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THE ECTOPIC NEWLY-FORMED NERVE FIBRES WHICH REPOPULATE THE LONG-TIME DENERVATED AND ATROPHIC CHICK SKELETAL MUSCLE

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In previous papers we have stated that distal segments of mammalian nerves, completely separated from their proximal stumps 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 & Erhart [3]; Erhart [1]; Erhart & Rezze [4, 5, 6, 7, 8]). Moreover, as a practical application of our researches, we have stated that proper nerve suture and 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 150 human patients have been operated on by different surgeons under this new orientation and in no case did complete clinical failure occur when the necessary precautions were taken (Erhart & Rezze [6]).

While working in collaboration with Zyngier [14] on denervated caudal bellies of chick biventer cervicis muscles as isolated nerve-muscle preparations, for distinguishing neuro-muscular blockers and other depressors of the skeletal muscle, we had the opportunity to study whole muscle innervation.

The biventer cervicis muscle of the chick (Fig. 1) is innervated by the dorsal ramus of C_1, from which a single nerve trunk penetrates into the muscle, through its deep surface, on the upper third of the cranial belly. The nerve trunk runs distally giving off collaterals for the motor units of the belly, traverses the intermediate tendon and reaches the distal belly (Fig. 2). The nerve trunk is completely included within the intermediate tendon (Fig. 4), accompanied by an artery and a vein. In the distal belly, the single nerve trunk bifurcates at the upper third of the

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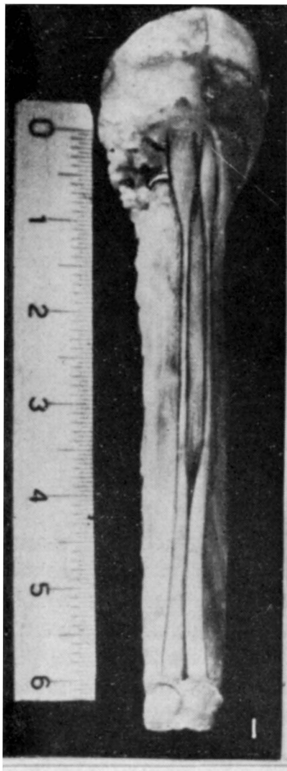

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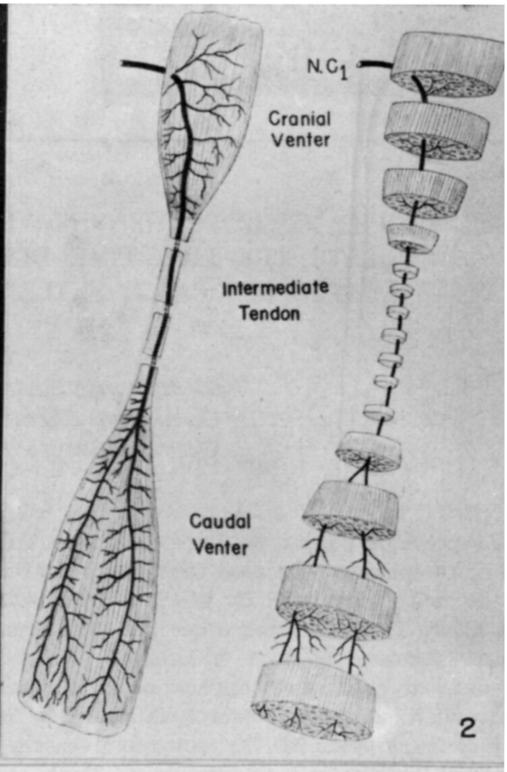
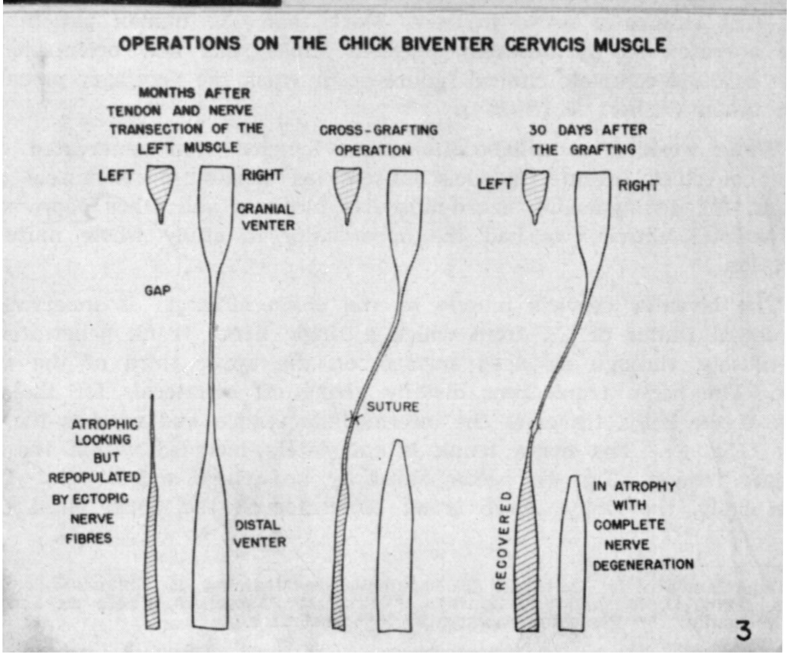
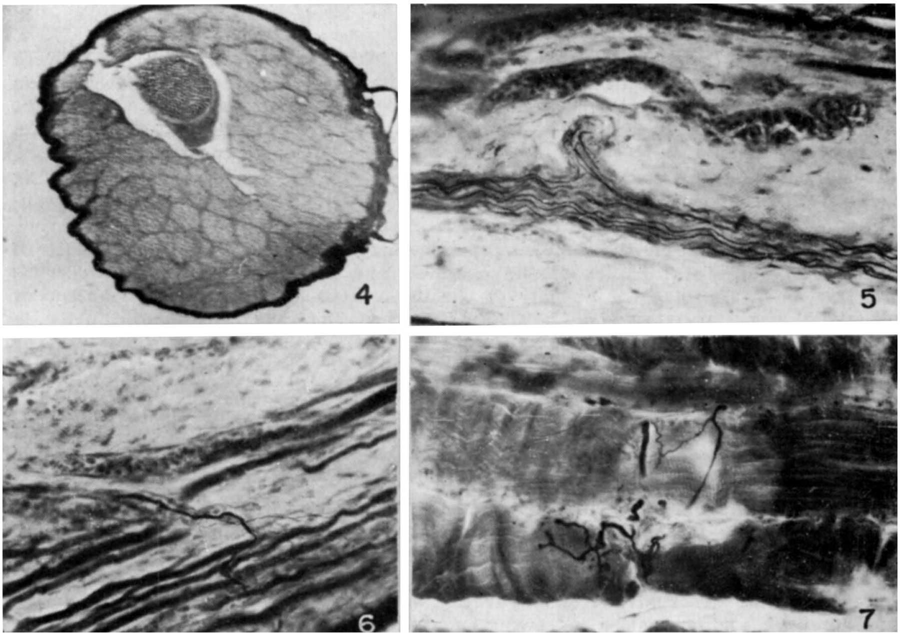

N.C1
Cranial Venter
Intermediate Tendon
Caudal Venter

2


OPERATIONS ON THE CHICK BIVENTER CERVICIS MUSCLE
MONTHS AFTER TENDON AND NERVE TRANSECTION OF THE LEFT MUSCLE
LEFT
RIGHT
CRANIAL VENTER
GAP
ATROPHIC LOOKING BUT REPOPULATED BY ECTOPIC NERVE FIBRES
DISTAL VENTER
CROSS-GRAFTING OPERATION
SUTURE
30 DAYS AFTER THE GRAFTING
LEFT
RIGHT
RECOVERED
IN ATROPHY WITH COMPLETE NERVE DEGENERATION

3



belly and later on trifurcates; from the main trunks collaterals spread off for the motor units of the belly (Fig. 2).

Experimental total transections of the intermediate tendon resulted in complete denervation of the distal belly. Thirty to sixty days post-operatively, histological silver preparations showed that all nerve fibres distal to the transection level were completely degenerated. Nevertheless, three or more months post-operatively, the distal bellies, although completely separated from their cranial bellies, showed an evident repopulation of ectopic nerve fibres (Erhart [2]).

These findings, confirming once more our previous results on transected peripheral nerves, provoked further investigations to clarify some still obscure facts.

Fig. 1 — Anatomical preparation of the biventer cervivis muscle of a 20 days old chick. Dorsal view.

Fig. 2 — Diagrammatic reconstruction of the biventer cervicis muscle innervation.

Fig. 3 — Scheme of the cross-grafting operation.

Figs. 4-7 are photomicrographs from 8 μ sections Cajal-De Castro silver impregnations of biventer cervis muscles of the chick.

Fig. 4 — Normal nerve trunk completely included within the intermediate tendon. (× 100).

Fig. 5 — Fascicle of ectopic newly-formed nerve fibres repopulating a 8 months previously denervated distal belly. (× 480).

Fig. 6 — Ectopic newly-formed nerve fibres repopulating a 9 months previously denervated distal belly. (× 480).

Fig. 7 — Motor endplate from a 8 months previously denervated distal belly, 30 days after the cross-grafting operation. (× 900).

MATERIAL AND METHODS

The biventer cervicis muscles of thirty chicks, four to five days old, were exposed under ether anesthesia, in order to perform a complete scissors transection of the intermediate tendon of the left muscle, at its upper third. The right muscle, not operated, was used as control and for further experiments. The skin wound was sutured.

Reopening of the neck skin of the chicks were performed at times varying between three and twelve months after the tendon and concomitant nerve transection.

At each reoperation the following particulars were noted: *a* — The state of the cranial and distal bellies of the operated and non operated biventer cervicis muscles; *b* — The state of atrophy of the denervated distal belly and whether or not there was fibrillation; *c* — The response of the denervated distal belly to electrical stimulation, 1.5 V, on the remaining tendon stump and direct on the muscle fibres.

Immediately after these records, twenty out of the thirty chicks were sacrificed and both bellies of the transected muscle, as well as some of the contralateral normal right muscles, were carefully dissected out and fixed for Cajal-De Castro silver impregnation. Serial histological preparations, longitudinally and transversally sectioned 8 μ were mounted.

The ten remaining chicks, operated nine to ten months previously, were reoperated. Cross-grafting between the proximal part of the right normal biventer cervicis muscle and the denervated distal belly of the left muscle were performed (Fig. 3) by common suture — nylon six zero. Naturally the scar tissue from the distal denervated stump was excised in order to allow a better end to end approach and an easier ingrowing of nerve fibres.

Three chicks died during or immediately after this operation.

Thirty days after the grafting operation, the biventer cervicis muscles of the seven remaining chicks were exposed and the following particulars were noted: *a* — The state of the suture site and of the cross-grafted muscle bellies; *b* — The state of atrophy of the recently transected distal right belly; *c* — The response to electrical stimulation, 1.5 V, of the cross-grafted muscles bellies on the cranial right normal belly and corresponding intermediate tendon, on the grafted region and on the distal left previously denervated belly.

The animals were then killed and the cross-grafted muscle bellies, as well as the recently transected right distal bellies, were carefully dissected out and fixed for Cajal-De Castro silver impregnations. Serial histological preparations longitudinally and transversally sectioned 8 μ were mounted.

RESULTS

The transected biventer cervicis muscles of the chick when examined three to twelve months post-operatively had the cranial belly retracted, greatly diminished in volume, but with normal appearance. The distal extremity of most of these cranial bellies showed small neuromas which fact was further confirmed by the histological silver preparations.

The distal denervated belly with the remainder of the intermediate tendon was always widely separated from the cranial venter because of (i) the initial retraction subsequent to the transection and (ii) the natural development and growth of the chick during these months. All the distal bellies were pale and had the appearance of atrophic muscles. Fibrillation was noted in some cases, most of which were three to four months post-operatively. Although having an atrophic appearance, most of the distal bellies, especially those that had been denervated for longer time, responded with evident contractions when stimulated with 1.5 V on the remaining tendon distal stump and directly on the denervated muscle venter.

Since these so considered atrophic muscle fibres were responding to indirect and direct stimulations, by contraction, it was assumed, based on previous histological observations on equivalent material, that newly-formed nerve fibres were conducting to muscular structures.

The histological serial silver preparations proved this to be so. Ectopic nerve fibres and nerve fascicles were seen repopulating the remainder of the intermediate tendon and the long-time denervated distal bellies (Figs. 5 and 6) which responded by contraction when electrically stimulated. Motor endplates were also seen in these long-time denervated distal bellies, chiefly in those eight to twelve months post-operatively. Moreover, in many of these cases, a typical "neuroma-like structure" of the nerve distal stump, as described by Erhart & Rezze[5], was seen included within the remaining intermediate tendon, close to its long-time sectioned distal extremity.

The few distal denervated bellies which did not respond to electrical stimulations, although being well silver impregnated, did not show any nerve repopulation. Perhaps they had some kind of negative biological reaction.

The reoperated seven chicks when reopened thirty days after the cross-grafting operation showed the following: The grafts were normal-looking. The cranial belly and the intermediate tendon of the right biventer cervicis muscles of the grafts were normal. The formerly "atrophic" long-time denervated left distal bellies were now pink-colored and had lost their atrophic appearance, although being smaller in volume when compared to normal distal biventer cervicis bellies of chicks at the same ages. The recently-severed distal right bellies which became useless because of the cross-grafting operation were pale and atrophic.

Electrical stimulations 1.5 V on the cranial right belly, on the intermediate tendon of the right muscle, on the graft suture region and on the distal previously denervated left belly, produced evident contractions of the left distal belly muscle fibres.

The histological serial silver preparations confirmed the afore mentioned observations. Nerve fibres and nerve fascicles from the normal cranial right belly and correspondent intermediate tendon, growing through the suture, penetrated and repopulated the already partially repopulated distal left belly. Typical motor endplates were again evident in these histological preparations of the long-time denervated left distal bellies (Fig. 7). In the distal right bellies severed thirty days before, during the grafting operation, no remnants of nerve fibres could be seen. They showed a complete nerve degeneration.

DISCUSSION

The presence of ectopic nerve fibres in long-time denervated chick skeletal muscles confirms once more our previous statements on distal segments of peripheral mammalian nerves completely separated from the proximal stump for more than six months, but maintained undisturbed in their natural connective tissue beds (Erhardt & Rezze[4, 5, 6, 7, 8]).

The former and present findings concur partially with those of Gwyn & Aitken's[10, 11] observations, during their discussion of the formation of new motor endplates in mammalian skeletal muscle. They have stated: New ectopic motor enplates form and mature. The various stages through which the new motor endplates pass to mature form are shown. The relationship of the new motor endplates and the regenerating nerve was followed. A number of the original motor endplates were re-innervated but there appeared to be no preferential attraction for the original endplates.

Discussing the subject Gwyn & Aitken[11] write the following: "In the absence of mechanical injury, it is the availability of the denervated muscle fibres which would appear to be the deciding factor in the production of the new motor endplates".

Kirsche [12] comments that "the processes of regeneration show characteristic features of the living, the investigation of which is an integral part of our striving for cognition".

What is the "availability of the denervated muscle" of Gwyn & Aitken and the "features of the living" of Kirsche? Would it be the same "deciding factor" that reorganises the ectopic nerve fibres observed by us? Technical difficulties still constitute limiting factors for studying these ectopic structures. We have discussed fully this problem in a former paper (Erhart & Rezze [8]) in which is presented a progressive sequence of histological findings which suggest a possible gradual reorganization and regeneration of the referred ectopic nerve fibres in distal nerve segments completely separated from the proximal stump for more than six months, but maintained undisturbed in their natural connective tissue beds.

Motor endplates were also identified by us in the silver preparations of the long-time denervated distal bellies of the transected biventer cervicis muscles of the chick. Nevertheless, we cannot state as Gwyn & Aitken [11] did, whether they were new ectopic motor endplates or re-innervated ones. Our experimental conditions were differently oriented.

Considering the response to electrical stimulation, our results were equivalent to those of Gwyn & Aitken. The denervated distal bellies, although seeming to be atrophic, responded to indirect and to direct stimulation by contraction, because nerve fibres and motor endplates were viable. The histological silver preparations confirmed this. Since our results differed from the general belief that long-time denervated skeletal muscles, atrophic-looking, are practically useless, we performed experiments of cross-grafting nerve-muscle implants.

Various experimental methods have been utilized.

A detailed study on the re-innervation of muscles after various periods of atrophy was done by Gutmann & Young [9]. The implantation attempt of nerves into denervated muscles are generally performed by inserting the nerve between separated muscles fascicles. Miledi [13] has even suggested that damage to muscle fibres makes them receptive to a foreign regenerating nerve.

Our cross-grafting experiments were successful in chick biventer cervicis muscles which, after being denervated for a long time (up to ten months), showed them to be well-recovered in about thirty days after the grafting operation.

Whether these results may be repeated in mammalian skeletal muscles, man included, is a matter for future investigation.

SUMMARY

1. The whole biventer cervicis muscles of the chick, being innervated by a branch of the dorsal ramus of C., presents structural peculiarities

which recommend it as good skeletal muscle for embryological, anatomical, physiological and pharmacological neuro-muscular investigations.

2. The nerve trunk responsible for the innervation of the distal belly runs completely included within the intermediate tendon; therefore, a tendon transection determines complete denervation and nerve fibre degeneration of the distal belly of the muscle.

3. Long-time experimentally denervated distal bellies (from three up to twelve months) are repopulated by ectopic nerve fibres which must have arisen from a source other than the proximal stump, neighbour nerves or nervi-vasorum.

4. Motor endplates appear in these long-time (eight or more months) denervated biventer cervicis distal bellies.

5. Although atrophic-looking such muscle bellies responded to indirect and to direct electrical stimulation — 1.5 V — by contraction.

6. The long-time denervated distal bellies of the biventer cervicis muscle of the chick, when properly reoperated by cross-grafting suture with the normal contralateral muscle, lost their atrophic appearance and showed to be successfully recovered in about thirty days.

RESUMO

Fibras nervosas neo-formadas que repopulam músculo estriado desnervado e atrófico de "Gallus domesticus".

Em trabalhos anteriores, foram analisadas e discutidas as fibras nervosas que aparecem nos segmentos distais de nervos de mamíferos, homem inclusive, lesados e separados do côto proximal há mais de seis meses. Neste, é estudado o comportamento dessas fibras no ventre distal do músculo *biventer cervicis* do *Gallus domesticus* desnervado experimentalmente por três até doze meses.

Transecções totais, experimentais do tendão intermédio do músculo *biventer cervicis* do *Gallus domesticus* determinam atrofia por desnervação do ventre distal, porque todo músculo é inervado apenas pelo ramo dorsal do primeiro nervo cervical (Figs. 1 e 2). Todavia, decorridos 3 ou mais meses pós-operatórios, é demonstrado, confirmando trabalhos anteriores, que tais ventres distais, embora totalmente separados dos correspondentes proximais, apresentam evidente repopulação de fibras nervosas (Figs. 5 e 6) que restabelecem, inclusive, suas conexões com fibras musculares estriadas (Fig. 7).

A despeito do aspecto atrófico, êsses ventres distais desnervados experimentalmente há 3 ou mais meses, quando estimulados com 1,5 V, respondem com evidentes contrações, e, quando nêles são implantados, com técnica cirúrgica adequada, o correspondente músculo homônimo, contralateral, íntegro (Fig. 3), há possibilidade de recuperação funcional em cêrca de 30 dias.

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