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PROPER MICROSURGICAL NERVE SUTURE MAY IMPEDE WALLERIAN DEGENERATION OF COMPLETELY TRANSECTED NERVES

AN ELECTRON MICROSCOPIC STUDY

EROS ABRANTES ERHART *
CIRO FERREIRA DA SILVA **
CLAUDIO FAVA CHAGAS ***

In previous papers we have stated that distal segments of mammalian and chick nerves, completely separated from their proximal stump for more than six months, are repopulated by newly-formed isolated nerve fibres which must have arisen from a source other than the proximal stump, neighbour nerves or nervi vasorum (Erhart and coll. [1-10]). Moreover, as practial application of our researches, we have stated that proper nerve suture and/or neurolysis should be highly recommended, even after delay of many years, for the motor and sensory rehabilitation of patients with long-term traumatic nerve injuries. More than 400 human patients have been operated on by different experienced surgeons under this orientation, and in no case did complete clinical failure occur when the necessary precautions were taken (Erthart [10]). Furthermore, fascicular nerve sutures performed with microsurgical technique improve reinnervation of the affected segments and, when adequately done short-time after the transection, may impede the complete Wallerian degeneration that normally occurs in the nerve segment distal to the transection (Erhart [10]).

In order to confirm once more the statement — impede the Wallerian degeneration — we have again completely transected the nerve of the biventer cervicis muscle of the chick, repeating experiments we had conducted [7] in 1968, this time for electron microscopic purposes.

Departamento de Anatomia, Instituto de Ciências Biomédicas da Universidade de São Paulo: * Professor Adjunto Aposentado; ** Professor Assistente Doutor; *** Auxiliar de Ensino. *Acknowledgements*: Equipments used in this research have been donated by FAPESP (grants nº 69/913, 70/1445 and 78/1375) and CNPq (grants nº 2222.0723/78 and 2222.1694/78).

MATERIAL AND METHODS

The material examined comprised the nerve of the biventer cervicis muscle of 26 chicks. Data for the descriptions of what was observed in the distal stump were obtained from 18 operated left or right biventer cervicis muscle nerves. This nerve, a dorsal ramus of C_1, penetrates into the muscle through its deep surface, at the upper third of the cranial belly. Then it runs distally giving off collaterals for the motor units of this belly, traverses the intermediate tendon and reaches the caudal belly (Fig. 1). The nerve trunk is completely included within the intermediate tendon, accompanied by an artery and a vein. This single nerve trunk bifurcates at the upper third of the caudal belly and later on trifurcates; from these main trunks, collaterals spread off for the motor units of this belly (Fig. 1).

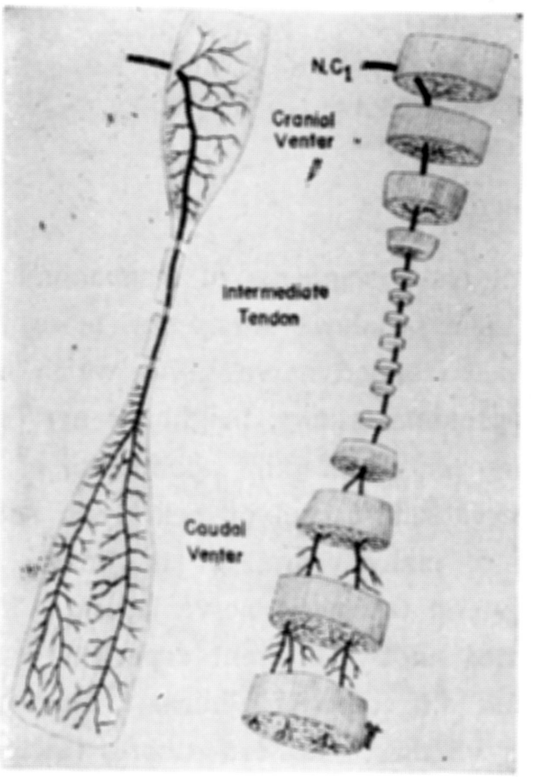

Fig. 1 — Diagramatic reconstruction of the chick biventer cervicis muscle innervation pattern.

The chicks, ten to fifteen days post-hatching, were anesthetized by ether inhalation. The biventer cervicis muscle with its intermediate tendon was surgically exposed; immediately after the tendon and nerve complete transection, an adequate microsurgical tendon and nerve suture was performed, with 10 zero nylon monofilament and atraumatic needle, in order to have an end-to-end fascicle coaptation, with no tension, as recommended by Millesi [13]. Nerves were re-exposed at intervals of 7.1/2, 8, 9.1/2, 11, 17, 23, 25 and 30 hours, and 2 months post-operatively. Cold (4ºC) 900 mOsm Karmowsky fixative (2% glutaraldehyde, 2% paraformaldehyde, 0.1 M cacodylate buffer at pH 7.2 and 0,25 mg% $CaCl_2$) was dripped over bellies and tendon, just before and during the excision of the proximal and distal nerve segments, as well as on the nerve suture level. Next, the three excised segments of each nerve were left in the fixative for two hours at 4ºC. They were then post-fixed in 1% osmium tetroxide in 0.1 M cacodylate buffer (pH 7,2) for one hour. After being rinsed

in a 0.9% NaCl solution they were transferred to 0.5% uranyl acetate for 12 hours at 4°C. Dehydrated through a graded series of ethanol and propylene oxide, they were embedded in araldite in shallow troughs. The blocks were trimmed and 1μm sections were cut and stained with methylene blue — Azur II (1:1), for orientation purposes. The blocks were cut on an LBX Ultratome, the sections picked up on 200 mesh grids, stained with uranyl acetate and lead citrate, and examined in a Philips E.M. 300 electron microscope. Much of the examination and micrography was conducted at relatively low magnification with the specific intention of recording topographical relationships of the nerve as a whole. The same procedure was used for transected but not sutured nerves and testimony normal nerves. From these we have studied just the distal segments. Furthermore, nerve material and caudal muscle bellies from the 2 months post-operatively chicks were fixed for Cajal — De Castro silver impregnation. Serial histological preparations, longitudinally and transversally sectioned 8μm, were mounted.

Fig 2 — Myelinated axons and clusters of non-myelinated axons from chick biventer cervicis muscle nerves, which were transected and immediately afterwards sutured through adequate microsurgery 7 1/2 hours post-operatively (3,300 X).

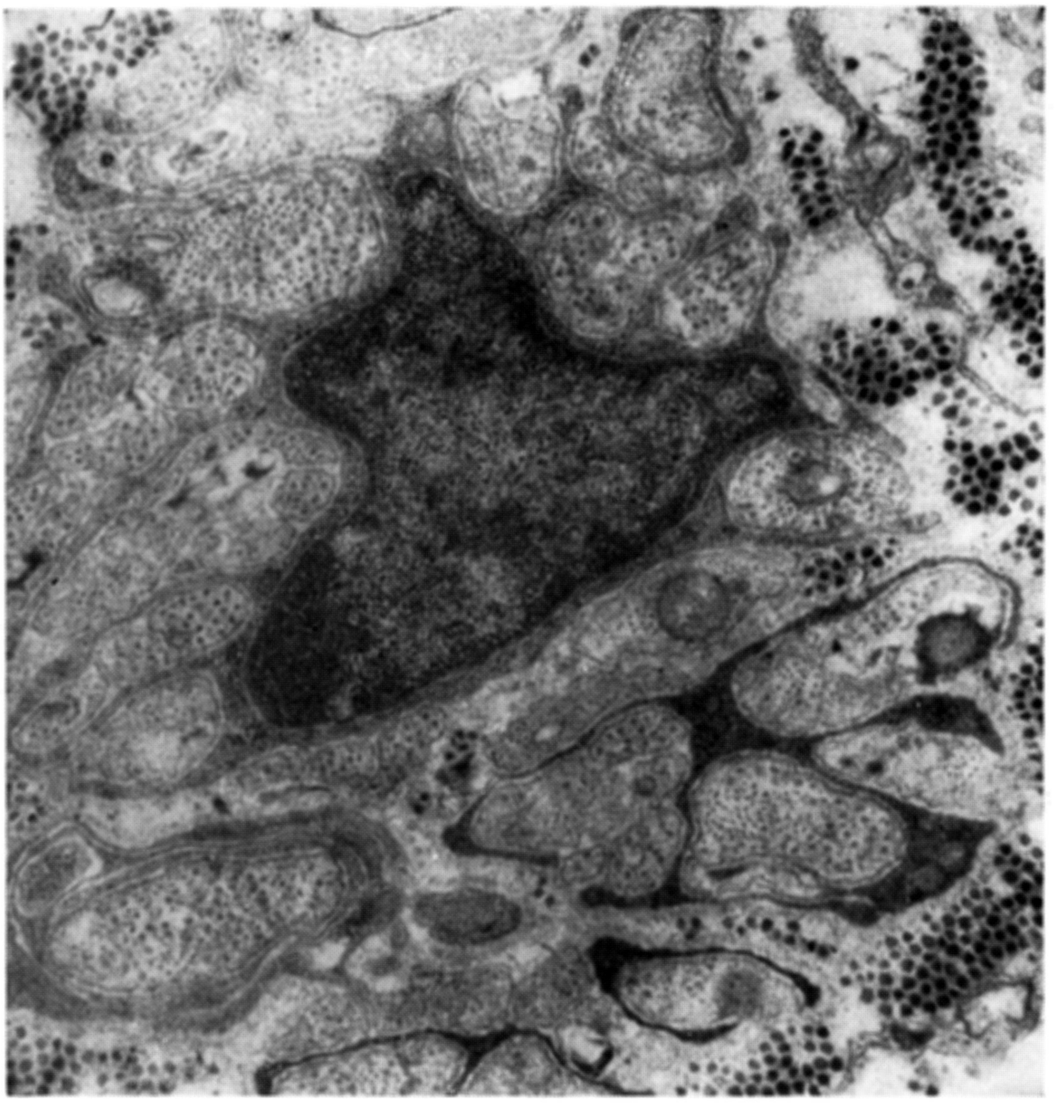

Fig. 3 — Same as figure 2. 7 1/2 hours post-operatively (15,000 X).

Fig. 4 — Same as figure 2. 9 1/2 hours post-operatively (4,200 X).

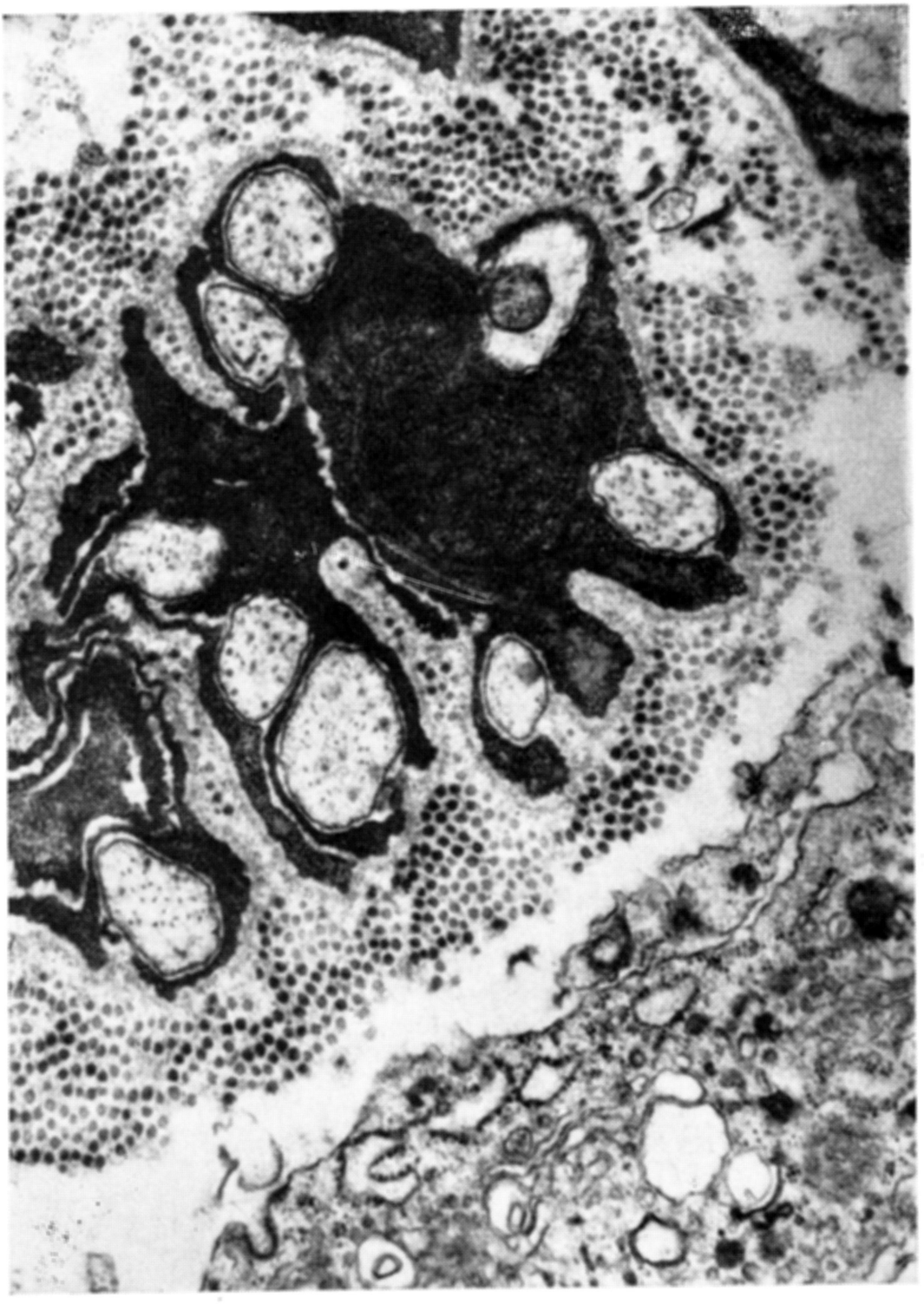

Fig. 5 — Same as figure 2. 30 hours post-operatively (3,300 X).

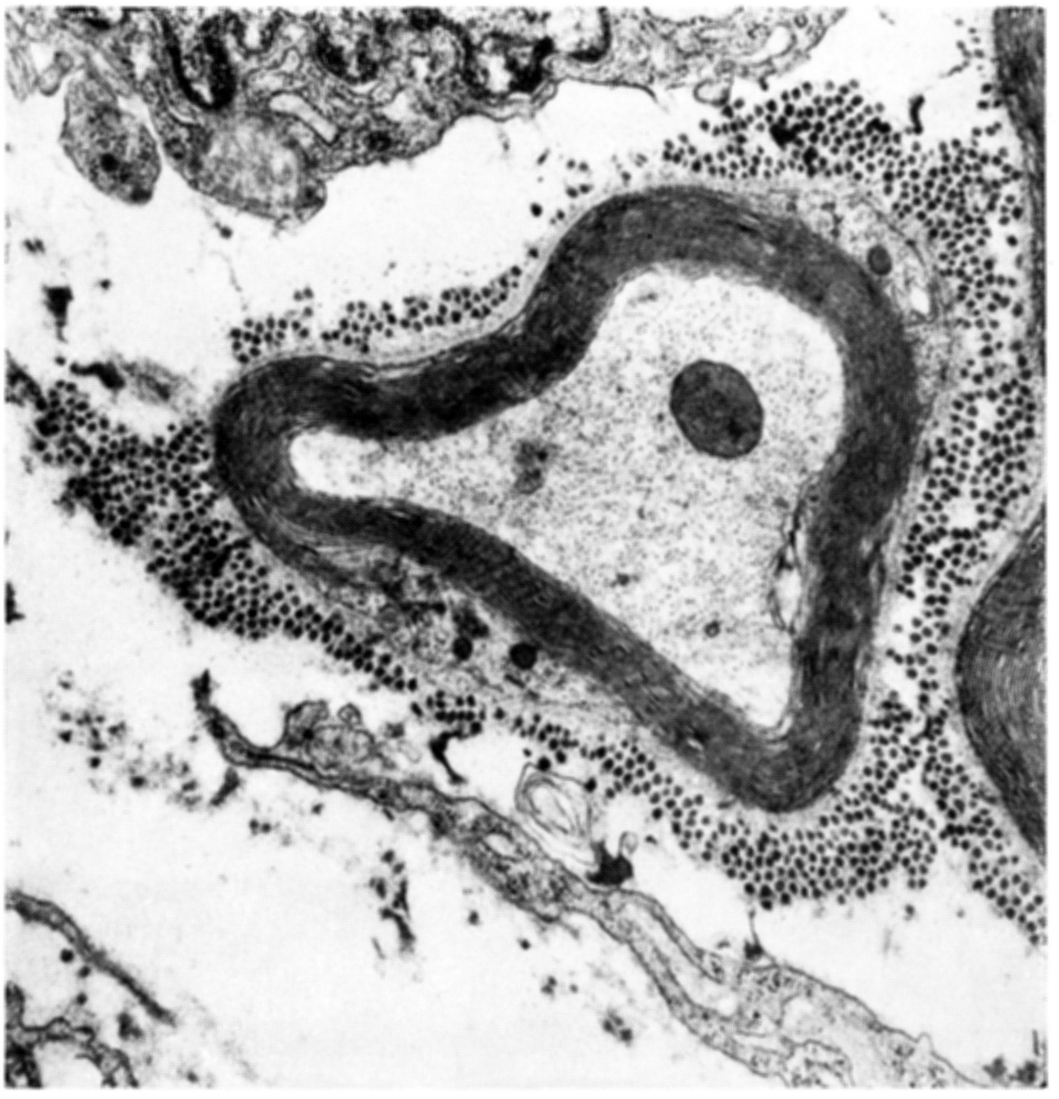

Fig. 6 — Same as figure 2. 30 hours post-operatively (4,200 X).

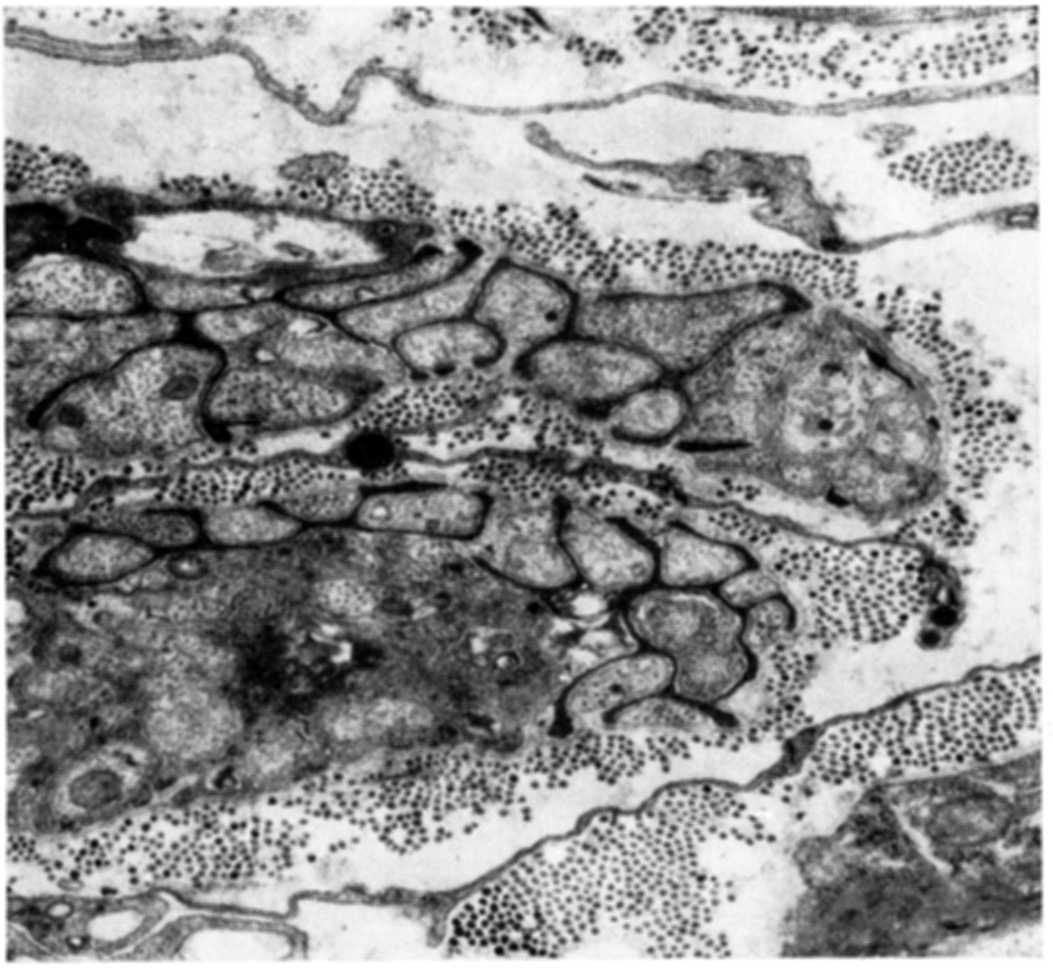

Fig. 7 — Same as figure 2. Two months post-operatively (3,300 X).

Fig. 8 — Same as figure 2. Two months post-operatively (4,200 X).

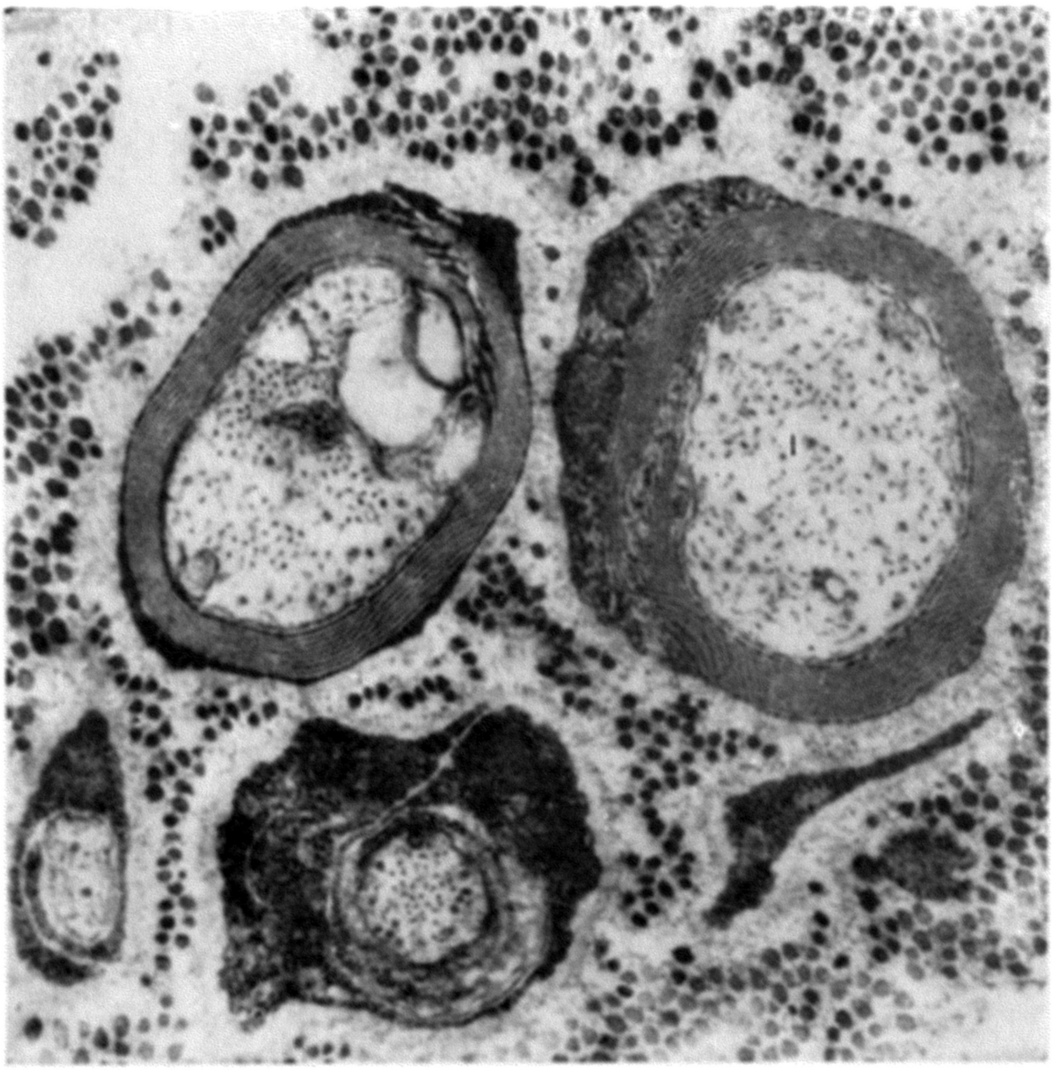

Fig. 9 — Same as figure 2. Two months post-operatively (7,200 X).

RESULTS

Experimental total transections of the intermediate tendon of the biventer cervicis muscle of the chick, with its nerve included, determined, as expected, complete denervation of the caudal belly and, 11 hours, post-operatively, Wallerian degeneration of the nerve distal segment could be observed.

When immediately after the nerve transection an adequate microsurgical fascicular nerve suture was performed, the following could be demonstrated: **A** — From 7.1/2 hours up to 2 months post-operatively, numerous normal-looking myelinated and non-myelinated axons were identified (Figs. 2 to 9). Most of the non-myelinated axons formed clusters associated with a single Schwann cell. **B** — Alterations in the ultrastruture of the «stained» elements of the axoplasm, as described by Morris et al. [15], were not seen in our material. **C** — Furthermore, the detailed analysis of the electronmicrographs showed that myelinated nerve fibres are generally more affected and damaged than non-myelinated ones. The latter showed being practically undisturbed by the nerve transection when an immediate fascicular nerve suture was well performed through microsurgery. **D** — Two months after successful nerve sutures, the caudal bellies of the biventer cervicis muscles of the chicks were normal-looking and functioning. No signs of a denervated muscle were observed and, in the histological serial silver preparations, numerous normal-looking motor endplates were seen. These data re-inforce the findings of Gorio & Carmignoto [11].

DISCUSSION

Electron microscopic findings confirmed our previous statement that proper microsurgical nerve suture may impede the Wallerian degeneration that normally occurs in the distal segment of a completely transected nerve (Erhart et al.[8,9,10]).

On the basis of the available information it is clear that the elements which comprise a nerve have a much greater plasticity than expected (Morris et al.[15]). The nerve must be considered a tissue in a functional sense (Ochs [16]), rather than just a collection of independent axons and their supporting cells. It thus becomes necessary to consider every damage on a nerve in the context of all its elements, including the vasa nervorum (Miyamoto & Tsuge [14]), and not just from the point of view of its effect on the axons. One must also emphasized importance of the axon's environment, i.e., the endoneurial connective tissue in which it is embedded.

From the present results and former data it becomes evident that two points must be embodied in any technique if it is to be effective in enabling a transected nerve to regenerate: firstly, the connective tissue in which the nerve is embedded must be maintained; secondly, the technique must ensure the reconstitution of the endoneurial environment (Morris et al. [15] and Lundborg & Hansson [12]). The fulfillment of these two principles should enable the axons to act biologically under the most favourable environmental conditions. In such cases the axons will be able to express their regenerative ability, with a corresponding improvement in functional recovery. The adoption of these concepts is fundamental for the successful surgical treatment of nerve injuries.

SUMMARY

Electron microscopic findings on the nerve of the biventer cervicis muscle of the chick, which was completely transected and immediately after submitted to an adequate microsurgical nerve suture, confirmed our previous statement that proper microsurgical nerve suture may impede the Wallerian degeneration that normally occurs in the distal segment of a completely transected nerve.

RESUMO

Neurorrafias com técnica microcirúrgica adequada podem evitar a degeneração Walleriana de nervos completamente transeccionados: estudo ao nível de microscopia eletrônica.

Neste trabalho experimental foi utilizado, como o fizemos [7] em 1968, o nervo do músculo biventer cervicis do pintainho (Fig. 1). Os nervos transeccionados ao nível do tendão intermédio do músculo e propositadamente não suturados, ou acidentalmente mal suturados, apresentaram, como esperado, degeneração Walleriana total do segmento distal e conseqüente desnervação do ventre distal do músculo. Os nervos, transeccionados e imediatamente suturados com técnica microcirúrgica fascicular adequada, apresentaram, em nível de microscopia eletrônica, de 7 1/2 hs. a 2 meses pós-operatório, numerosas fibras nervosas mielínicas e amielínicas íntegras (Figs. 2 a 9), além, naturalmente, de fibras nervosas em diferentes e progressivas fases de degeneração Walleriana. Os ventres distais dos músculos cujos nervos foram operados com sucesso apresentavam coloração e aspecto normais, respondiam a estímulos mecânicos e, em cortes histológicos para microscopia óptica, exibiram numerosas placas motoras íntegras. A afirmação que fizemos em trabalhos anteriores — "a degeneração Walleriana total que normalmente ocorre em segmentos distais de nervos completamente transeccionados pode ser parcialmente evitada através de neurorrafias, com técnica microcirúrgica fascicular adequada" — é agora documentada em nível de microscopia eletrônica.

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Departamento de Anatomia — Faculdade de Medicina da Universidade de São Paulo — Caixa Postal 2921 — 01000 São Paulo, SP — Brasil.