INFLUENCE OF THE NERVOUS SYSTEM ON TRANSPLANTATION REGENERATION OF SKELETAL MUSCLE

R. P. Zhenevskaya, I. L. Novoselova, and M. M. Umnova

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The gastrocnemius muscles of turtles (*Testudo horsfieldi*) were completely detached and reimplanted in the same position with the nerve led up to the graft or with denervation. The recovery of contractile activity and innervation of the grafts from the approximated nerve were studied from their beginning 1 month after the operation until their still incomplete state 6 months after the operation. The grafts had a muscular type of structure only if their innervation was restored; otherwise, connective tissue developed in them. Two months after the operation a significant difference was found in the weight of the innervated and denervated grafts. As regards the time for recovery of innervation and of the gravimetric indices, grafts in turtles occupy an intermediate position between those studied previously in rats and frogs.

KEY WORDS: *innervation of muscles; transplantation; denervation.*

The question of the nervous control over regeneration of organs in animals has attracted considerable attention of research workers and has been widely discussed in the literature [1, 7, 11, 13]. The influence of the nervous system on regeneration and transplantation of skeletal muscles in mammals [2, 3, 6, 8, 9] and amphibians [4, 5] has been demonstrated in A. N. Studitskii's laboratory. The present writers [10] showed that free transplantation of whole muscles is possible in reptiles (*Testudo horsfieldi*).

The object of the present investigation was to determine the role of the nervous system in transplantation regeneration in a grafted muscle and its role in the secondary morphogenesis of the muscular organ after transplantation in turtles.

EXPERIMENTAL METHOD

Experiments were carried out on 72 turtles (*T. horsfieldi*) weighing 300-500 g. The experiments were divided into two series. In series I the right gastrocnemius muscle was divided at the distal and proximal tendons, separated from its bed, and then replaced, reattached by ligatures to the residual stumps. The tibial nerve was divided and its central ends sutured to the middle part of the muscle. In series II the right gastrocnemius muscle was reimplanted in the same way, but the central end of the tibial nerve was deflected from the graft and was sutured to the sartorius muscle. Material was investigated between 2 weeks and 6 months after the opeation. A physiological control of the contractile response of the graft to sciatic nerve stimulation and to direct electrical stimulation by means of a cardiac stimulator of the ES-3M type was used. The grafts and the gastrocnemius muscle of the opposite limb were weighed. The muscles were fixed and treated by histological methods.

EXPERIMENTAL RESULTS

When the nerve was sutured to the graft the transplanted muscles did not respond by contraction either to direct stimulation or to stimulation of the nerve during the first month. From 1 to 1.5 months after the operation solitary grafts began to contract weakly in their proximal part. The strength of contraction and the number of contracting grafts increased

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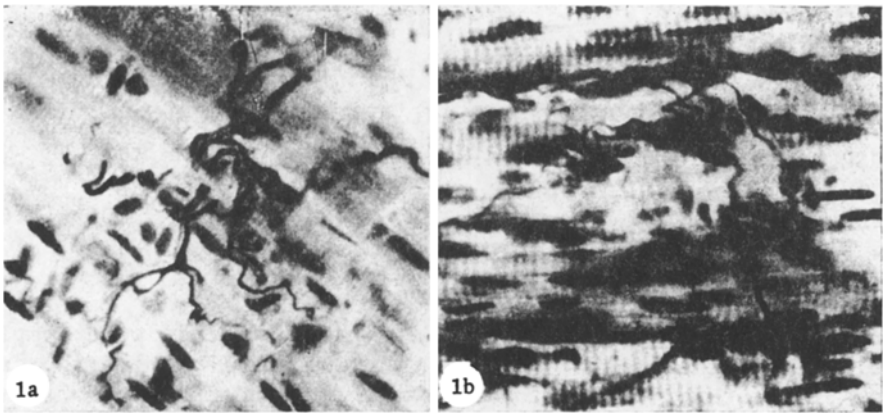

Fig. 1. Axo-muscular synapse in gastrocnemius muscle of *T. horsfieldi*. a) In intact muscle; b) in transplanted muscle 4.5 months after grafting. Formalin. Impregnation by the Bielschowsky–Gros–Lavrent'ev method, 200×.

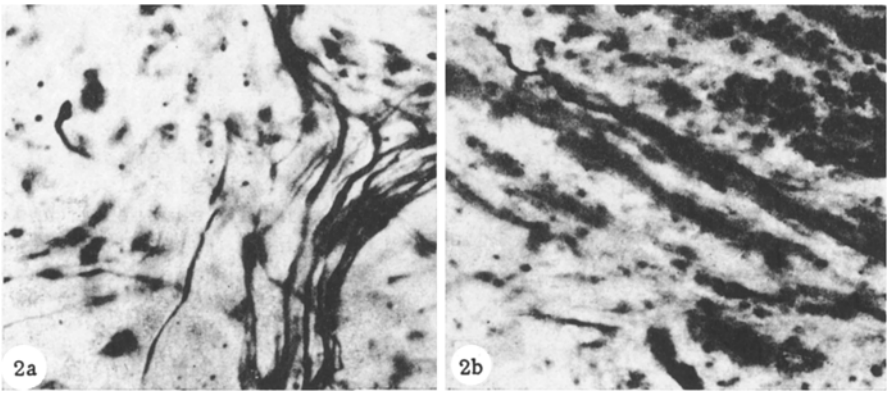

Fig. 2. Regenerating nerve fibers in transplanted turtle muscle 1 month after grafting. a) With nerve approximated to graft; b) with nerve deflected to neighboring muscle. Formalin. Impregnation by the Bielschowsky–Gros–Lavrent'-ev method. a) 200×, b) 100×.

after 2 months, and after 3 months all the transplanted muscles contracted in response to stimulation of the nerve. After reimplantation of muscles and displacement of the nerve from them, macroscopic examination showed that in many cases nerve fibers were growing from the deflected nerve into the graft. The physiological control 2.5 months later showed that the grafts responded by contraction to direct stimulation. After 3 months only 1 of the 5 transplanted muscles contracted in response to electrical stimulation of the deflected nerve, whereas after 5-6 months 50% of the grafts responded to this stimulation.

Weighing of the reimplanted muscles showed that in both series of experiments the weight of the grafts 2 weeks after transplantation was much greater than the weight of the control muscles. After 1 month the weight of the denervated grafts was below that of the control (84 ± 4%), whereas the weight of the grafts with the approximated nerve remained high (119 ± 8%). By 2 months, the weight of the latter was equal to that of the control, whereas the weight of the denervated transplanted muscles showed an even greater reduction (68 ± 2%). Later, evidently because of spontaneous reinnervation of some of the denervated grafts and the appearance of contractile activity in them, the decrease in their weight was arrested (72 ± 1% after 6 months), but it still remained lower than their weight in the experiments with approximation of the nerve to the graft (77 ± 4%). In cases of permanent denervation, however, the weight of the grafts continued to fall and by 6 months was down to 54% of the weight of the control muscles.

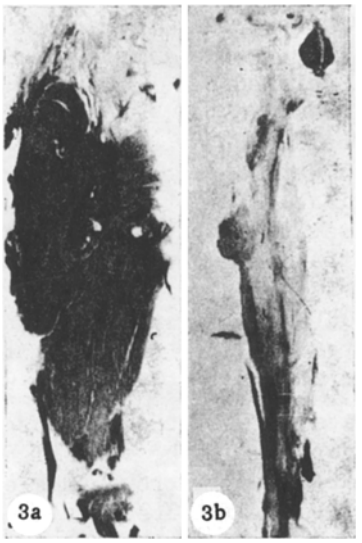

Fig. 3. Longitudinal section through graft of whole muscle of *T. horsfieldi* 6 months after transplantation. a) With nerve approximated to graft; b) with nerve deflected to neighboring muscle. Formalin. Impregnation by the Bielschowsky–Gros–Lavrent'ev method, 4×.

In the intact turtle gastrocnemius muscle the type of axo-muscular synapse most commonly found is as follows: the motor nerve fiber, leaving the intramuscular trunk, divides at an angle of 180° into two long terminals, which run along the muscle fiber. Along the course of the terminals and at their end small loops can be seen. Sometimes the primary terminal gives off very short and thin secondary terminals. At the turning points of the terminals round nuclei can be seen (Fig. 1a).

The reimplanted muscles 2 weeks after the operation were totally denervated in both series of experiments and no axons could be found in them. Edema was observed in the grafts. Degeneration of some muscle fibers and the formation of myogenic elements were observed. The process of reinnervation of the grafted muscles evidently began in the 3rd week after the operation. After 1-1.5 months, a whole stream of thin unmyelinated nerve fibers ran out from the nerve trunk, sutured to the graft, into the muscle, and on entering it they formed a plexus (Fig. 2a). The axons were either pointed at their ends or formed spherical or oval bulbs. Two months after transplantation the regenerating nerve fibers penetrated deeper into the substance of the grafts, which consisted chiefly of young cross-striated muscle fibers, containing numerous round nuclei. Small nerve trunks filled with axons could be seen in the proximal and central parts of the grafts. Sometimes single nerve fibers departed from the trunks and ran along the muscle fibers without forming typical terminals. Restoration of the innervation of the grafted muscles showed appreciable progress at the subsequent times. By 5-6 months, when the newly formed muscle fibers were thicker and their nuclei longer, the whole graft reflected a process of reinnervation. Nerve fibers approaching the muscle fibers formed irregularly shaped synapses: the long terminal ran along the muscle fiber without dividing or giving off secondary terminals, or the axons branched into interweaving terminals (Fig. 1b).

Experiments in which the nerve was deflected from the graft showed that 1 month after the operation the transplanted muscles were denervated. Only in one case could a thin nerve trunk be found in the preparations, from which solitary axons ran toward the graft (Fig. 2b). In the transplanted muscles which contained many old muscle fibers, the newly formed muscle cells were separated by wide gaps filled with loose connective tissue and a few cells. After 2.5-3 months the grafts from which the nerve was deflected at the operation could be divided into two groups: nerve filaments were growing toward some grafts and had started to reinnervate them, whereas others still remained almost totally denervated. In the first case the grafted muscles, as in the experiments of series I, consisted mainly of young cross-striated muscle fibers, with round and oval nuclei. Nerve fibers were growing into the graft at its proximal end. Small nerve trunks and single nerve fibers ran across or along the muscle

fibers without forming typical synapses. In other cases, only single axons could be seen in the proximal part of the grafts. The muscle fibers of these grafts were thin and winding and their cross-striation was ill-defined. Bundles of muscle fibers were loosely arranged and separated by wide bands of fibrous connective tissue, containing many blood vessels and sometimes foci of infiltration. Besides the young muscle fibers, old thick muscle fibers could be seen, the muclei of which were degenerating. Differences in the structure of the grafts which the nerve was approximated (or which underwent spontaneous reinnervation) and those left denervated became clearer still later. After 5-6 months, in the experiments with deflection of the nerve, three different types of results were obtained: 1) grafts reinnervated spontaneously and contracting distinctly in response to nerve stimulation; 2) grafts into which nerve fibers had grown but which did not respond by contraction to nerve stimulation; 3) grafts remaining denervated and with no contractile activity. Grafts of group 1 were similar in structure to those obtained after nerve suture (Fig. 3a).

They consisted of differentiated cross-striated muscle fibers, the diameter of which was much larger than at previous periods of the investigation. The muscle fibers lay compactly, bundles of them being separated by narrow bands of connective tissue. The graft contained many nerve trunks and single nerve fibers, giving axo-muscular synapses of irregular shape. The grafts of type 2 differed from those described above in the fact that the diameter of the muscle fibers was irregular, the bands of connective tissue were fairly wide, and they occasionally contained small foci of infiltration. The grafts were innervated, but less abundantly than in group 1. The axons very rarely reached the muscle fibers and they terminated in small glomeruli. The basis of the grafts of group 3 was composed of loose fibrous connective tissue, which contained single thin muscle fibers with dull sarcoplasm and indistinct cross-striation (Fig. 3b). Single axons, not giving synapses, were very rarely found on the muscle cells.

These results demonstrate the role of the nervous system in transplantation regeneration of the reptilian skeletal muscle. Regenerating nerve fibers grow into the graft and form axo-muscular synapses. However, the recovery of the innervation of the grafted muscle takes place only in turtles and is not complete even after 6 months, for the architectonics of the nerve net and the structure of the axo-muscular synapses at that time are abnormal. Comparison of these data with those obtained previously in mammals and amphibians shows that the reptiles investigated occupy an intermediate position as regards the speed of the process: in rats regenerating axons are found in the grafted muscle after 1-2 weeks, in frogs after 2 months, but in turtles after 1 month. The same pattern is found with the formation of axo-muscular synapses and the appearance of a contractile response of the graft to nerve stimulation.

The experiments in which the nerve was deflected from the graft at the time of transplantation show clearly how the result of the morphogenetic process in the grafted muscles depends on its nervous control. The grafts had a "muscular type" of structure only if they recovered their innervation. When reinnervation was delayed, grafts of "mixed type" were obtained, with a roughly equal content of muscle and connective tissue. If no reinnervation took place, the grafts consisted of a band of connective tissue. Similar results were obtained in rats and frogs. However, comparison of the weight of the grafted muscles in the various animals showed that grafts in frogs and turtles attained a greater relative weight than in rats. Furthermore, differences in the weight of the grafts in the experiments with reinnervation and denervation appeared in rats after 3-4 weeks, in frogs after 4 months, and in turtles after 2 months, i.e., the turtles again occupied an intermediate position.

The authors' views regarding changes in interaction between muscular and nervous tissues in the course of evolution have thus received further confirmation.

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IMPLANTATION GROWTH OF NEPHROGENIC TISSUE AND TUBULAR EPITHELIUM OF THE RENAL NEPHRON CULTURED *in vivo*

I. V. Dunaev, G. S. Solov'ev, and S. M. Panteleev

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Implantation growth of nephrogenic tissue of a 17-day rat embryo and of the epithelium from the nephron of animals aged 1 month was compared. Nephrogenic tissue in implants *in vivo* showed appearances characteristic of its histogenesis. The tubular epithelium from the nephron of month-old animals showed manifestations of tissue growth and formed atypical kidney structures, reflecting its ability to undergo tissue and organotypical differentiation. It is concluded that the epithelium of the renal nephron has a wide range of reactive and plastic properties and is capable of organotypical determination.

KEY WORDS: *nephrogenic tissue; nephron; implantation growth.*

Organotypical growth of epithelia of different origin cultured *in vivo* by Lazarenko's method [7] has been interpreted by some workers as evidence of the existence of the organ-specific determination of these epithelial tissues. However, there is as yet no general agreement as to how the epithelial growths observed in implants should be interpreted: as ordinary tissue differentiation (histoblastic growth) or as the result of realization of the organ-specific determination of epithelia of different organs. To obtain one of the possible alternative answers to this question, it was decided to compare implantation growth of the epithelium of formed organ structures and the implantation growth of the epithelium of their anlagen.

EXPERIMENTAL METHOD

Implantation growth of the epithelium of the renal nephron of rats aged 1 month and the nephrogenic tissue from the kidneys of 17-day rat embryos was studied. Homoimplantation was carried out by Lazarenko's classic technique [7]. Between the first and the 60th days of the experiment the implants were extirpated, fixed in Carnoy's fluid, and embedded in paraffin wax. Histological sections were stained with Mayer's hematoxylin and eosin. Nucleic acids (after Brachet and Feulgen), acid and neutral mucopolysaccharides (after McManus and Hale), and total protein (after Danielli and Pearse) were detected histochemically, with appropriate controls. In all series of experiments the recipients were male rats aged 3 months. Altogether 60 implants were studied. The choice of donors was determined by the fact that nephrogenic tissue predominates considerably in the kidneys of 17-day rat embryos, whereas in the kidneys of normal month-old rats no undifferentiated aggregates of cells of the nephrogenic tissue are found [1, 3, 7, 10].

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