Transplantation of an Entire Muscle in Mice

Skeletal muscles have been considered to be incapable of surviving after transplantation from one animal to another[1–4]. There may be several factors responsible for the failure of muscle transplantation. Among these are oxygen and nutrient deficiency for the transplant, immunological reaction between transplant and host tissues, disturbances of trophic effect of nerve on muscle and deleterious effects by infection[1–13]. Taking these factors into consideration, we attempted to reinvestigate transplantation of a whole muscle between mice.

Material and method. Under ether anesthesia an entire extensor digitorum longus (EDL) muscle was excised, with its complete attachment of tendons, in each of paired normal mice (strain C57 BL/6J) which were littermates of the same sex 25–40 days after birth. The excised muscles were exchanged between the paired animals and transplanted into the area vacated by removal of the corresponding muscle. The tendons were sutured to neighboring tissues, maintaining approximately the resting length of the muscle. This operation was performed under a dissecting microscope, and care was taken to minimize injury of the EDL muscle and surrounding muscles. The EDL muscle excised was transferred as quickly as possible. The cut ends of nerve fibres and blood vessels were put back in place after transplantation, and the skin was closed. The operation was performed under aseptic condition, and antibiotics (penicillin) was given for a few days afterwards.

Results and discussion. On the 3rd day after the operation, some transplant still retained contractile ability when stimulated directly (Figure 1B). Nerve stimulation could not induce any muscle contraction. The earliest sign of contraction induced by nerve stimulation was observed on the 5th postoperative day in 4 out of 6 cases. On the 11th to 12th day, nerve stimulation invariably produced muscle contraction. The amount of muscle tension produced by nerve stimulation was comparable to that by muscle stimulation. Thus functional reinnervation appears to be almost complete within 2 weeks after transplantation (see ref. [4]). The abortive nuclear migration, demonstrated on 143-day transplant in Figure 2I (arrows, also see ref. [14,15] on a complete migration toward the periphery in the minced muscle), was not due to lack of innervation of the muscle fibres[16] (Figure 2J).

The transplant attained a normal range of contraction time quickly after transplantation (Figure 1C). Half relaxation time became rather slower shortly after the transplantation and was restored gradually to the normal range in 3 weeks (Figure 1D). Recovery of time course of

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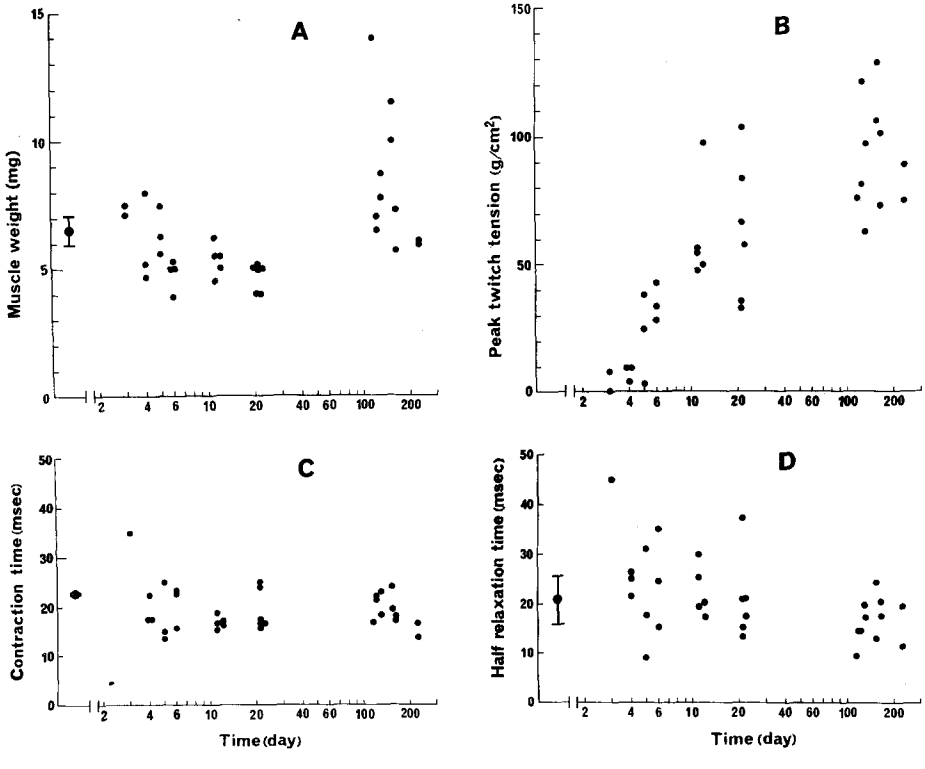

Fig. 1. Changes in muscle weight (A), peak twitch tension (B), contraction time (C), and half relaxation time (D) of EDL muscles at intervals of 3 to 226 days after transplantation. Under pentobarbital sodium anesthesia (70 mg/kg, i.p.), the EDL muscles were exposed and dissected free distally. Recording and stimulating conditions were all the same with those reported elsewhere[25]. Isometric twitch contraction was induced by directly stimulating the muscle (B, C, D) or indirectly by stimulating the cut sciatic nerve (text). Larger filled circles for A, C and D show mean values and standard deviations ($n = 11$) obtained from control mice of similar ages. Muscle weight was measured as wet weight after recording contractile properties. Cross-sectional area of muscles for B was calculated from ratio of muscle wet weight to length of superficial muscle fibres, assuming density of muscle to be unity.

twitch contraction appears to be too fast if the transplants follow the same processes in regeneration as that in postnatal development[10, 17, 18]. It seems likely that some surviving muscle fibres participated in this quick contraction within 2 weeks after transplantation[19]. The quick contraction after 3 weeks, however, might be attributed to the mature characteristics of innervating nerve fibres[10–12, 19].

Despite increasing restoration of contractile ability in the transplants, their weight decreased postoperatively until about the 21st day (Figure 1A, B). Three days after operation, some muscle fibres began to break into fragments. The sarcoplasm became amorphous and less eosinophilic (Figure 2A-E). The degenerative processes were more pronounced at the inner zone at first (Figure 2A–E). Preserved muscle fibres continue to an initial myotube (Figure 2D, arrow), while the long slender myotube is lying between the preserved fibres that still retained their own nuclei at the periphery and had clear cross-

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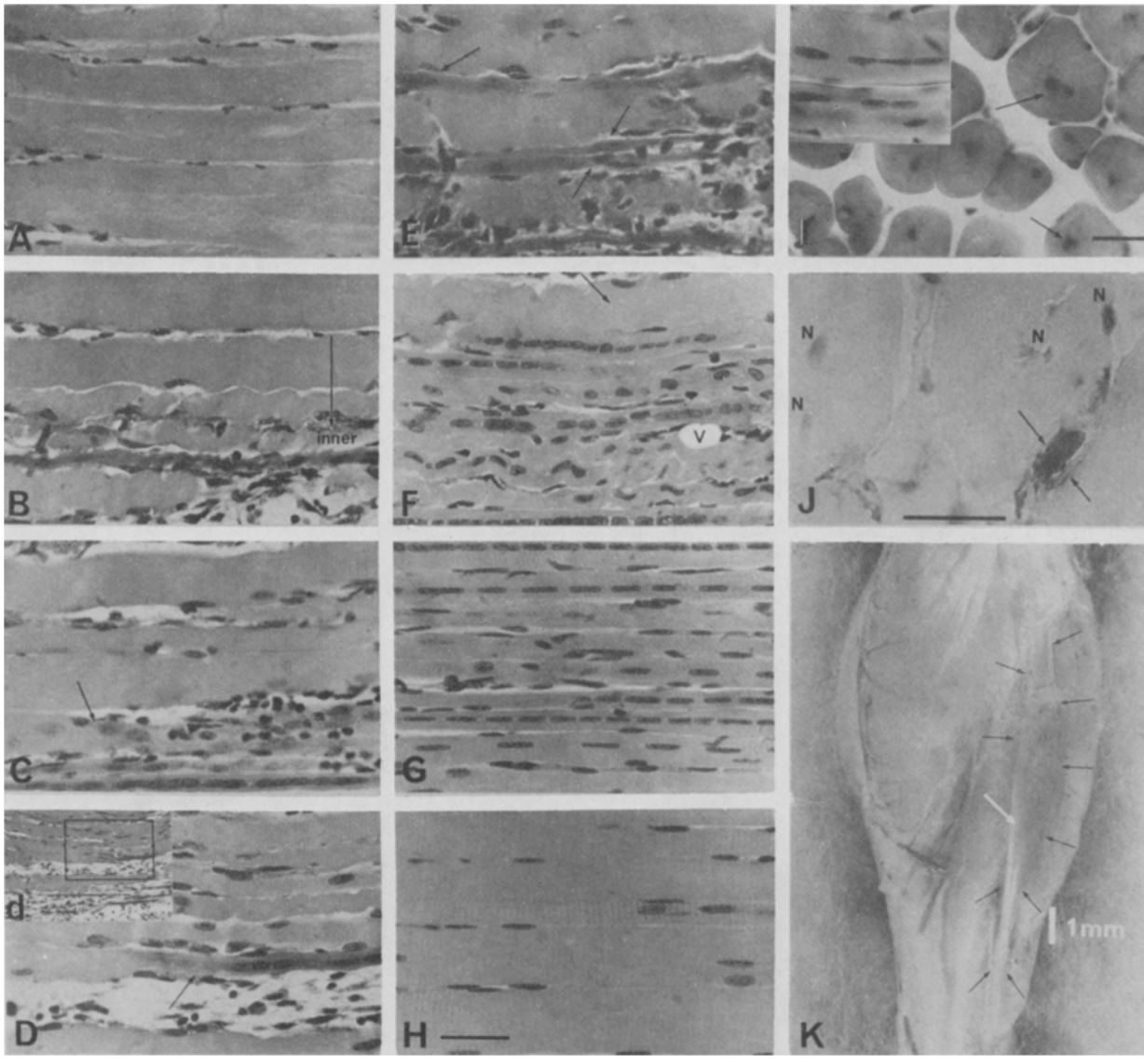

Fig. 2. Transplanted EDL muscle and its histological and histochemical features. H and E staining performed on longitudinal sections at 5 μm (A–H). A) Inner zone of 3-day transplant. Note, some nuclear pycnosis. B) Near inner zone of 4-day transplant. C) Intermediate zone of 5-day transplant. At bottom is inner zone (also for D, E and F). Arrows show continuity of a preserved muscle fibre with a newly formed polynucleated cell. D) Magnification of rectangular portion of inset (d) which was viewed through intermediate zone of a 6-day transplant. E) Intermediate zone of another 6-day transplant. F) Outer zone of 12-day transplant. Almost all muscle fibres have been replaced by myotubes with cross-striations. V, vacuole. G) Inner zone of 21-day transplant. Note slender and more basophilic muscle fibres compared with those of H with clear cross-striations. H) Nonoperated EDL muscle of contralateral side of 61-day-old mouse. Calibration bar, 30μm for A–H. I) Transverse section of the 143-day transplant, stained with H. and E., sectioned at 10 μm. Inset, longitudinal section of another muscle by same procedure. Note a relatively large number of nuclei and their central localization (arrows). J) Acetylcholine esterase activity on muscle fibres of 115-day transplant (arrows) with central and peripheral nuclei (N). Transverse section was stained after Koelle[26], and then with H. and E. Calibration bar, 30 μm. K) Transplanted EDL muscle (arrows) exposed in situ after 143 days under anesthesia. Blood supply and distal tendon of muscle may be seen.

striations (Figure 2E, arrows). These features may reflect 2 different modes of regeneration, which may concomitantly occur under our experimental conditions[20] (for review of muscle regeneration, see[21–23]).

There remained relatively intact muscle fibres in the outer zone of the mass 3 to 12 days after transplantation (Figure 2B–F). These apparently intact muscle fibres seemed to be subjected to degenerative and regenerative processes 3 weeks after operation (Figure 2G). In some other transplants at this time, although the original muscle fibres were still definitely preserved, myoblasts or initial myotubes were scarcely found. From these findings it is likely that such slender fibres, especially those at the outer zone, may represent either less dedifferentiated fibres or simply atrophied fibres associated with temporary denervation[16]. Tissue reconstitution was practically complete 3 weeks after transplantation (Figure 2G). Infiltration of lymphocytes was not marked at any stage after transplantation.

Between 120 and 150 days after operation, the muscle suffered a 15% loss in number of fibres compared with the normal EDL muscle of contralateral side. Our transplant seems to be a fairly complete structural replica of the muscle removed (Figure 2K). In contrast with the results by other investigators[13,14], the tissue reconstitution of our transplant took place, keeping its internal architecture apparently intact. This may provide favorable conditions for studying the mechanisms of muscle regeneration or reconstitution[24].

Zusammenfassung. Bei Mäusegeschwistern führte homoplastische Transplantation kleiner Muskeln in toto zur Wiedererlangung ihrer vollen Funktion unter nervöser Kontrolle.

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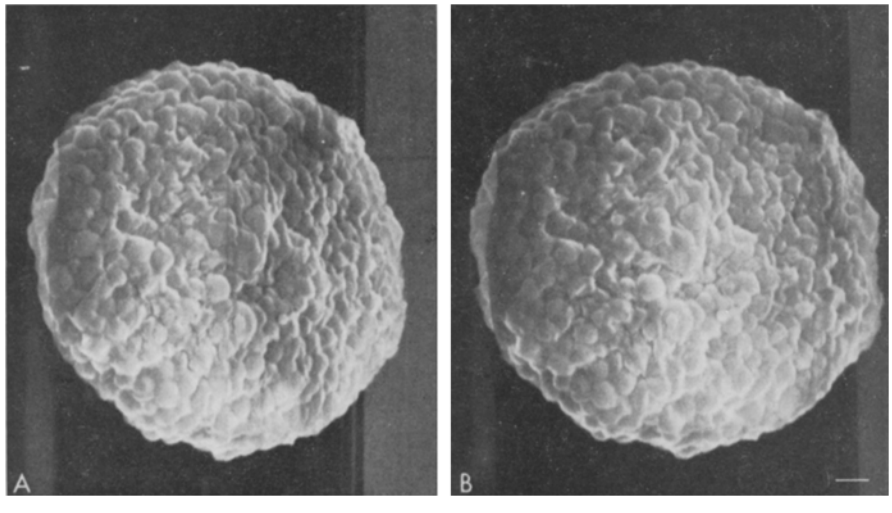
Viewing Stereopair Micrographs: Facilitated Alignment through Use of Color Filter

Stereopair micrographs are readily obtained with the scanning electron microscope. They are particularly useful at high magnifications, where small surface features are not always easily interpreted from single micrographs. Fuller advantage can be taken of the high resolution obtained with this instrument if some further optical magnification is used while viewing the prints. A commercially available viewer (Wild Heerbrugg ST 4 mirror stereoscope) in fact, affords a choice of 3× and 8× oculars as adjuncts to the basic nonmagnifying unit. With some images, particularly where a nearly-repeating pattern is present, proper alignment of the images with respect to each other becomes progressively more difficult as greater magnification is used because reference features are easily lost. Eye strain rapidly develops, perhaps because of involuntary efforts at focusing the images by eye.

It has been found that insertion of a lightly colored filter on one side of the optics greatly facilitates proper alignment by reducing the confusion between the two images and by emphasizing the individuality of the two members in paired reference features without interfering with perception of the three-dimensional effect. The filter can be removed after alignment.



Rat peritoneal fluid mast cell air-dried from 95% ethanol after fixation in mixed aldehydes and post-fixation in OsO_4. Cambridge Stereoscan S4-10 SEM, secondary electron mode, 5,000× direct magnification. (These are contact prints.) Tilt angles: A = 42°; B = 28°. Marker = 1 μm.