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# EFFECT OF FERRIC CHLORIDE ON DENERVATED SKELETAL MUSCLES

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The transferrin-like glycoprotein sciatin [6, 8], the iron-transporting protein transferrin [6, 7, 10], and even trivalent ferric ions [5, 9] have a differentiating effect on myogenic skeletal muscle cells developing *in vitro*. In particular, 10-100 μM of $Fe^{+++}$ increases the number of clustered acetylcholine receptors on the surface of muscle tubes [5]. Injection of transferrin bound with $Fe^{+++}$ into a denervated skeletal muscle *in situ* also changes the expression of acetylcholine receptors [11], thereby partially preventing the development of a denervation syndrome with respect to this feature. These facts suggested that the factor responsible for neurotrophic control of skeletal muscle is transferrin. To determine the mechanism of the effect of transferrin and also of $Fe^{+++}$ on muscle, it was decided to study the action of these factors on skeletal muscles.

This paper givea the results of a study of the effect of inorganic iron on some histochemical and morphometric characteristics of denervated skeletal muscles.

## EXPERIMENTAL METHOD

Experiments were carried out on 20 non-inbred male albino rats in which the sciatic nerve was divided unilaterally under ether anesthesia under sterile conditions, and on 10 rats which, after division of the nerve, received an intraperitoneal injection of $10^{-6}$ M $FeCl_3$ (1 ml/100 g body weight) daily for 3 days. The animals were weighed at the beginning and end of the experiments. The rats were decapitated under deep ether enesthesia. The soleus and plantaris muscles were isolated and weighed, and myosin ATPase activity [4] and succinate dehydrogenase (SDH) activity, with the aid of nitro-BT [2], were determined in frozen sections 10 μ thick. The relative number of muscle fibers of different types was counted and the area of their cross section measured in histological sections. Muscle homogenates with equal protein content were subjected to polyacrylamide gel disc electrophoresis [1]. Lactate dehydrogenase (LDH) activity was determined and the gels were photographed. Densitometry of the negatives was done on an IFO-451 dual-beam recording microphotometer. The results were subjected to statistical analysis by the t test [3]. A 0.05 level of significance was adopted.

TABLE 1. Relative Number (in %) and Area of Cross Section (in $\mu^2$) of Muscle Fibers in Control and Experimental Animals ($X \pm S_X$)

| | Soleus muscle | | Plantaris muscle | |
|---|---|---|---|---|
| | I | II | I | II |
| Control | 78,2±2,6<br>2400±112,8 | 21,8±2,6<br>2960±75,3 | 16,8±1,7<br>2722,6±92,9 | 83,2±1,7<br>3162,4±89,5 |
| Experimental | 80,6±3,1<br>2594,4±102,6 | 19,8±3,2<br>3562,8±86,2* | 27,6±3,2*<br>2612,6±78,4 | 72,3±3,2<br>3224,1±149,5 |

I) Type I of muscle fibers, II) type II.
Note. Asterisk indicates significant differences compared with control.

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TABLE 2. Activity of LDH Isozymes (in %) in Control and Experimental Animals ($X \pm S_X$)

| Isozyme | Soleus muscle | | | | Plantaris muscle | | | |
|---|---|---|---|---|---|---|---|---|
| | experiment | | control | | experiment | | control | |
| | A | B | A | B | A | B | A | B |
| $LDH_1$ | 19,8±5,6 | 26,8±4,2 | 19,6±2,6 | 25,9±1,5 | 14,5±3,7 | 12,8±3,8 | 12,3±1,2 | 14,7±0,6 |
| $LDH_2$ | 27,4±1,1 | 31,8±1,9 | 29,4±7,5 | 28,9±2,3 | 20,8±2,1 | 16,1±1,6 | 24,4±1,7* | 18,5±0,8 |
| $LDH_3$ | 22,3±2,4** | 24,1±3,3 | 30,2±1,6 | 22,8±1,6* | 28,0±2,5 | 20,2±1,4 | 24,7±1,1* | 20,4±1,2 |
| $LDH_4$ | 15,2±2,3 | 11,8±1,7 | 17,5±1,7 | 17,3±1,8 | 23,5±2,9 | 25,9±2,3 | 30,7±3,4 | 24,1±1,1 |
| $LDH_5$ | 8,2±1,1** | 5,4±1,6 | 3,1±0,5 | 4,5±1,9 | 13,2±2,7 | 21,3±3,7 | 8,4±2,8** | 22,2±1,4* |

Note. A) Denervated muscle; B) homonymous contralateral muscle. *) Significant differences on comparison of denervated and contralateral muscles in control; **) differences between homonymous muscles in control and experiment.

## EXPERIMENTAL RESULTS

No difference was found in the weight of the rats of the control (muscle denervation only) and experimental (denervation + $FeCl_3$) groups. The weight of the muscles in the intact limb of animals of both groups was significantly greater than the weight of the muscles on the side of denervation; although the difference was greater in the experimental animals than in the controls, it was not statistically significantly greater.

No differences were found between the control and experimental groups as regards the relative number of muscle fibers of types I and II in the soleus muscle (Table 1). The relative number of type I muscle fibers was increased, and of type II fibers reduced, in the denervated plantaris muscle of the experimental animals (Table 1). Muscle fibers of types B and C were identified in the soleus muscle of both groups of animals on staining for SDH, whereas in the denervated pantaris muscle, by contrast with the contralateral plantaris muscle and with the same muscle in animals receiving $FeCl_3$, all the muscle fibers had about equal SDH activity, corresponding to its level in type B muscle fibers.

The area of the type II muscle fibers in the soleus muscle of animals of the experimental group was significantly greater than in the control (nerve division only), but the area of the type I muscle fibers in the experimental group did not exceed that of the control statistically significantly. The area of the muscle fibers of the plantaris muscle was the same in the experiment and control (Table 1).

After division of the nerve changes took place in the content of the LDH isozymes: The $LDH_3$ level rose in the soleus muscle and the $LDH_2$ and $LDH_3$ levels in the plantaris muscle, but the $LDH_5$ content decreased. After injection of $FeCl_3$ the $LDH_3$ level in the soleus muscle fell while the $LDH_5$ level rose. In the plantaris muscle the LDH spectrum after injecton of $FeCl_3$ was the same as that in the control (Table 2).

The intact rat soleus muscle is characterized by an H type of LDH spectrum (predominance of $LDH_1$ and $LDH_2$), whereas the plantaris muscle has the M type of spectrum (predominance of $LDH_4$ and $LDH_5$). Denervation did not affect the content of H and M subunits in the soleus muscle, whereas in the plantaris muscle the content of H and M subunits after denervation was equal. In animals receiving $FeCl_3$ the soleus muscle preserved its intrinsic type of H and M subunits, whereas in the plantaris muscle the content of the H subunit was increased, but not significantly.

The results of this investigation are evidence that these two muscles respond differently to injection of $FeCl_3$. The parameters chosen (the number of different types of muscle fibers, of H and M subunits, the area of cross section of the fibers, LDH isozyme activity) are criteria by which the limits of the phenotypic features of muscle fibers can be estimated quantitatively. In the denervated soleus muscle features of the denervation syndrome was abolished after injection of $FeCl_3$, as could be judged from the increase in area of cross section, especially of the type II muscle fibers, and from the LDH isozyme spectrum. In the plantaris muscle there was no difference between the LDH isozyme spectra of the control and experimental animals, but the fact as noted that during demonstration of SDH activity in that muscle, it was impossible to identify the three types of muscle fibers characteristic of the intact muscle. These findings indicate that a change takes place in metabolism of the muscles. SDH is the marker enzyme of the mitochondria, and a change in its activity may evidently indicate structural changes in the mitocondria. The results also suggest that structural

changer may take place not only in the mitochondria, but also in the contractile system and in the satellite cells, as is confirmed by the change observed in the relative numbers of different types of muscle fibers in the plantaris muscle. Our data are evidence that $FeCl_3$ participates in the mechanism of neurotrophic control of skeletal muscles by partially preventing the development of a denervation syndrome in the soleus (slow) muscle.

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