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Takuro Ogata*

Masahiko Mori†

*Okayama University,

†Osaka University,

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Takuro Ogata and Masahiko Mori

Abstract

Histochemical studies of several oxidative enzymes of the muscle spindles in mouse limbs were carried out. Observations elucidated the existence of the three types of intrafusal fibers which can be distinguished from each other by the difference in the activity of oxidative enzymes as in the muscle fibers, i. e., the first one is the "red intrafusal fibers" and has the most intense activity of oxidative enzyme, the second one the "white intrafusal fibers" with a minimal activity, and the third, "medium intrafusal fibers" being distinguished by their moderate activity of oxidative enzymes from the others.

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HISTOCHEMICAL DEMONSTRATION OF THE THREE TYPES OF INTRAFUSAL FIBERS OF MUSCLE SPINDLES, A STUDY ON OXIDATIVE ENZYMES

Takuro OGATA and Masahiko MORI*

*Department of Surgery, Okayama University Medical School
Okayama, (Director: Prof. D. Jinnai)*

**Department of Oral Surgery, Osaka University, Osaka
Director: Prof. K. Kawakatsu*

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In the previous papers¹⁻⁴ it has been reported that the striated muscle fibers of mammals can be divided into three types from their activities of the histochemically demonstrable oxidative enzymes, i. e. the first one having the highest activity is the smallest in diameter, which corresponds to the red muscle fibers, the second one is the lowest in activity, larger in size, and the third having the moderate enzyme activity and being called "medium fibers" by the authors, medium in size as compared with the other two. Correlated with these the authors have studied the oxidative enzyme activity of intrafusal fibers of the muscle spindle and it has been demonstrated that the intrafusal fibers can also be classified into three types from their activities of the oxidative enzymes as in the striated muscle fibers of mice.

MATERIALS AND METHODS

The striated muscles of limbs in healthy adult mice served as materials. The muscles were removed after sacrificing the animals under ether anesthesia, and cut into sections 20—30 μ thick in a cryostat at -16°C and then mounted on a slide glass. For the histochemical demonstration of succinoxidase, lactic dehydrogenase, malic dehydrogenase, α -glycerophosphate dehydrogenase, and DPNH-diaphorase and TPNH-diaphorase were employed, as in the method described by PEARSE⁵. After the incubation at 37°C for 30 minutes, the slides were mounted by glycerin without dehydration.

RESULTS

The intrafusal fibers of mouse muscle spindles were divided into three types according to the activities of oxidative enzyme reaction as the case with striated muscle fibers (Fig. 1). The smaller intrafusal fibers showed a higher activity of succinoxidase and contained a greater amount of formazan, while

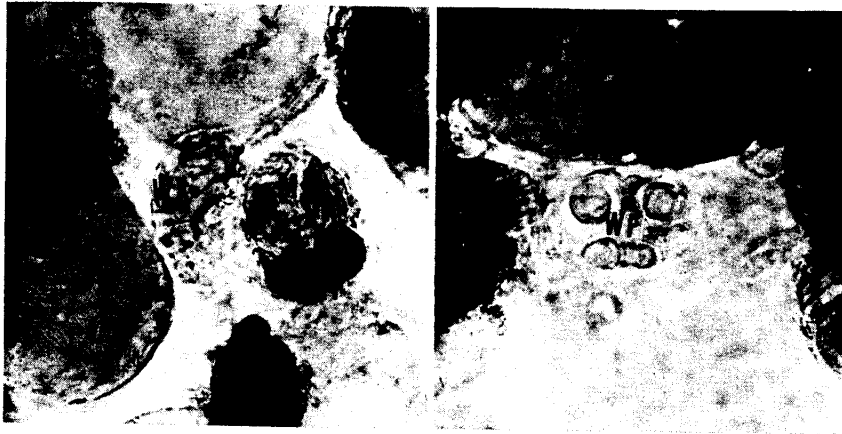


Fig. 1

Fig. 2

Fig. 1. The Muscle Spindle of M. Gastrocnemius in Mouse ($\times 400$).
Showing succinoxidase reaction. Note the muscle spindles composed of the red intrafusal fibers (intense activity, RF) and the medium intrafusal fibers (intermediate activity, MF) and the left side muscle spindle is composed of the white intrafusal fibers (minimal activity, WF).

Fig. 2. The Muscle Spindle of M. Gastrocnemius in Mouse ($\times 400$).
Showing succinoxidase reaction. Note the muscle spindle composed of the white intrafusal fibers only.

the larger intrafusal fibers a lower activity. And the medium sized intrafusal fibers showed an intermediate activity between the smaller intrafusal fibers and the larger fibers. The activity of succinoxidase of the smaller fibers corresponded to that of the red muscle fibers, and the medium and larger intrafusal fibers showed the same degree of activity as the medium and white muscle fibers.

Thus the smaller intrafusal fibers with a marked activity were designated as "red intrafusal fibers", the larger ones as "white intrafusal fibers" and the medium ones as "medium intrafusal fibers" in the present paper.

Most intrafusal fibers had shown such a relationship as mentioned above between the size and succinoxidase reaction, but some intrafusal fibers revealed no such a relationship, e. g. some white intrafusal fibers were small in size.

Some of the muscle spindles were composed of only one type of the intrafusal fibers, while the others were of different types of the intrafusal fibers.

Similar findings were observed in the histochemical reactions of lactic dehydrogenase, malic dehydrogenase, α -glycerophosphate dehydrogenase, DPNH-diaphorase and TPNH-diaphorase, i. e., the red intrafusal fibers showing a higher activity of these enzymes, the medium intrafusal fibers a moderate activity and the white intrafusal fibers the lowest.

DISCUSSION

Muscle spindles were known as the sensory receptors in the striated muscle (COOPER)⁶. Recently, KONDO (1957)⁷ reported that two types of intrafusal fibers were distinguished in cat muscle spindles by Sudan black B staining. In our previous papers (OGATA¹⁻³, 1958; OGATA and MORI⁴, 1961, and TSUKAMOTO and MORI⁸, 1962) it was reported that three types of muscle fibers, namely, the red, white and medium muscle fibers were distinguished in mammalian muscle fibers. In the present study, the three types of intrafusal fibers are differentiated in the mouse muscle spindles by oxidative enzyme reactions. And the three types of intrafusal fibers have been designated as "red intrafusal", "medium intrafusal" and "white intrafusal fibers", respectively.

The metabolism of intrafusal fibers has been left almost unclarified. KARL and BRZENSKI (1961)⁹ stated that the spindle space contained a high amount of hyaluronic acids and it showed no lymphatic system on either PAS or Hale-PAS stain. But the present study revealed that the three types of intrafusal fibers showed the same oxidative enzymatic activity as that of the three types of the muscle fibers already described. From the present results, it may reasonably be assumed that the metabolic activity in intrafusal fibers is similar to that of muscle fibers. Since mitochondria contain a larger amount of oxidative and reducing enzymes, it may be safe to assume that the red intrafusal fibers contain a considerable number of mitochondria, while the white intrafusal fibers contain a little or none of them. As the other vertebrates possess three types of muscle fibers, it is likely that they may have three types of the intrafusal fiber as well. This problem, however, needs further investigation.

SUMMARY

Histochemical studies of several oxidative enzymes of the muscle spindles in mouse limbs were carried out. Observations elucidated the existence of the three types of intrafusal fibers which can be distinguished from each other by the difference in the activity of oxidative enzymes as in the muscle fibers, i. e., the first one is the "red intrafusal fibers" and has the most intense activity of oxidative enzyme, the second one the "white intrafusal fibers" with a minimal activity, and the third, "medium intrafusal fibers" being distinguished by their moderate activity of oxidative enzymes from the others.

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