## Differential Response of Myosin – ATPase Activity and Contraction Properties of Fast and Slow Rabbit Muscles Following Denervation

Fast and slow muscles differ not only in contraction properties, but also in dependence on aerobic and anaerobic mechanisms in generation of cellular energy. Denervation produces a multiplicity of changes depending on morphological and biochemical type of muscle, see[1]. However a comparison of strictly defined fast and slow muscles, concerning both metabolic and contraction properties, after denervation has so far not been undertaken.

*Material and methods.* Adult male or female rabbits (about 3 kg body weight) were used in the experiments. The muscles of the hind limb were denervated unilaterally by sectioning the sciatic nerve in the middle of the thigh and removing 2 cm of the nerve to avoid reinnervation of the muscles. The contralateral side served as a control.

Contraction properties. The extensor digitorum longus (EDL) and the peroneus longus muscles innervated by the peroneal nerve are both fast muscles with identical contraction properties. In the study of contraction properties we have therefore used the peroneus longus muscle, methodologically advantageous because of its smaller size and thickness. The soleus muscle is known to be an exceptionally slow muscle. Contraction time (time to peak) was measured in vitro at 36°C with the mass stimulation method[2] and recorded by an automatic analyzer[3]. The stimuli were square pulses of 1.0 msec in duration of supramaximal intensity. Optimal resting tension (tension producing maximal twitch tension) was determined before each experiment.

Biochemical properties. Myosin was prepared from the fast EDL and the slow soleus muscle by dilution and precipitation procedure[4] with 10 *mM* sodium pyrophosphate and 1 *mM* magnesium chloride included in the extraction medium. Protein was determined by the biuret method, inorganic phosphate according to FISKE and SUBAROW[5]. Low molecular weight proteins were dissociated from myosin by treatment with *p*-chloromercuribenzoate[6] and isolated by the procedure used by SAMAHA et al.[6]. Polyacrylamid gel electrophoresis was carried out as described by ORNSTEIN[7] and DAVIS[8]. The stocking and separating gels were made up in 8 *M* urea.

*Results.* Figure 1 shows that the rate of muscle atrophy is slightly higher in soleus muscle in later denervation periods. The Table shows the changes in contraction time in the peroneus and soleus muscles following denervation. In the course of denervation, the peroneus shows prolongation, the soleus muscle shortening of contraction time. 8 weeks after denervation, the denervated peroneus muscle is 23% slower, the denervated soleus muscle 44% faster than the control muscles. Figure 2 shows that the yield of myosin/g of muscle (wet wt.) decreases progressively during denervation. The amount of myosin obtained from the denervated soleus muscle is much lower than that from the denervated EDL muscle. The method used for isolation of pure myosin cannot be quantitative and only about $^1/_4$ of the total myosin present was extracted from both control EDL and soleus muscles. However, as all preparations are done under strictly identical conditions, the data indicate that the relative amount of myosin decreases with denervation. During the denervation period, the relative content of sarcoplasmic proteins apparently increases; but part of the moysin molecule may change as a consequence of denervation, and thus the isolation procedure may not involve this modified myosin. Figure 3 shows the changes in ATPase activity of myosin during denervation. As expected in view of prolongation of contraction time in the fast, and its shortening in the slow muscle, ATPase activity of myosin decreases in the fast EDL and increases in the slow soleus muscle. Specific ATPase activity of the soleus muscle is almost doubled 8 weeks after denervation. The relation of ATPase activity of myosin and speed of contraction is well known for normal muscles[9].

The spectrum of low molecular weight proteins of myosin was also studied, as it was shown that these proteins are specific and differ in fast and slow muscles, e.g.[10]. It is assumed that some of these low molecular weight proteins are located in or near the active site of the myosin ATPase and thus changes in pattern of these small proteins might suggest how ATPase activity is regulated in vivo. Figure 4 shows the results of an electrophoretic study of proteins released from myosin by *p*-chloromercuribenzoate treatment. The pattern of low molecular weight proteins of myosin from the denervated EDL muscle is partially changed to the pattern of the normal soleus muscle. An opposite trend is observed when proteins of normal and denervated soleus are compared.

*Discussion.* The changes observed in specific ATPase activity demonstrate that changes in contraction prop-


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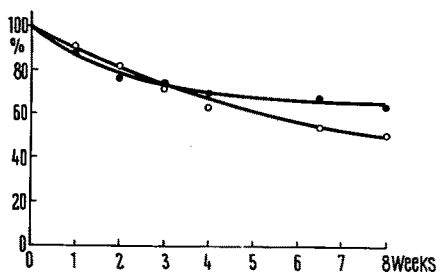


Fig. 1. Wet weight of muscles after denervation expressed in percent of control muscle. ○, EDL; ●, soleus muscle.

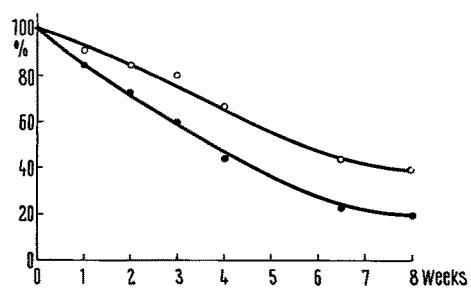


Fig. 2. The yield of myosin per g of muscle after denervation expressed in percent of control muscle. ○, EDL; ●, soleus muscle.

Contraction time of control and denervated rabbit muscles (36°C, in msec)

| Muscle | Control | 1 week after denervation | $p$ | Control | 4 weeks after denervation | $p$ | Control | 8 weeks after denervation | $p$ |
|---|---|---|---|---|---|---|---|---|---|
| | Mean ± SE | | | Mean ± SE | | | Mean ± SE | | |
| No. of animals | 5 | | | 5 | | | 5 | | |
| peroneus | 24.5 ± 1.47 | 20.2 ± 0.97 | < 0.025 | 25.1 ± 1.02 | 30.1 ± 1.63 | < 0.025 | 27.5 ± 0.72 | 33.9 ± 1.42 | < 0.005 |
| soleus | 87.6 ± 8.95 | 63.4 ± 8.51 | < 0.05 | 91.6 ± 8.04 | 54.6 ± 1.93 | < 0.001 | 88.2 ± 4.37 | 48.9 ± 4.76 | < 0.001 |

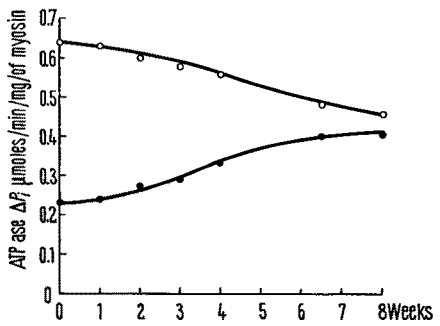


Fig. 3. Adenosine triphosphatase activity of myosin from control and denervated muscles, ○, EDL; ●, soleus muscle. Assayed at 25 °C in 0.05 *M Tris*/pH 7.5/0.025 *M* KCl, 10 m*M* $CaCl_2$, and 5 m*M* ATP.

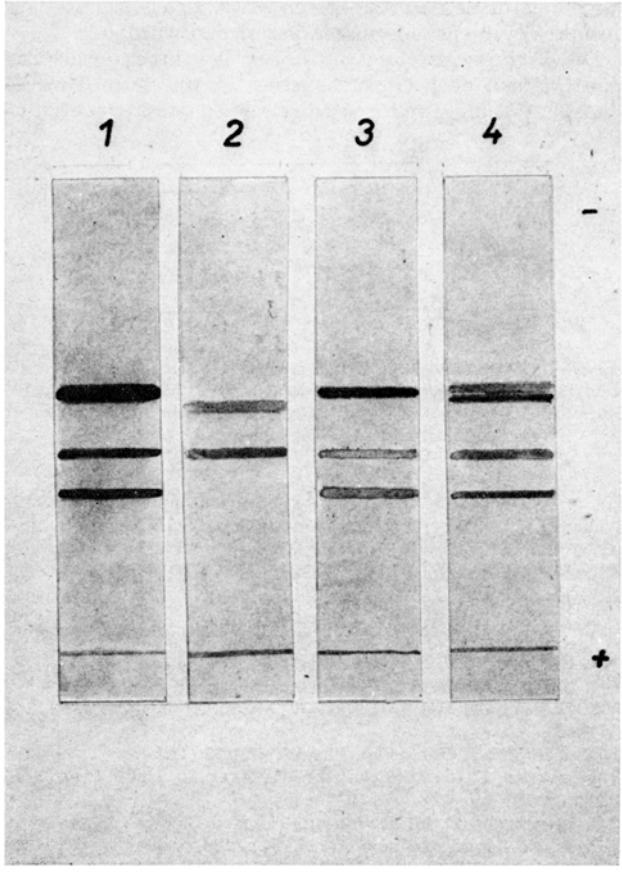


Fig. 4. Effect of denervation (8 weeks) on low molecular weight proteins of slow soleus and fast EDL-muscles myosin. 1. Control EDL. 2. Control soleus. 3. Denervated EDL. 4. Denervated soleus muscle.

erties in denervated muscle are due to alterations in the myosin molecule and not only to a change in the amount of myosin. Moreover, fast and slow muscles react differently to denervation. These are several possibilities of explaining this differential response, which can be generally regarded as a process of dedifferentiation due to loss of nervous regulation. In explaining the mechanisms of the differential behaviour of fast and slow denervated muscles, it must be remembered that both muscles are not homogeneous, containing fibres with high (type II) and low (type I) ATPase activity in different proportions, see[11]. The following mechanisms may be responsible: a) relative independence of slow fibres on neural control, b) preferential atrophy of one type of muscle fibres[12], c) formation of a new type of muscle fibres, and d) shortening of the denervated soleus muscle producing a shortening of contraction time analogous to tenotomy[13].

More data are necessary to answer the question which mechanism is responsible for the differential response of fast and slow muscle to denervation and consequently for the regulation of contractile and enzymatic properties of muscle. The rabbit soleus muscle contains few type II fibres only and preferential atrophy of slow fibres might occur. However, a lower ATPase activity of myosin is observed in the denervated EDL muscle and other factors apparently also operate. Nevertheless, it can be concluded that alterations in the myosin molecule occur after denervation and are reflected by changes of specific ATPase activity of myosin and by the pattern of low molecular weight proteins, related apparently to the synthesis of a different type of myosin molecule or part of it.

*Zusammenfassung.* Nachweis unterschiedlicher Kontraktionsgeschwindigkeiten bei schnellen und langsamen Kaninchenmuskeln sowie entsprechende Unterschiede in der Myosin-ATPase-Aktivität und im Spektrum niedermolekularer Proteine nach Denervation werden gezeigt.

I. Syrový, E. Gutmann and J. Melichna[14]

*Institute of Physiology,*
*Czechoslovak Academy of Sciences,*
*Praha-4-Krc (Czechoslovakia), 3 May 1971.*

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