Motor Endplates in Autologous Muscle Transplants[1]

For a long time it has been taken for granted that striated muscle does not tolerate free transplantation even within the same organism. Several authors have reported a nearly total degeneration of the muscle due to the initial ischemia during the first days after transplantation. STUDITSKY[2] and his group in Russia were the first to succeed in transplanting whole muscles or fragments of muscles in the early fifties. He denervated the gastrocnemius muscle of rats 1 month prior to transplantation. Then the muscle was autografted to the site of the removed contralateral gastrocnemius muscle and the tibial nerve was brought into contact with the graft. Within 3 months the muscle regained normal weight and contractility. STUDITSKY suggested that denervation makes the muscle less sensitive towards ischemia, which is inevitable during the first days following free transplantation.

Based on similar assumptions, successful transplantation experiments in dogs were made by THOMPSON[3,4] in London about 1970. The pronator teres muscle was transplanted to the surface of the sartorius and gracilis muscle. Thus nerve fibres and blood vessels could invade the graft from the underlying muscles. The best results were achieved when the muscle had been denervated 2–3 weeks before transplantation. THOMPSON also succeeded in autografting a small muscle of the dorsum pedis into the face in human patients. Relatively little attention, however, had been dedicated to the fate and localization of the motor endplates. THOMPSON only describes small multiple motor endings on the fibres of the transplanted muscles in dogs, whereas in human transplants he suggests reinnervation of the original endplates.

Materials and methods. In 15 young male rats (average weight 100 to 150 g) the extensor digitorum longus muscle (EDL) was transplanted 1 week after denervation and fixed longitudinally on the surface of the chest (Figure 1). In this way blood vessels and nerve fibres could invade the graft from 2 to 3 intercostal spaces. The animals were sacrificed after various intervals ranging from 1 to 11 weeks. In 5 control animals the EDL was denervated but left in situ. Prior to the morphological examination electrophysiological stimulation experiments were made in all animals. After excision of the grafts, they were freed from scar and connective tissue and their weight was determined. The muscles were fixed in 6% paraformaldehyde and small fibre bundles were stained in toto for cholinesterase after KOELLE and FRIEDENWALD[5]. Single muscle fibres were isolated by teasing under the stereomicroscope. In addition, frozen sections were made from every specimen and stained by the same method.

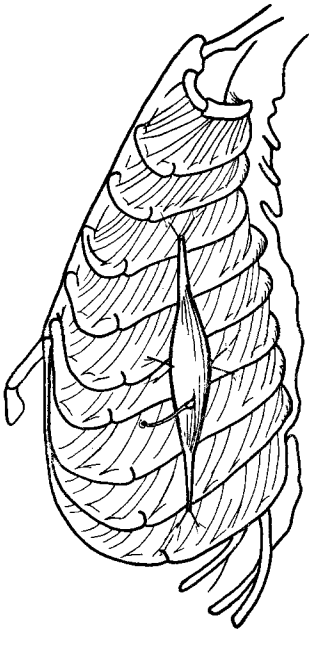

Fig. 1. Position of the transplanted EDL muscle on the chest.

Results and discussion. In all animals, the graft could be identified as a small muscle belly on the surface of the chest. The weight of the grafts turned out to be of rather questionable value because of the formation of reasonable amounts of scar tissue. Therefore we cannot give quantitative data on the amount of surviving muscle tissue.

One week after transplantation, especially in deep fibre layers, the muscles undergo ischemic destruction[6], whereas the motor endplates exhibit the well-known features of degeneration caused by the preceding denervation. In normal endplates (Figure 2a), the axon terminals lie on the folded postsynaptic membrane. The folds are best seen in palisade-like manner on both sides of the axon branches. In denervated endplates, the synaptic folds are well preserved, whereas the withdrawal of the nerve fibres causes certain shrinkage and fragmentation of the endplate subunits. Figure 2b shows a denervated endplate 7 days after transplantation. In our material, fragmentation was a rather common characteristic of degenerating endplates. In order to work out the morphology of the denervated endplates, the incubation periods had been prolonged from 20 min for normal endings to 4 h for denervated endplates. Already 2 weeks after transplantation, reparative processes are observed in the muscle fibres. During this phase of regeneration and formation of new muscle fibres, the endplates are still subject to progressive degeneration. It seems that this process of degeneration is even accelerated in the transplants in comparison to control muscles which were denervated but left in situ. This is probably due to the ischemic alteration of the transplanted muscles.

At this stage, no nerves could be detected by macroscopic inspection of the exposed graft. All attempts of indirect stimulation from the surrounding tissue were of no success. Therefore contractions of the grafted muscles could be elicited only by direct stimulation. The spontaneous fasciculation of the muscles at this stage was an additional criterion that the muscle fibres had not yet received motor innervation.

In stages of 2–10 weeks after transplantation, new nerves could be observed emerging from the intercostal muscles and penetrating the graft, usually together with blood vessels. Electrical stimulation of these nerves resulted in visible contractions of the transplanated muscles. No spontaneous fasciculation was observed. The histochemical examination of muscle grafts at this stage revealed new cholinesterase-positive endings in addition to the old degenerated endplates.

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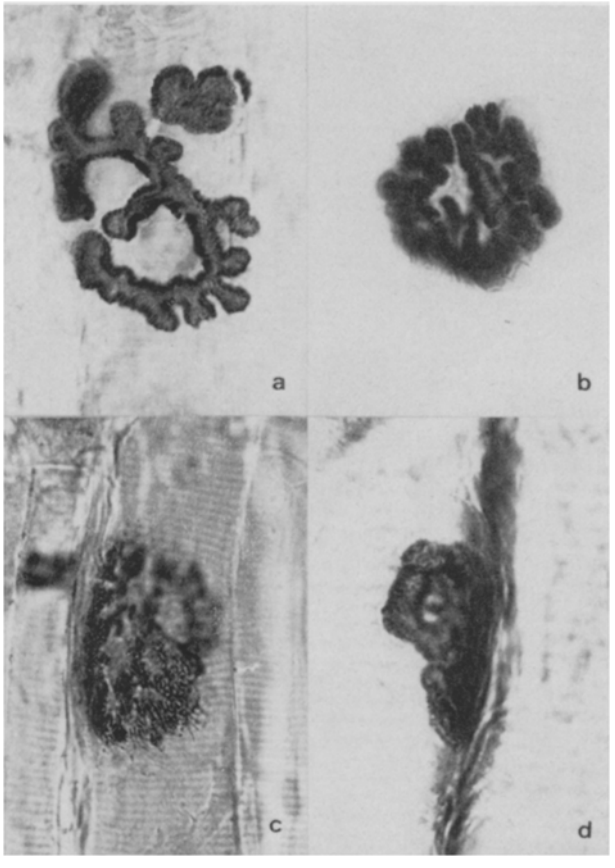
Small cholinesterase-positive sites of round shape are arranged in rows or groups and are the early representatives of the new endings. Later on the endings become more compact. But, even 11 weeks after transplantation, they still differ in shape from normal endplates. In longitudinal sections of the muscles, two or more zones of endplates could be distinguished: the band of degenerated ones and at least one additional and less distinct region with the new, innervated endings.



In spite of great difficulties due to large amounts of connective tissue, it was possible, in some cases, to isolate fragments of muscle fibres showing 1 old, degenerated, non-innervated endplate (Figure 2c), and at some distance from it, a new innervated motor ending (Figure 2d). The distance of the 2 endings obviously depends on the site at which the new nerve enters the muscle. If the nerve reaches the muscle near its tendon, the endings are situated excentrically on the muscle fibres.

The formation of new ectopic endplates indicates that in at least a high percentage of fibres, the ingrowing axons did not reach the original endings to reinnervate them. Probably the large time-lag between denervation and the formation of new neuromuscular contacts in free muscle transplantation without implantation of a nerve is one of the reasons for the de novo formation of motor endings. An additional factor might be the severe damage of the muscle.

Zusammenfassung. Ungefähr 7 Wochen nach Transplantation zeigte sich eine Reinnervation der Muskeln durch kollaterale Sprossung aus den darunterliegenden Interkostalnerven. Die Transplantate reagierten auf indirekte Stimulation. Histochemisch waren neugebildete motorische Endplatten nachweisbar. In einigen Fällen konnte an Isolationspräparaten an ein- und derselben Muskelfaser zusätzlich zur alten, degenerierten eine neugebildete motorische Endplatte beobachtet werden.

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Fig. 2. Photomicrographs of motor endplates in EDL muscles. a) Normal endplate in control muscle. b) Denervated endplate 7 days after transplantation. c) Denervated endplate 70 days after transplantation. d) New endplate 70 days after transplantation. Modified Koelle-technique, Magnification: ×500 (a–c); ×800 (d).

Effects of Incubation Temperature on the Kinetics of Cultured Lymphocytes from the Opossum, *Didelphis virginiana*

The common opossum (*Didelphis virginiana*) possesses a body temperature which is considerably lower than that of the majority of marsupials and eutherian mammals[1–3]. In ambient temperatures of 14–26 °C, the rectal temperatures of opossums ranged from 32–34.5 °C[1]. In in vivo experiments[4], the mean durations of the G_2 and S phases for cells from opossum stomachs were longer than those of eutherian mammals[5–7]. It was suggested that this was caused by the lower body temperature of opossums as the result of a Q_{10} phenomenon[4]. Day-old rats lacking thermoregulatory mechanisms demonstrated increases in G_2 and S phase durations at temperatures substantially below normal for those animals[8]. In contrast, a short S phase for chicken intestinal epithelia has been related to the higher body temperature (40.5 °C) of this species[6]. A number of investigators have demonstrated that incubation of in vitro cell systems at temperatures above or below the optimum range (generally 37–39 °C) resulted in prolonged durations of individual phases and/or the total cell cycle[9–12]. This study was undertaken to determine if incubation of opossum lymphocytes at 34 °C

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