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# Achilles tendon injury

## Tendon elongation and soleus muscle fine structure in rabbit after different therapies*

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**Summary.** Unilateral tenotomy of the Achilles tendon was carried out in 60 rabbits. The limb was then either mobilized directly or immobilized for 10 to 35 days using a plaster usually after tendon suture. In certain cases the plaster was removed early (on the 7th or 16th day) and the animals were than allowed to use this leg.

Separation between tendon ends was apparent from steel markers, placed close to each cut end of the tendon and examined by X-ray. The separation curve was biphasic and both the first and the inactive phase reflected the degree of tension over the tendon suture. However, during the second separation phase, which began between the 17th and 21st day, the separation gradually reached the same level in all groups. Enzyme histochemistry and electron microscopy revealed severe degenerative changes in immobilized and in shortened muscles. Furthermore, a gradual shift in fibre type characteristics from type 1 slow-twitch fibres towards type 2 fast-twitch fibres occurred. Rapid recovery followed removal of the plaster.

The findings indicated that both degenerative and regenerative processes and adaptive processes had been initiated in all experimental muscles when the tendon continuity was broken. The adaptive processes progressed gradually during the five-week post-operative period and might have been responsible for the second phase of the tendon end separation. The fibre adaptation, i.e. the transformation, may be accounted for by changes in structure of the myofibrils and composition of the myosin molecules.

**Key words:** Achilles tendon – Tendon injuries – Muscles – Myofibrils – Ultrastructure

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Rupture of Achilles tendon is a common sports injury, especially among badminton players. This injury is treated either surgically or conservatively. These two methods of treatment are equally favoured (Smart et al. 1980). In principle, the surgical alternative is advantageous since it allows immobilization of the lower leg with the triceps surae muscle in a stretched position (Tabary et al. 1972; Tomanek and Lund 1974; Edgerton 1975; Goldspink 1977; Fugl-Meyer et al. 1979; Sjöström et al. 1979). However, experimental data showing a post-operative lengthening of the sutured Achilles tendon (Nyström and Holmlund 1982) suggests that shortening of the immobilized muscle occurs relatively soon after suturing the tendon. This event may reduce or eliminate the advantageous effects of muscle immobilization in the stretched position and may also explain the small differences between the therapeutic effects of various treatments. The basic events underlying this tendon elongation are not sufficiently understood to permit an adequate discussion of this problem. Previous authors of studies on calf muscles when investigating alternative therapeutic methods, have not considered tendon elongation in performing and interpreting their work. The present study attempted to determine whether the tendon elongation was due to events in the muscle.

## Materials and methods

*Animals.* Native breed adult white albino rabbits of both sexes, weighing between 3.5 and 5.0 kg, were used. They were kept in ordinary cages (0.5 × 0.6 m) at room temperature with free access to food and water.

*Surgical procedure.* The rabbits were anaesthetized by intravenous injection of 0.03 g pentobarbital per kg body weight. In addition, local anaesthesia (Xylocain® 0.5%) was given subcutaneously. The operations were performed under aseptic conditions. The lower leg that was to be operated on was shaved and washed with a 0.1% solution of benzethonium chloride. The calcaneal tendon was exposed by a curved incision on the lateral aspect of the lower leg. The tendon was freed from mesotenon and completely divided 15 mm from its insertion on the calcaneus. Two tied stitches of 5-0 stainless steel sutures were made 2–3 mm from the cut tendon ends to allow X-ray documentation (see below) of the position of the tendon ends during the follow-up of tendon healing (Cowan and Courtemanche 1959; Ejeskär 1981). The ends of the tendon were brought together and sutured with a single loop of 3–0 Prolene® (Nyström and Holmlund 1982).

*The experimental model.* In twelve groups, each consisting of five animals, tenotomy was carried out on the left Achilles tendon and in eleven of these groups the tendon was sutured (Table 1). The limb was then either mobilized or immobilized. In the latter case, a padded plaster bandage was applied, embracing the whole paw, the lower leg and half the thigh, with the paw and the knee joint in a semiflexed position. In some cases, the leg was mobilized on the 7th or 16th day after the operation by removal of the plaster.

The animals were killed by an overdose of mebumal sodium and their soleus muscles from both the left (*experimental*) and the right (*control*) side were removed and examined by enzyme histochemistry and electron microscopy. Samples were taken 40 mm from the insertion of the tendon.

*X-ray.* Immediately after operation, every second day for three weeks and subsequently, twice a week, the distance between the two steel stitches close to the tendon ends was assessed

**Table 1.** The experimental model. Rabbits, divided into 12 groups with 5 animals each, were subjected to Achilles tendon injury (tenotomy) and the tendon was sutured in eleven groups. The limb was immobilized using a plaster and/or mobilized. Sampling occurred after various periods of post-operative days

| Group number | Plaster | Number of post-operative days before | |
|---|---|---|---|
| | | removal of plaster | sampling |
| Immediate dynamic work | | | |
| 1 | | | 10 |
| 2 | | | 20 |
| 3 | | | 35 |
| Immobilization followed by mobilization | | | |
| 4 | × | 7 | 10 |
| 5 | × | 7 | 20 |
| 6 | × | 7 | 35 |
| 7 | × | 16 | 20 |
| 8 | × | 16 | 35 |
| Immobilization | | | |
| 9 | × | | 10 |
| 10 | × | | 20 |
| 11 | × | | 35 |
| 12 (no suture) | × | | 35 |

by X-ray. For this procedure, the rabbits (not anaesthesized) were placed on the right side and the legs were X-rayed using cassette film Kodak PE 4006 and a film-focus-distance of 0.58 m.

*Light microscopy*. Specimens from the left and right legs were placed together and frozen in liquid propane cooled by liquid nitrogen. Serial cross-sections, about 10 micron thick and cut at −20° C, were treated for acid and alkaline stable myofibrillar adenosine triphosphatase (mATPase), succinic dehydrogenase (SDH) and with haematoxylin-eosin (Dubowitz and Brooke 1973). On basis of the staining characteristics for mATPase at pH 9.4, the fibres were termed as type 1 (lightly stained) or type 2 (heavily stained). From each specimen, 400 fibres were classified into these two categories. For determination of fibre diameter, i.e. the mean of the two orthogonal diameters (Schmitt 1976), an ocular micrometer scale was used. Two hundred fibres were measured per fibre type and specimen.

*Electron microscopy*. Muscles mounted at their in situ-length were treated for two hours at 4° C with 2.5% glutaraldehyde in Tyrode's solution, pH 7.3, 270 mOsmol. During rinsing in Tyrode's solution, the muscles were cut into smaller pieces, postfixed for two hours in 1% osmium-tetroxide, rinsed again, dehydrated in acetone and then embedded in Vestopal W. Semithin (1 μm) sections were cut and stained with toluidine blue for examination in the light microscope. Selected areas were then trimmed and ultrathin (60–80 nm) sections were cut and stained with uranyl acetate and lead citrate.

**Table 2.** Postoperative separation (mm; mean ± SEM) of steel markers placed close to each cut end of rabbit Achilles tendon

| | Number of postoperative days | | |
|---|---|---|---|
| | 10 | 20 | 35 |
| Immediate dynamic work (groups 1–3) | 24 ± 0.6* | 29 ± 0.7* | 40 ± 3.5 |
| Immobilization followed by mobilization (groups 4–8) | | | |
| Removal of plaster after 7 days | 13 ± 1.5 | 21 ± 1.7 | 38 ± 3.9 |
| Removal of plaster after 16 days | 13 ± 1.5 | 20 ± 2.1 | 38 ± 6.6 |
| Immobilization (groups 9–11) | 14 ± 2.1 | 18 ± 1.5 | 36 ± 2.7 |
| Immobilization (no suture) (group 12) | 23 ± 1.3* | 28 ± 0.8* | 38 ± 3.2 |

The different results have been compared with data on the immobilized groups (9–11; cf. Table 1) from the same experimental period (Student's *t*-test for paired observations, * = $P < 0.05$)

## Results

### *Macroscopical findings*

All experimental muscles, except for those used after removal of the plaster (groups 4–8), decreased markedly in size and often showed haemorrhagic spots. They also appeared to be paler and harder than the control muscles. Muscles mobilized for two weeks or more after removal of the plaster (groups 5, 6 and 8) were essentially normal, but smaller in size than the controls. The sutured tendons, although initially swollen around the suture line, moved freely when examined by flexion of the ankle. In contrast, the unsutured tendons (group 12) had abundant scar tissue. Following plaster removal, the diameter of the tendons increased and after about two to four weeks there was no major difference between the experimental and control tendons.

Separation of the tendon steel stitches were seen in all groups (Table 2). The initial separation occurred during the first five days followed by a period during which none or very little separation occurred until about the 20th day when a new period of separation started. This phenomenon was more pronounced, especially at the early stages, in the groups which had been left without plaster immediately after the tendon suture (groups 1–3) and in the group immobilized without suture (group 12). However, 35 days after the operation, irrespective of treatment, the degree of tendon end separation was essentially the same.

### *Microscopical findings*

*Control muscles.* The appearance of the control muscles was essentially the same as that described in previous studies (Wählby et al. 1978, Sjöström et al. 1979), and will therefore not be considered further in this work. Data

**Table 3.** Fibre type occurrence and fibre diameter (mean ± SEM) in the soleus muscles

| Group number[a] and number of postoperative days | Type 1 fibres | | Diameter Type 1 fibres | | Diameter Type 2 fibres | |
|---|---|---|---|---|---|---|
| | experimental (%) | control (%) | experimental (μm) | control (μm) | experimental (μm) | control (μm) |
| Immediate dynamic work | | | | | | |
| 1. 10 | 80±1* | 93±6 | 62±1* | 84±1 | 70±5* | 86±4 |
| 2. 20 | 84±2* | 90±5 | 54±4* | 90±3 | 45±4* | 86±4 |
| 3. 35 | 71±5* | 92±5 | 47±4* | 89±7 | 41±4* | 81±3 |
| Immobilization followed by mobilization | | | | | | |
| 4. 7+3 | 62±4* | 90±2 | 63±5 | 71±4 | 72±8* | 93±5 |
| 5. 7+13 | 77±2* | 91±2 | 73±4 | 81±4 | 68±4 | 73±5 |
| 6. 7+28 | 85±2 | 94±4 | 40±5* | 81±4 | 45±7* | 79±6 |
| 7. 16+4 | 76±3 | 87±6 | 48±6 | 50±5 | 50±7* | 87±6 |
| 8. 16+19 | 82±1* | 97±5 | 36±1* | 53±1 | 47±6* | 84±2 |
| Immobilization | | | | | | |
| 9. 10 | 88±4 | 93±3 | 62±7 | 70±5 | 69±5* | 94±7 |
| 10. 20 | 69±2* | 96±2 | 38±3* | 67±3 | 37±4* | 96±2 |
| 11. 35 | 77±3* | 95±5 | 27±3* | 85±4 | 31±5* | 95±1 |
| 12. 35 (no suture) | 75±8* | 99±5 | 49±6* | 85±5 | 44±5* | 81±2 |

[a] Cf. Table 1

The left (experimental) muscles have been compared with the right (control) muscles from the same animals (Student's *t*-test for paired observations, * $P<0.05$)

on the relative number and diameters of different control muscle fibres are presented in Table 3.

*Experimental muscles.* A wide range of degenerative changes were found in all muscles, although the extent to which they occurred varied between animals. Thus, the changes were particularly pronounced, at least initially, in muscles mobilized directly after suture (group 1) and in muscles immobilized without suture (group 12). In later stages, the abnormalities were more obvious in the strictly immobilized groups (groups 10 and 11). However, no major qualitative difference was found between the different muscles. Ten days after operation (groups 1 and 9), the fibres were irregular in profile and organized in poorly defined fascicles (Fig. 1a). A number of type 1 fibres were necrotic and infiltrates of inflammatory cells as well as phagocytosis was observed. In contrast, although there was evidence for fibre splitting in many type 2 fibres, none of these fibres were necrotic. In the muscle fibres, the myofibrils were poorly defined, the sarcoplasmic reticulum (SR) was disorganized and triads and pentads were frequently seen (Fig. 2a). The mitochondria were small and longitudinally oriented or rounded. Autophagic vacuoles were seen containing mitochondrial remnants and granular debris. In many fibres, membrane bound vesicles, probably of SR-origin,

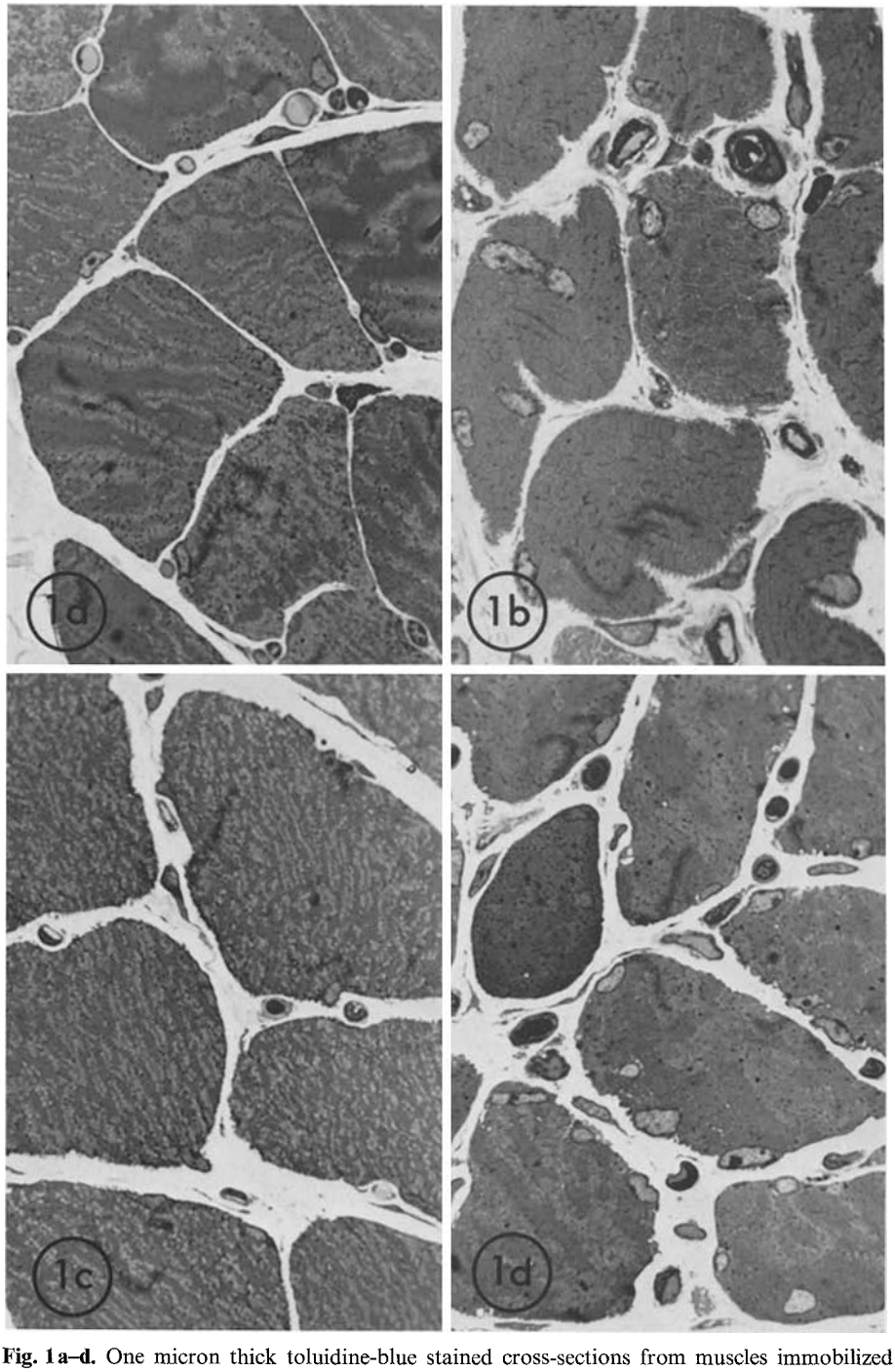


**Fig. 1a–d.** One micron thick toluidine-blue stained cross-sections from muscles immobilized for 10 days (**a**); showing fibres with irregular profile organized in poorly defined fascicles, or 35 days (**b**); showing small and uneven fibres interspersed with abundant connective tissue. **c** Shows muscle, immobilized for one week and thereafter used for dynamic work for four weeks, with essentially normal morphology. **d** Shows muscle, immobilized for 16 days and used for four days, with distorted structure. Magnification $\times 325$

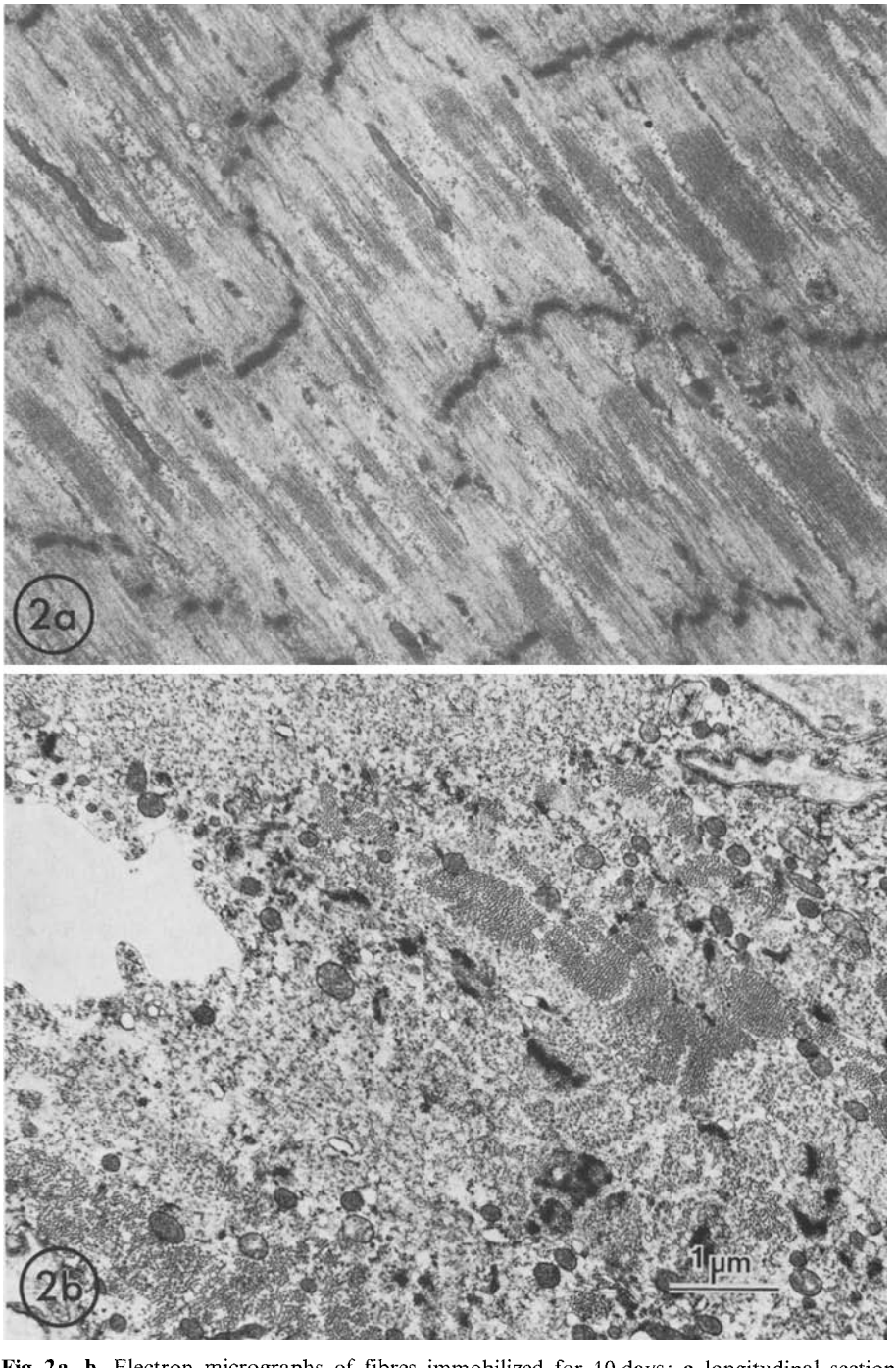


**Fig. 2a, b.** Electron micrographs of fibres immobilized for 10 days; **a** longitudinal section, **b** cross-section. The myofibrils are poorly defined, the sarcoplasmic reticulum is disorganized and the mitochondria are small. Membrane bound vesicle is seen. Magnification $\times 15{,}000$

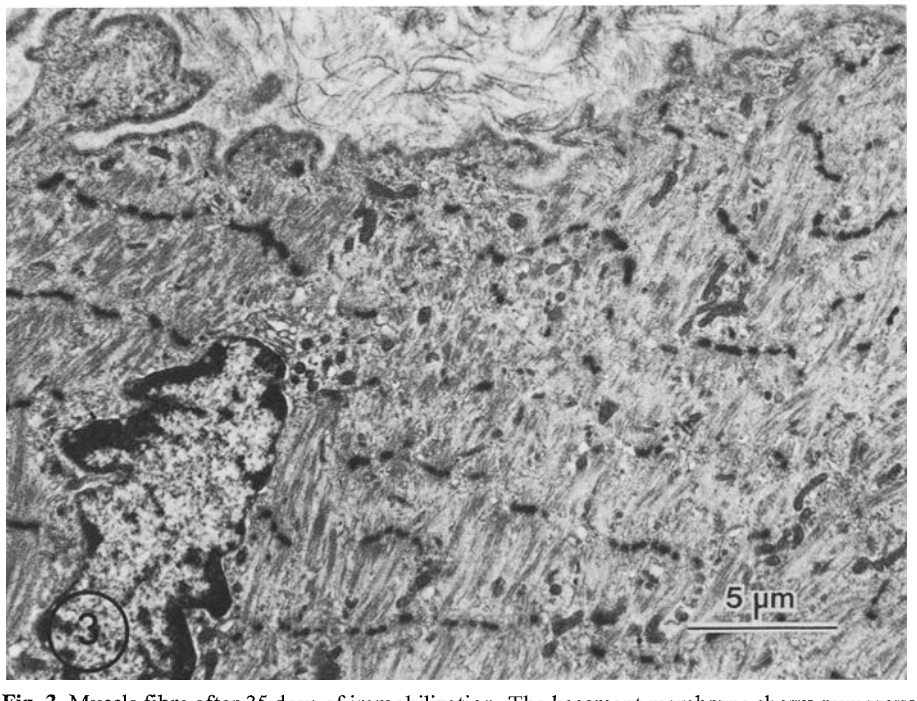


**Fig. 3.** Muscle fibre after 35 days of immobilization. The basement membrane shows numerous irregular projections filled with cytoplasmic evaginations. The myofibrillar material is almost totally disintegrated and disoriented. Magnification × 4,000

were seen (Fig. 2b). They contained scanty, flocculent material, membrane remnants and granular debris. Regenerative activity was marked in places with undifferentiated mononucleated cells and cells with thin and thick filaments and definite myofibrils seen in proximity to the basement membrane.

Twenty or 35 days after operation (groups 2, 3, 10 to 12), the specimen consisted of small, uneven fibres interspersed with abundant connective tissue and fat cells (Fig. 1b and Fig. 3). Numerous scattered nuclei were seen and the relative number of type 1 fibres had decreased (Table 3). Type 2 fibres showed a wide range of staining intensities after acid preincubation. At ultrastructural level, the basement membrane showed numerous irregular projections filled with cytoplasmic evaginations. The myofibrillar material was sometimes observed to be totally disintegrated and disoriented. The ratio of thin and thick myofilaments varied between fibres. Frequent abnormal Z-band configurations were noted. The number of mitochondria was low. The nuclei were round or irregular in profile with sparse chromatin and weakly stained nucleoli. Membrane bound vesicles, with or without electron dense material, of varying sizes and shapes, were found in many fibres. In contrast to the muscles analyzed ten days after operation (groups 1 and 9), very few, if any, signs of fibre necrosis and fibre regeneration were seen in muscles which had longer postoperative periods.

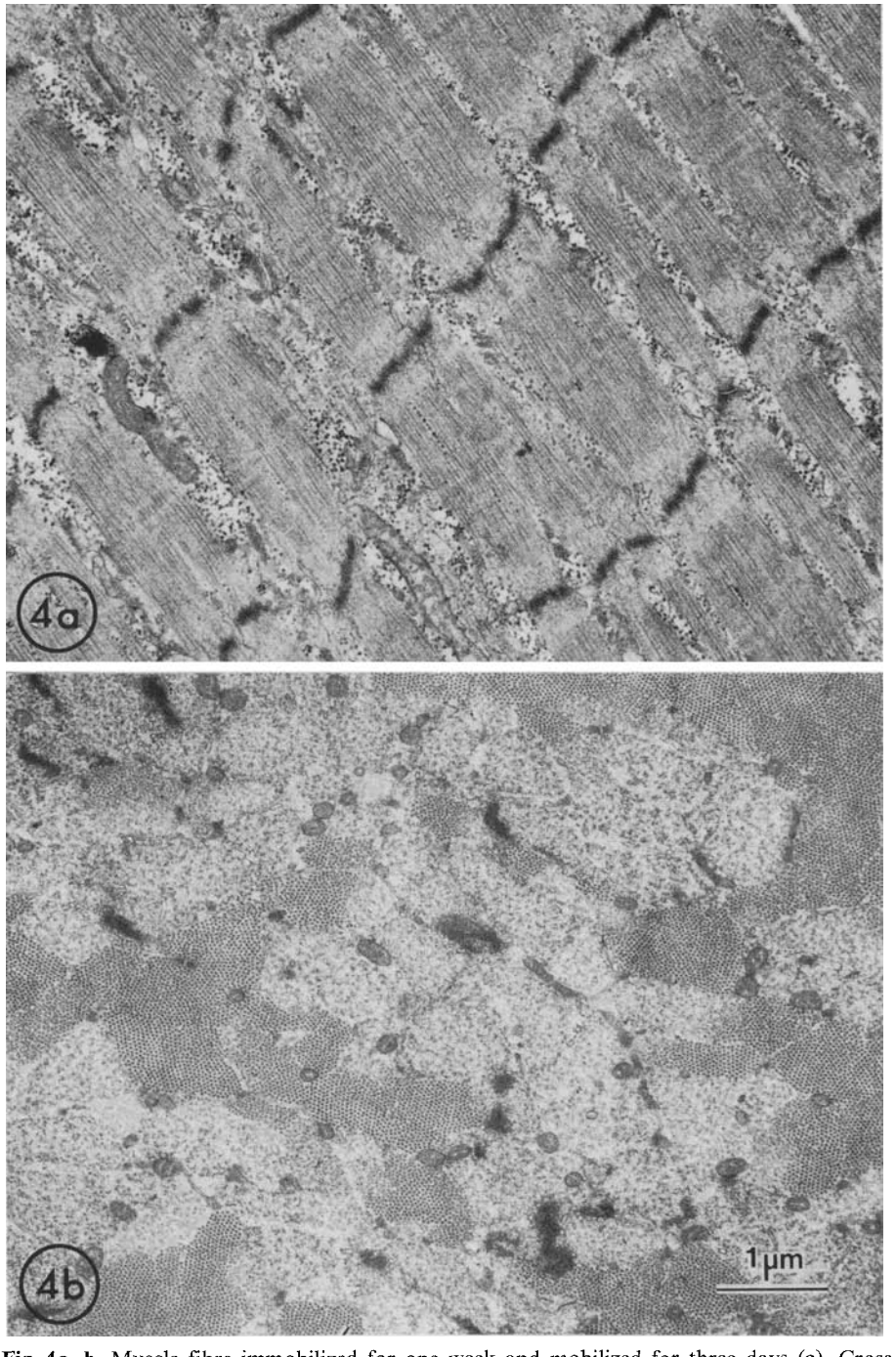


**Fig. 4a, b.** Muscle fibre immobilized for one week and mobilized for three days (**a**). Cross-sectioned fibre from muscle used for dynamic work for two weeks after plaster removal (**b**). Particulate structures are frequent and the myofilamentous material is organizes in tightly packed myofibrils. Magnification ×15,000

*Muscles after removal of plaster.* The earlier the plaster was removed the more rapid and extensive was the improvement in morphology. Thus, muscle immobilized for one week and involved in dynamic work for two or four weeks (groups 5 and 6) although smaller in size than corresponding controls, had essentially normal light microscopical morphology (Fig. 1c). Muscles immobilized for 16 days and used for less than one week (group 7) in contrast, were seriously distorted in structure (Fig. 1d). However, the different fibre types in these muscles (Group 7) were more easily distinguished and more rounded or polygonal in shape than those in muscles obtained immediately after immobilization (e.g. groups 9 and 10). Proportion of type 2 fibres was relatively high throughout the observation period.

Three to four days after plaster removal (groups 4 and 7), irrespective of the length of the preceeding immobilization period, the muscle fibres were, at the ultrastructural level, morphologically similar to those in immobilized muscles (Fig. 4a). However, two to three weeks after plaster removal (groups 5 and 8), particulate structures were frequent and myofilamentous material was organized in tightly packed myofibrils (Fig. 4b). The thick myosin-containing filament material was especially well organized whilst the arrays of thin filaments were incompletely built up. Broadening and streaming of the Z-bands was a common feature. Essentially normal ultrastructure was observed four weeks after plaster removal.

## Discussion

*Effects of immediate dynamic work or immobilization*

Immediate use of the triceps surae muscle after tendon suture resulted in a dramatic early separation of the two tendon ends. This was probably due to the tension initially developed during active, dynamic contractions by muscle fibres that were relatively unaffected in function. As a result, an extensive deformation of the suture material and the suture canal was observed in addition to slipping of the knot. The muscles were thus slacker than immobilized unsutured muscles. Concomitantly, the muscle fibres, especially the slow-twitch type 1 fibres, became undifferentiated and degenerated. These latter events are known to take place in calf muscles immobilized in a slackened position, i.e. with the ankle in a plantar flexion (Sjöström et al. 1979). In view of this, the situation in muscles mobilized immediately after Achilles tendon suture or immobilized without suture is, in practice, similar to that in muscles immobilized in a slackened state. The degree of muscle fibre degeneration, reflected by the degree of tendon end separation during the initial postoperative phase, was found to vary. The underlying pathophysiological process should, however, be the same in all muscle fibres. This assumption is further supported by the fact that all these muscles had a similar morphology 35 days after the tenotomy.

*Effects of removal of plaster*

Muscles engaged in dynamic work after a period of immobilization regained normal morphology relatively soon after removal of the plaster. Within

less than a week signs of active protein synthesis were seen and the overall morphology was essentially normal within two to three weeks. These findings are in agreement with those of Booth and Seider (1979), who found that several biochemical values, including muscle wet weight and protein content, and physiological variables of rat soleus muscles immobilized for 90 days returned to normal levels by the 14th recovery day. The present findings also confirm a series of other reports showing that skeletal muscle has a remarkable capacity to regenerate (e.g. Carlson 1973). During the early postoperative days, immbolization was found to be essential for recovery. Thereafter, however, the shorter the immobilization time, the more rapid and complete became the structural improvement. The question that is raised by these findings is whether the immobilization period in man, in the treatment of ruptured Achilles tendon which usually lies in the range of three to six weeks, should be shortened.

*Degeneration and regeneration*

The final tendon end separation was the same irrespective of the therapeutic method used. This fact indicated the existence of an adaptive process common to all the experimental animals. The process might have been initiated when the tenotomy was performed resulting in a break in the tendon continuity. Regenerative activity is induced whenever muscles are subjected to pathological conditions (Carlson 1973; Studitsky 1974). Satellite cells may be activated and in the case of death of the existing muscle fibre, a population of myoblasts will be established. Within a week, myotubes may be formed which then, after a further one or two weeks, can be innervated. These new muscle fibres, whose contractile material is adapted to the abnormal functional demands, may then maintain a high resting tension or may actively contract isometrically and thereby be responsible for the second separation phase starting by the 14th to 17th postoperative day. In the present work, there was indeed evidence for muscle fibre necrosis and regeneration. However, the extent of these changes may be too small to account for the dramatic second phase of tendon end separation.

*Fibre adaptation*

Since myofibrillar material has a remarkable capacity to change its internal structure and composition, functional adaptation of existing fibres is also possible. A rapid reduction (within a week) in the number of sarcomeres has been demonstrated after tenotomy of cat Achilles tendon and immobilization of the soleus muscle (Hayat et al. 1978; Huet et al. 1979). However, for such changes, intact innervation was essential. Biochemical analysis of the myosin molecules of rabbit and human skeletal muscle suggest a high degree of flexibility in the myosin subunit composition (Rubinstein et al. 1978; Hoh and Yeoh 1979; Lutz et al. 1979, Billeter et al. 1980). Thus, intermediate forms, e.g. between type 1 and 2, may rapidly be formed. The time necessary for the altered synthesis of these proteins lies in the range

of five to seven days. After tenotomy, in our experiments all muscles were subjected to a shortening and/or immobilization. Signs of qualitative transformation were then present in that a greater proportion of fibres showed type 2 properties. These structurally and functionally altered fibres might then have been responsible for the second phase of tendon end separation (cf. Table 2). This second phase may have started as early as about one week after operation.

In conclusion, the findings indicated that *both* degenerative and regenerative processes *and* adaptive processes had been initiated in *all* experimental muscles when the tendon continuity was broken. The adaptive processes progressed gradually during the five-week postoperative period and might have been responsible for the second phase of the tendon end separation. Fibre transformation may be accounted by changes in structure of the myofibrils and composition of the myosin molecules.

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