myocardial function in the adult individual is typically maintained not by stimulation of proliferation, but by hyperplasia of the structures and hypertrophy of the cytoplasm in nondividing myocytes [6].

## LITERATURE CITED


1. L. N. Belov, M. E. Kogan, E. A. Leont'eva, et al., Tsitologiya, No. 11, 1332 (1975).
2. V. Ya. Brodskii, The Development and Regenerative Potential of Cardiac Muscle, ed. by J. Oberpriller, New York (1991), pp. 254-290.
3. V. Ya. Brodskii, A. L. Chernyaev, and I. A. Vasil'eva, Virchow's Arkh. (1991).
4. V. Ya. Brodskii, I. A. Vasil'eva, N. V. Panova, et al., Byull. Éksp. Biol. Med., No. 2, 393 (1989).
5. P. P. Rumyantsev, Int. Rev. Cytol., **51**, 187 (1977).
6. D. S. Sarkisov, V. D. Arutyunov, L. D. Krymskii, et al., Hypertrophy of the Myocardium and Its Reversibility [in Russian], Moscow (1966).
7. C. P. Adler and H. Friedburg, J. Molec. Cell. Cardiol., **18**, 39 (1986).
8. H. Kondo, Shikoku Acta Med., **37**, 281 (1981).
9. W. Sandritter and G. Scomazzoni, Nature, **202**, 100 (1964).
10. J. M. Tas, J. P. Ploeg, and N. S. Cohn, J. Microscop., **119**, 295 (1980).


# EFFECT OF SENSITIZATION ON GUINEA PIG SLOW MUSCLE WITH DISTURBANCE OF NEUROTROPHIC CONTROL


**V. V. Valiullin, A. M. Devyataev, R. R. Islamov, M. E. Valiullina, and A. Yu. Teplov**



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The influence of neurotrophic control on various structural and functional characteristics of muscle fibers (MF) has been studied quite adequately. Repeated investigations have shown that disturbance of neurotrophic control modifies the phenotype of the skeletal muscles [4, 13]. It has been found, for instance, that division of the motor nerve (denervation) affects the set of myosins in MF, and that in fast muscles the content of the slow light chain 1 (LC1) increases, whereas in slow muscles, on the contrary, the concentration of LC3, characteristic of fast myosin, is increased. Unfortunately, virtually all studies of the protein composition of MF when neurotrophic control is disturbed had been undertaken by electrophoretic methods [9], but when isoforms of contractile proteins with closely similar electrophoretic parameters are present, interpretation of the results can be very difficult. The immunologic approach, using antibodies (AB) to concrete proteins or to their fragments [6] must therefore be regarded as the most adequate approach for identification of contractile proteins. Besides neurotrophic control, phenotypes of skeletal muscles are determined also by hormonal influences. There have been many investigations into the endocrine regulation of skeletal muscle function, which have shown that both hormone excess and hormone deficiency can modify various characteristics of skeletal muscles [2, 14]. For instance, hypo- and hyperthyroid-


Department of Histology and Embryology and Department of Pathological Physiology, S. V. Kurashov Kazan' Medical Institute.


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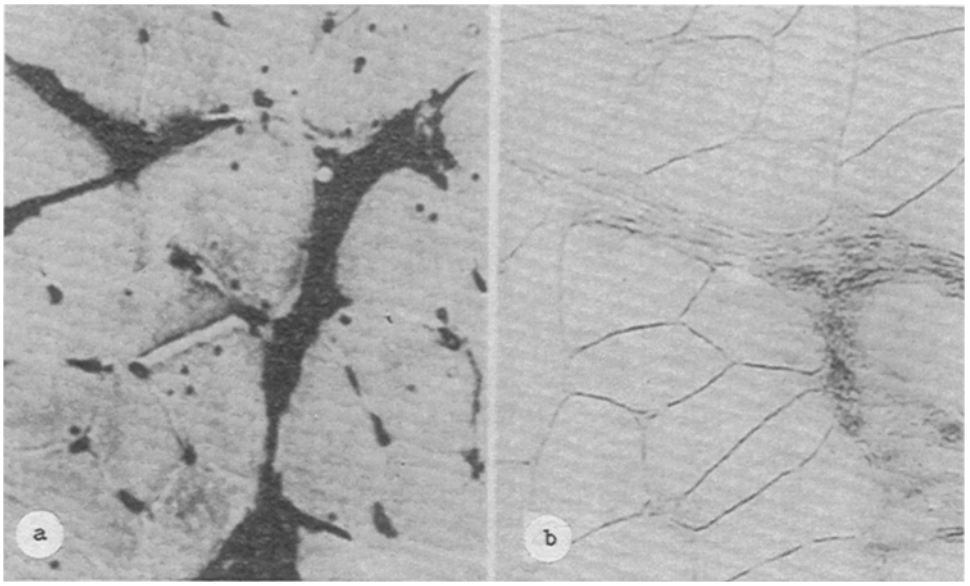


Fig. 1. Soleus muscle of intact guinea pig: a) ATPase activity of myosin (pH 9.4); b) immunohistochemical staining (PAP method) with monoclonal AB to fast myosin heavy chains.

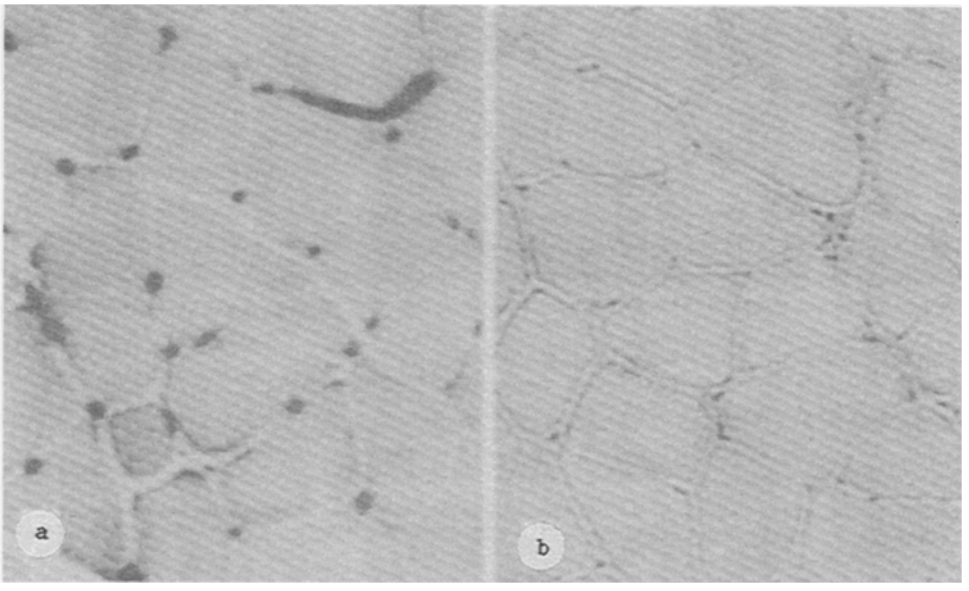


Fig. 2. Soleus muscle of sensitized guinea pig. Legend as to Fig. 1.

ism alter the LC complement of myosin in skeletal muscle fibers [12], and contractile proteins of muscles are sensitive to the glucocorticoid level [7]. Incidentally, the contractile muscle proteins in these investigations were studied mainly by electrophoretic methods. Whereas the role of nervous and humoral factors in the regulation of skeletal muscle phenotypes has been studied sufficiently fully, interaction between these two controlling systems during maintenance of the differentiated state of skeletal muscles still remains unclear [2]. The role of the humoral system likewise is not confined to the working only of the endocrine glands, but it may also include factors unconnected with the action of particular hormones. Thus virtually nothing is still known on how sensitization affects skeletal muscle [11], although it is difficult to suggest that muscle is completely insensitive to processes taking place in the body during allergy.

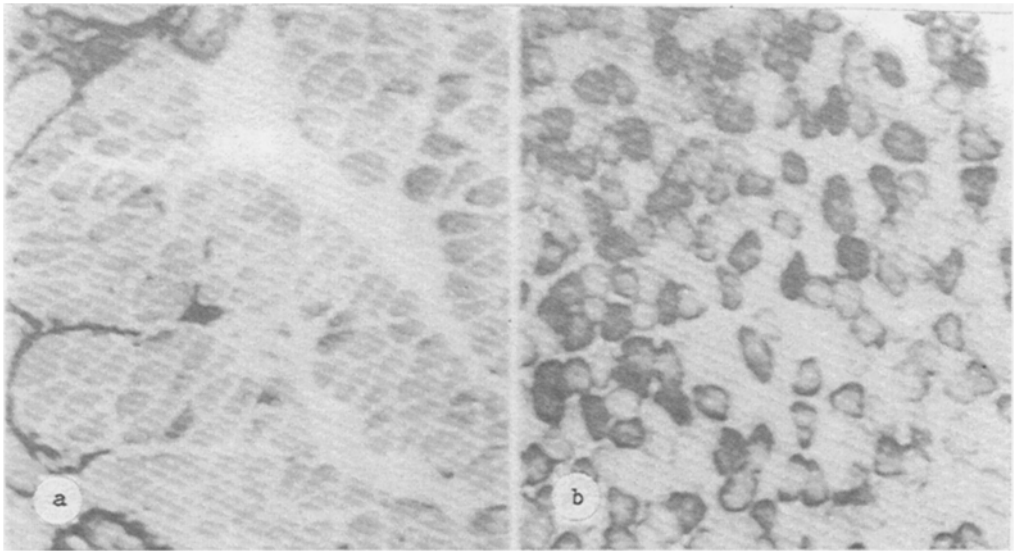


Fig. 3. Denervated soleus muscle of guinea pig after protein sensitization. Legend as to Fig. 1. Pale MF are slow, dark MF are fast.

The aim of this investigation was to study the effect of sensitization on guinea pig slow muscle when neurotrophic control is disturbed.

## EXPERIMENTAL METHOD

The slow soleus muscle of adult male guinea pigs weighing 350-400 g was studied. Activity of myosin ATPase [10] was detected histochemically in frozen sections 8 $\mu$ thick, and direct immunohistochemical staining (by the PAP method) with monoclonal AB to fast myosin heavy chains (Sigma) also was carried out. The sciatic nerve was divided in the animals of one group (n = 6) and guinea pigs of the other group received two subcutaneous injections of antigen (AG) into the left thigh (the second injection was given 14 days after the first). Animals of the 3rd group began to be sensitized 1 week after division of the nerve. Sensitization was carried out with solution containing 10 $\mu$g ovalbumin with 1 mg of dry aluminum hydroxide gel in 1 ml of physiological saline per animal. The level of sensitization was monitored by the passive cutaneous anaphylaxis test [5], and by thin-layer immune analysis [8], and on the 21st day of the experiment the AB titer was: 1/256-1/1024 and 1/256-1/512 respectively. The muscles were studied 3 days after the experiment began. The methods of the operations performed and details of the controls were described by the writers previously [1, 2].

## EXPERIMENTAL RESULTS

The soleus muscle of the intact guinea pig consists entirely of slow type I MF with low ATPase activity (Fig. 1a) and not reacting with AB to fast myosin (Fig. 1b). Denervation did not change the histochemical profile of the muscle, as reflected both in the level of ATPase activity and the results of staining with monoclonal AB [3].

Sensitization likewise did not affect the histochemical characteristics of the muscle: all MF were identified as type I slow fibers (Fig. 2a, b). Injection of AG when neurotrophic control was disturbed led to the appearance of MF in the soleus muscle that reacted with AB to fast myosin (Fig. 3a), whereas histochemical staining for ATPase demonstrated the homogeneity of the muscle – all MF had weak enzyme activity, and must accordingly be classed as type I slow MF (Fig. 3b).

The results are evidence that neither sensitization nor denervation separately changed the parameters of MF which we studied, but after division of the nerve the muscle became sensitive to the action of AG; under these conditions, moreover, induction of fast myosin synthesis took place in some MF. This action of AG on denervated muscle is difficult to explain in terms of present-day knowledge of the processes taking place in a muscle during sensitization. We again recorded disparity between the ATPase activity of myosin and its qualitative composition, which is not in agreement with

data indicating that such correlation exists [15]. This fact is further confirmation of the hypothesis which we put forward previously [3], according to which ATPase activity is not always a reliable marker for histochemical typing of MF.

## LITERATURE CITED


1. V. V. Valiullin and N. P. Rezvyakov, Byull. Éksp. Biol. Med., **96**, No. 9, 38 (1983).
2. V. V. Valiullin and N. P. Rezvyakov, Byull. Éksp. Biol. Med., No. 11, 521 (1986).
3. V. V. Valiullin, R. R. Islamov, M. E. Valiullina, and G. I. Poletaev, Byull. Éksp. Biol. Med., No. 2, 201 (1991).
4. E. M. Volkov, Usp. Fiziol. Nauk, **20**, No. 2, 26 (1989).
5. P. I. Anderson and H. K. Bernstad, Brit. J. Phys., No. 4, 601 (1981).
6. C. Cecarelli, V. Eusebi, and G. Bussolati, Basic Appl. Histochem., **30**, No. 2, 139 (1986).
7. A. F. Clark and P. G. Vignos, Muscle Nerve, No. 2, 265 (1979).
8. H. Elwing, S. Lange, and H. Hygren, J. Immunol. Meth., **38**, No. 3/4, 257 (1980).
9. M. C. Gardahaut, A. Khaskiye, P. Rouaud, et al., Med. Sci. Res., **15**, 1525 (1987).
10. L. Guth and F. J. Samaha, Exp. Neurol., **28**, 365 (1970).
11. J. B. Harris, Trends Neurosci., **3**, No. 9, 224 (1980).
12. C. D. Ianuzzo, P. Patel, V. Chen, et al., Nature, **270**, 74 (1977).
13. R. Matsuda, D. Spector, and R. C. Strochman, Proc. Nat. Acad. Sci. USA, **81**, 1122 (1984).
14. T. P. Seene and K. Alev, J. Steroid Biochem., **22**, 767 (1985).
15. R. S. Staron and D. Pette, Histochemistry, **86**, 19 (1986).