Histochemical Studies on the Disorders of Muscle.


Masanori Uono, M. D., Hitoshi Tanabe, M. D., Satoshi Ueda, M. D. and Masanori Tomonaga, M. D.

The Third Department of Internal Medicine, (Director: Prof. S. Okinaka, M. D.)
Faculty of Medicine, University of Tokyo.

Nowadays it is not sufficient to account for the etiology of muscular disorders by morphological study alone. According to many experimental results, metabolic especially enzymological changes of the muscle in several disorders are so different from normal's.

On the other hand, it has been divided that the striated muscles consist of two different kinds of muscle fibers, macroscopically red and white muscles, since Ranvier 1874. And it was clarified recently that the white muscle was a rapid-contracting, kinetic, pyramidal motor fiber, while the red muscle was a slow-contracting, tonic, extrapyramidal fiber.

And the white, thick muscle fibers, rich in sarcoplasm, mitochondria and glycogen, have contained a lot of phosphorylase (PhR) in related with anaerobic glycogenolytic pathway; while the red, thin muscle fibers, poor in sarcoplasm and mitochondria, rich in lipid, have contained succinic dehydrogenase (SD) in related with TCA-cycle.

Then enzymological changes of red and white muscles in various muscular disorders have been investigated by means of proving histochemically the existence of PhR and SD activity in muscle fibers; and the changes of cholinesterase (ChE) activity in the muscular endplates were investigated simultaneously.

In this report, we showed our results and discussions with regard to change of these enzymes in experimental atrophied muscles after denervation of sciatic nerve and in a few muscular diseases.

Materials and methods

Anesthetized dogs were operated, one side of sciatic nerve was cut about 3 cm length on major trochanter. After operation, they were sacrificed successively among a week to 9 months. Then we applied the histochemical methods on the materials: gastrocnemius-, soleus- and anterior tibial muscles, sweat glands of paw, sciatic nerve and spinal cord (L1-S3).

PhR activity was demonstrated by using Takeuchi & Kuriaki's jodine method, SD was by Rutenburg & Seligman's neotetrazolium method and ChE was by Koelle's thiocholine method respectively.
Results

1) M. gastrocnemius

PhR activity was markedly reduced for a month, and was completely disappeared after 6 months, exclusive of healthy thick muscle fibers (Fig. 1, 2). On the other hand, SD activity was fairly remained in the red muscle fibers, and about 4 months after denervation it was gradually diminished in the atrophied muscle fibers (Fig. 3, 4). ChE activity in the muscular end-plates was also reduced from about 5 months and morphological changes (atrophy, elongation, elaboration etc.) were observed (Fig. 5, 6).

2) M. soleus

Reduction of PhR activity in the atrophied muscle fibers was observed at about 4 months (Fig. 7, 8), SD and ChE activities were changed as same as in gastrocnemius (Fig. 9, 10).

3) M. tibialis anterior

PhR activity was reduced by moderate speed between gastrocnemius and soleus, and it was observed the same tendency to change in SD and ChE activities (Fig. 11, 12, 13, 14).

4) Sweat glands in the denervated paw

![Fig. 1 M. gastrocnemius of the dog (normal), cross-section, phosphorylase. (×100)](image1)

![Fig. 2 M. gastrocnemius of the dog 4.5 months after denervation of sciatic nerve, cross-section, phosphorylase. (×100)](image2)

![Fig. 3 M. gastrocnemius of the dog (normal), cross-section, succinic dehydrogenase. (×100)](image3)

![Fig. 4 M. gastrocnemius of the dog, 8 months after denervation of sciatic nerve, cross-section, succinic dehydrogenase. (×100)](image4)
PhR and SD activities in sweat glands were kept better than in the muscles, but ChE activity in the nerve fibers and terminal apparatus were markedly disappeared from 4~5 months after denervation.

5) Spinal cord (L₄-S₈)

Each enzymatic activities in anterior horn cells were diminished and
atrophy of the cells were observed in varying grades.
6) Central cutting portion of sciatic nerve

Internal (stored) ChE in the cutting portion of sciatic nerve was observed within a week and gradually disappeared in a month or more.

**Comment**

1) PhR reduction in the white muscles after denervation was rapidly found in gastrocnemius (rich in white muscle) and was more slowly in tibialis and soleus (rich in red muscle), whereas SD activity after denervation was relatively well kept in long time in each muscle.

Based on this assumption, it is possible to think white muscle groups (pyramidal) take place in dysfunction prior to red muscle groups (extrapyramidal).

By the way, PhR-defect in dystrophied muscles was found since about a month after sympathetic ganglionectomy in infant dogs²⁴,¹⁰).

2) ChE reduction and deformities in the muscle endplates were found from 3~4 months after denervation, but it is not so intense as in myasthenic muscles¹,³,¹⁷).
Summary

Metabolic disturbances of the muscle fibers and morphological changes of the muscle endplates show so many different attitudes from each disorder of the muscle that these findings are clues to reveal the etiology, diagnosis and treatment of these diseases.

Acknowledgement

Authors’ acknowledgement is due to Prof. S. Okinaka and Ass. Prof. M. Yoshikawa for their valuable guidances and advices.

References


Discussion

Dr. Takeuchi: I’m very glad for your practical application of the phosphorylase reaction. My coworkers, Dr. Kobayakawa et al, have reported that phosphorylase reaction of skeletal muscles promoted its intensity just after the denervation and then gradually diminished. Did you obtain such a finding in your observations?

Dr. Uono: We have not yet studied about the early reaction of phosphorylase after denervation (1 week) and will examine in future.

Effect of Adenosine-5-Phosphate, Adenosine Triphosphate, and Adenosine-3', 5'-Cyclic Phosphate Upon the Histochemical Phosphorylase Reaction

Tadao Takeuchi, Yasuya Hirata and Goyo Koya

Department of Pathology, Kumamoto University School of Medicine, Kumamoto, Japan

According to the biochemical knowledge\(^{1-3,7-12,19}\), there are two forms of phosphorylase, the one, phosphorylase a, being active in the absence of adenosine-5'-phosphate (AMP), the other, phosphorylase b, requiring the addition of AMP for the expression of its activity, and phosphorylase a and b