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Takuro Ogata*

*Okayama University,

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Abstract

From the histochemical studies of cytochrome oxidase on the striated muscles of the cats and mice, the following results have been obtained. The three types of muscle fibers are distinguishable by the activity of cytochrome oxidase from each other as by the succinic dehydrogenase activity, namely, the red muscle fibers show a high cytochrome oxidase activity, the medium fibers a moderate activity, and the white muscle fibers a low activity. And it is suggested that these three types of fibers are different in their respiration, metabolism and have the different functions.

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**A HISTOCHEMICAL STUDY OF THE RED AND WHITE
MUSCLE FIBERS
PART II ACTIVITY OF THE CYTOCHROME OXIDASE
IN MUSCLE FIBERS**

Takuro OGATA

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

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From the histochemical study of the striated muscles, the author reported in the former paper⁵ that the red muscle fibers show a high succinic dehydrogenase activity, while the white muscle fibers a low activity. In this report, the author will describe that the histochemical cytochrome oxidase reaction shows the same localization as the succinic dehydrogenase reaction.

MATERIALS AND METHODS

The striated muscle of healthy adult cats and mice of both sexes were used. The muscles of animals were removed immediately after the animals were killed, anesthetized with ether. For the histochemical demonstration of cytochrome oxidase system, the method established by GRAFF² was applied.

OBSERVATIONS AND RESULTS

M. gastrocnemius of the cat which belongs to the white muscle by naked eye, is shown to be composed of the three types of muscle fibers, which are distinguishable by the histochemical cytochrome oxidase reaction from each other as in the findings of the succinic dehydrogenase reaction reported in the former paper⁵. As shown in Fig. 1, the large fibers (W) show the low activity of the cytochrome oxidase, being stained blue slightly. Sudan black B staining indicates that these fibers can be stained faintly as indicated in the first paper⁵, and suggested that they are the white muscle fibers according to the concept presented by DENNY-BROWN¹. Besides these, there are small ones, almost half the white muscle fibers in diameter. They show a higher cytochrome oxidase activity and have an intense affinity to Sudan black B, showing they are the red muscle fibers.

The medium sized fibers (M) are distinguishable by their moderate size and moderate cytochrome oxidase activity, which is intermediate between those of the red and white muscle fibers.

M. soleus of cat which belongs to the red muscle by naked eye, is composed of the fibers giving an intense activity of cytochrome oxidase as shown in Fig. 2. But close observation proves that there are a few muscle fibers showing a rather low enzymatic activity, though they are almost the same in size as those showing an intense activity. Among those fibers of high activity, the two types of fibers can be distinguished from each other on the cytochrome oxidase activity, the comparatively strong fibers and the weak. The former may correspond to red fibers and the latter to medium fibers.

The muscle fibers composed of *diaphragma* of the cat proves to be distinguished by their activity in the cytochrome oxidase reaction as in the case of *M. gastrocnemius*.

M. gastrocnemius of mouse, as shown in Fig. 4, also shows to be composed of three types of muscle fibers by this reaction.

DISCUSSION

In the former paper⁵ it was shown that the red muscle fibers show a high succinic dehydrogenase activity, the medium fibers a moderate activity, and the white muscle fibers a low activity. It was further shown that the difference in the succinic dehydrogenase reaction observed among the three types of fibers, is to be attributed to the difference in number and activity of mitochondria contained in them. As described above, by the histochemical cytochrome oxidase reaction, too, these three types of muscle fibers are distinguishable from each other as in the findings of the succinic dehydrogenase reaction, showing the highest activity in red muscle fibers, moderate in medium fibers and the lowest in white fibers. The granules appear as small round or rod particles as observed in succinic dehydrogenase reaction.

Since, as is well known, the cytochrome oxidase is the enzyme or enzyme complex which catalyzes the oxidation of the reduced cytochromes by molecular oxygen and closely related with the succinic dehydrogenase cytochrome system as a member of succinoxidase system, it is a reasonable result that the cytochrome oxidase reaction shows the same localization as that of the succinic dehydrogenase reaction on tissue sections. Consequently this histochemical reaction shows the actual site of the intracellular localization of the enzyme. According to GREEN³ cytochrome

oxidase and the entire succinoxidase system are exclusively located in mitochondria. Then this reaction indicates the localization and the activity of the mitochondria in muscle fibers. Morphologic picture of the granules giving positive reaction supports this view.

HAUROWITZ and HARDIN⁴ showed that the difference of color between the red and white muscle ascribed to myoglobin and cytochromes within the muscle fibers. The author's observation proves that the red muscle fibers show the high activity of cytochrome system, suggesting that they are rich in cytochromes, though their myoglobin contents are unknown. Therefore, it is reasonably inferred that the three types of muscle fibers which are different from each other in the contents or activity of cytochromes as well as in the number of mitochondria have the different respiratory activity and metabolic process, i. e., the different function specific to each types of fibers. The smooth contraction of the muscle bundle as a whole will be brought about by the harmonized co-operation of the fibers of these three types in the metabolism and the mobility.

SUMMARY

From the histochemical studies of cytochrome oxidase on the striated muscles of the cats and mice, the following results have been obtained.

The three types of muscle fibers are distinguishable by the activity of cytochrome oxidase from each other as by the succinic dehydrogenase activity, namely, the red muscle fibers show a high cytochrome oxidase activity, the medium fibers a moderate activity, and the white muscle fibers a low activity. And it is suggested that these three types of fibers are different in their respiration, metabolism and have the different functions.

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EXPLANATION OF FIGURES

- Fig. 1. M. gastrocnemius of the cat, cross section, cytochrome oxidase reaction. Note the three types of muscle fibers, namely, the small fiber (R) has a higher enzyme activity, the large fiber (W) a lesser activity and the medium sized fiber (M) a moderate activity. $\times 100$.
- Fig. 2. M. soleus of the cat, cross section, cytochrome oxidase reaction. Note the existance of two types of muscle fibers, namely, a higher active type of fiber (R) and slightly lesser active type of fiber (M). $\times 100$.
- Fig. 3. Diaphragma of the cat, cross section, cytochrome oxidase. Note the three types of muscle fibers. $\times 100$.
- Fig. 4. M. gastrocnemius of the mouce, cross section, cytochrome oxidase reaction. Note the three types of muscle fibers. $\times 400$.

