as compared with the uncompressed portions of the same nerve sample. The effect was clearly one of

[8] Weiss, Paul, *Arch. Surg.*, 1943, **46**, 525.

TABLE I.
Reduction of Degeneration Index by Arterial Constriction of Explanted Nerves.

No.	Nerve*	Artery†	Period of explantation, hrs	Compressed Zone: Total of fibers counted	Compressed Zone: Degeneration index (i_c) %	Uncompressed Zone: Total of fibers counted	Uncompressed Zone: Degeneration index (i_u) %	Compression effect $\Delta = i_u - i_c$ %	σ_Δ %	P	Significance
1462 A	Tib	car	24	299	32	277	46	+14	4.1	<.01	+
B	,,	,,	24	328	38	300	54	+16	4.0	<.01	+
C	,,	,,	48	318	27	253	45	+18	4.0	<.01	+
1463 A	Per	fem	24	103	48	93	43	− 5	7.1	.48	—
C	,,	,,	48	106	31	86	34	+ 3	6.8	>.50	—
1464 A	Tib	car	24	324	30	260	72	+42	4.2	<.01	+
B	,,	,,	24	293	34	306	49	+15	4.0	<.01	+
C	,,	aorta	48	305	39	364	53	+14	3.9	<.01	+
D	,,	car	48	308	26	254	50	+24	4.1	<.01	+
1465 A	Per	,,	24	164	47	148	70	+23	5.6	<.01	+
B	,,	fem	24	122	26	135	34	+ 8	5.7	.16	—
D	,,	,,	48	89	28	127	29	+ 1	6.3	>.50	—
1469 A	Br	,,	24	230	74	240	99	+25	3.2	<.01	+
B	,,	,,	24	189	87	179	96	+ 9	3.5	.01	±
C	,,	,,	48	244	77	297	97	+20	3.0	<.01	+
D	,,	,,	48	161	92	118	97	+ 5	3.5	.16	—
1470 A‡	Br	,,	24	396	52	354	92	+40	3.3	<.01	+
B	,,	,,	24	226	80	195	92	+12	3.4	<.01	+
C	,,	,,	24	187	89	159	92	+ 3	3.1	.32	—

* Tib = tibial; Per = peroneal; Br = brachial.
† car = carotid; fem = femoral; all arteries from 95 g donors except 1470 (150 g donor).
‡ Reproduced in Fig. 2a.

delay rather than of arrest, since the difference tended to disappear in specimens kept long enough for degeneration to run its course in both experimental and control stretches (*cca.* 48 hours). Fig. 1 shows the differential after 12 hours, Fig. 2a after 24 hours, while Fig. 2b proves the ineffectiveness of non-constricting sleeves.

As the fiber population of a nerve does not degenerate in strict synchronism, the progress of degeneration can be measured by the ratio of degenerated fibers (axon already fragmented) to preserved fibers (axon still continuous). The percentage of degenerated fibers may be referred to as the "degeneration index." In an average of 5 random sample sections of each of 19 nerves, the "degeneration index" was determined for the compressed and the uncompressed portions. Table I gives the results with statistical analysis. In 13 cases the difference between the compressed and uncompressed parts is of high statistical significance (9-42%; $P < 0.01$). Of the 6 statistically not significant cases, 4 are peroneal nerves which, owing to their smaller size, have suffered only minor constriction or none, and one is a 48-hour case too far advanced to show a differential.

Decompression experiments have demonstrated that the released fibers continue to degenerate, many apparently at a reduced rate, and evidence of their lag may still be present 24 hours later. Experiments in which explanted nerves were subjected to longitudinal stretch produced no obvious effects on degeneration.

Our results lead to the following conclusions. Pressure impairs, but does not destroy, the capacity for Wallerian degeneration. The impairment is not due to vascular interference since it can be reproduced *in vitro* in the absence of all circulation. Of the constricting and sheathing effects of an arterial sleeve, only the former affects degeneration, since a nerve segment insulated from the medium by a non-constricting artery degenerates without delay. Variations in the state of degeneration along a peripheral nerve course may be but the result of pressure fluctuations. Pressure could perhaps also account for the failure of

nerves to degenerate in plasma-clots[8] in contrast to liquid serum, as such clots develop strong contractile forces in their interior.[9]

Summary. Lateral compression of peripheral nerves delays Wallerian degeneration of the compressed zone *in vitro*.

[9] Weiss, Paul, *J. Exp. Zool.*, 1934, **68**, 393.