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Takuro Ogata*

*Okayama University,

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**Abstract**

An electon microscopic study on the structural differences among the red, white and intermediate muscle fibers of mice was made and the following results were obtained. 1. The red fiber contained very numerous mitochondria, the white fiber a few and the intermediate fiber a moderate number. The distribution of mitochondria was different in each type of muscle fiber. The cristae of mitochondria of the red fiber was quite well developed, that of the white fiber poorly and that of the intermedtate fiber moderately. 2. Sarcoplasmic reticulum of the white fiber was considerably well developed but that of the red and intermediate fibers poorly developed. 3. Glycogen particles were abundant in the white fiber, less in the intermediate fiber and least in the red fiber.

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# AN ELECTRON MICROSCOPIC STUDY ON THE RED, WHITE AND INTERMEDIATE MUSCLE FIBERS OF MOUSE

Takuro OGATA

*Department of Surgery, Okayama University Medical School, Okayama, Japan (Director: Prof. S. Tanaka), Present address: Yamins Laboratory, Beth Israel Hospital, Boston, Mass., U. S. A.*

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In the previous papers[1,2], it has been reported that the mammalian muscle fibers are divided into three types from their oxidative enzyme activities, i. e., the smaller red muscle fiber having the strongest activity of oxidative enzymes, the larger white fiber having a low activity and the "medium fiber" or "intermediate fiber" with a moderate activity.

The structural differences between the red and white muscle fibers have been observed under electron microscopic studies by some workers[3,4,5], revealing some different amount and distribution of mitochondria and sarcoplasmic reticulum between these two fibers. However, no detailed electron microscopic observation has been reported on the intermediate fiber. In the present paper, the structural differences among these three kinds of muscle fibers are reported.

## MATERIALS AND METHODS

For electron microscope study, the central part of the *caput mediale* of *M. triceps brachii* of mice was used as the red fiber specimen and the most superficial layer of the *caput longum* of *M. triceps brachii* was used as the white fiber specimen. From the observation of histochemical succinoxidase reaction, these two specimens were found to be composed of only the red or white fibers respectively. To select the intermediate fiber, thin muscle specimens having diameter of about 1 mm and 4 mm in length were removed from the central part of *caput longum* of *M. triceps brachii.* From these specimens very thin strips having diameter of about 0.1 mm were dissected as quickly as possible with two needles in physiologic saline solution. Each strip was placed lengthwise and cut into halves; one half of these strips was immediately immersed into fixative for electron microscopy and the other half was incubated in histochemical reaction mixture of succinoxidase at 35°C for 30 minutes. In examining the histochemical results of these strips, it was observed that a few of them were composed only of intermediate fibers and some of them had an occasional red or white fiber mixed in them. The other half of each selected strip was used as the intermediate fiber specimen for electron microscopy. How-

ever, by this method the fine structure of muscle fiber was somewhat destroyed. Therefore, the sections of the central part of *caput longum*, promptly fixed after removing from the muscle, were also used for electron microscope observation. By comparing the electron microscope picture made by the above two different methods, the structural features of the intermediate fibers were identified.

The mice were anaesthetized with ether, then the muscle was exposed. 1% osmium tetroxide aqueous solution (buffered at pH 7.4 with veronal acetate buffer) or 7% formaldehyde aqueous solution (unbuffered) was applied on the surface of exposed muscle for 5 minutes. Then the muscle was dissected into slice of 1 mm. When 1% osmium tetroxide solution was used as fixative, the slices were immersed in it at 3°C for 3 hours. When 7% formaldehyde solution was used as fixative, the slices were immersed in it at 3°C for 3 hours, then they were immersed in 1% osmium tetroxide solution at 3°C for 3 hours. The former method is better to observe sarcoplasm and the latter method to observe myofibril. The fixed blocks were washed quickly with distilled water and transferred to 50% ethanol, dehydrated and embedded in epoxy resin. The sections were stained according to Lawn's method.[6] The section was observed with the the HU-11P electron microscope of the Hitachi Co.

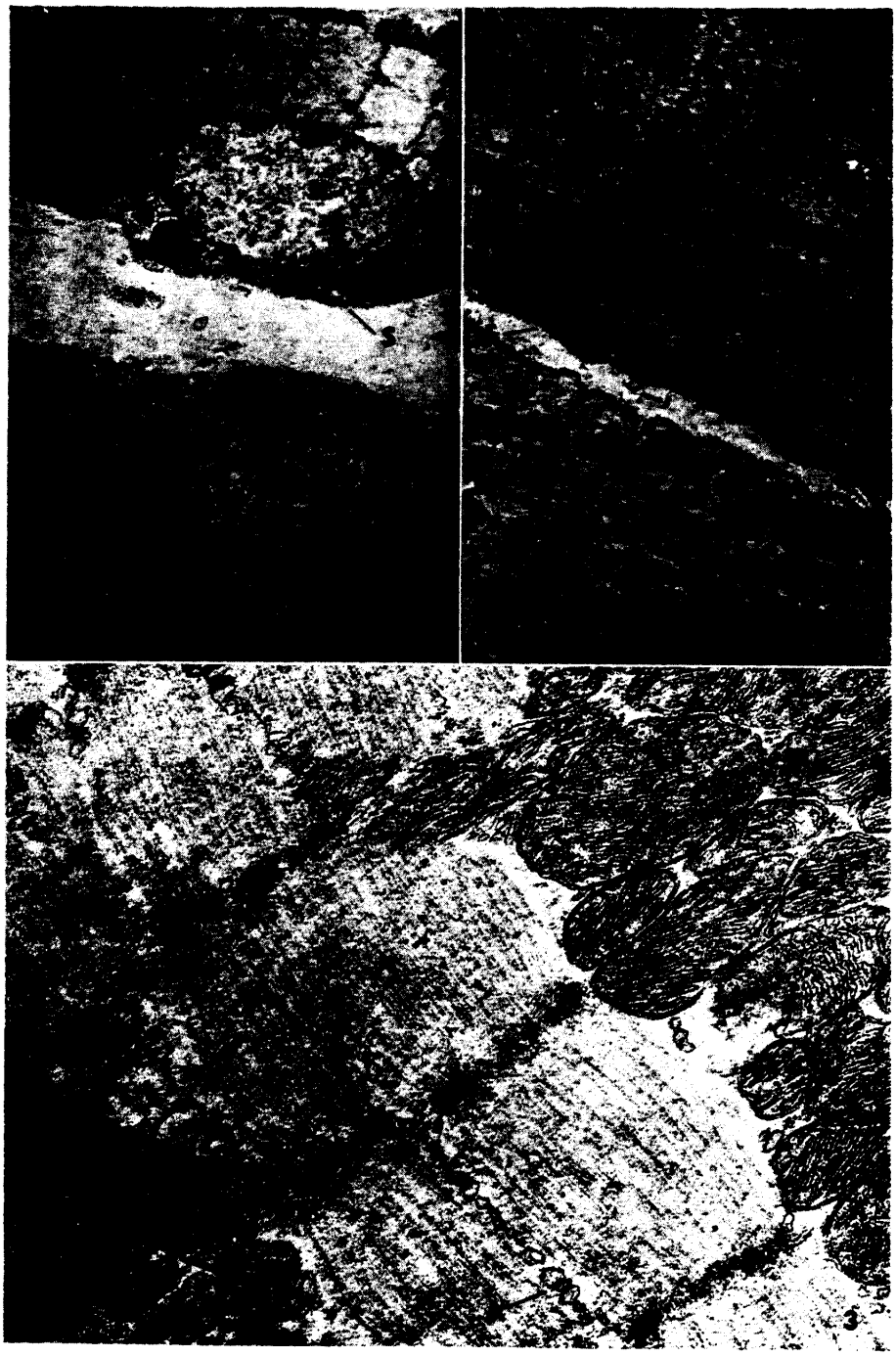
## RESULTS

In the red muscle fiber, there were many distinctly outlined accumulations of large mitochondria beneath the sarcolemma, especially near the nucleus (Fig. 1). The subsarcolemmal accumulation of mitochondria was nearly continuous throughout the long axis of muscle fiber; however, the amount of mitochondria was partly varied, i. e., in some parts especially in the area near the nucleus (Fig. 1) and in the area near the neuromuscular junction, many mitochondria were accumulated; however, in other parts mitochondria were lined up in a single row. In some parts of interfibrillar space, the accumulations of many larger internal mitochondria were observed preferentially towards the periphery of

Fig. 1 Red muscle fiber. Osmium fixation. In the subsarcolemmal space, especially in the perinuclear region, a prominent accumulation of large mitochondria is seen. ×8,000 M: mitochondria in subsarcolemmal space, N: nucleus, S: sarcolemma

Fig. 2 Red muscle fiber. Osmium fixation. In the subsarcolemmal spaces and the interfibrillar space, accumulations of large mitochondria (M1, M2L) are seen. On each side of Z-lines, small mitochondria (M2S) are seen. ×8,000

Fig. 3 Red muscle fiber. Osmium fixation. Cristae of large mitochondia (M1) are well developed and run parallel to each other. The cristae extend usually parallel or nearly parallel to Z-line (Z). The cristae of some mitochondria extend radially from the Z-line (M2). Sarcoplasmic reticulum (R) is not so well developed as that in white fiber. ×23,000



muscle fiber (Fig. 2). The mitochondria accumulated in the subsarcolemmal or interfibrillar space were usually large. They were usually oval in shape, however, some of them of an irregular shape. The matrix of mitochondria was moderately dense, its cristae were numerous and ran parallel to each other, although sometimes the parallel cristae pursued an undulating course. In each interfibrillar space on each side of Z-line, a pair of small internal mitochondria was usually seen. These mitochondria were of a small or medium size and an oval or elongated oval shape. Its cristae were well or moderately developed.

The sarcoplasmic reticulum was not so well developed as that in the white fiber (Fig. 3). The number of glycogen particles was variable but generally less numerous than that in the white fiber.

In the histochemical succinoxidase specimen, the heavy and almost continuous deposition of formazan was observed beneath the sarcolemma, however, the amount of the depostion was partly varied. In the interior of the fiber many large and fine formazan particles were observed; the large spherical particles were distributed preferentially towards the periphery of muscle fiber. The fine particles seemed to be located at the level of I-band.

In the white fiber, a few mitochondria were dispersed in the subsarcolemmal space and the interfibrillar space (Fig. 4). Although accumulations of a few mitochondria could be occasionally observed in some parts of the subsarcolemmal and interfibrillar space, there were no accumulations of numerous mitochondria as in the red fiber. Generally, internal mitochondria were sporadically located at the I-band level, and not on each side of Z-line as usually seen in the red or intermediate fiber. No striking differences in size and structure were observed between the mitochondria in subsarcolemmal space and that in interfibrillar space. The size of mitochondria was medium or small and its shape was oval, round oval or irregular. The matrix was less dense than in the red fiber. The cristae were poorly developed and some of them ran parallel to each other, but others separately in different directions (Fig. 6).

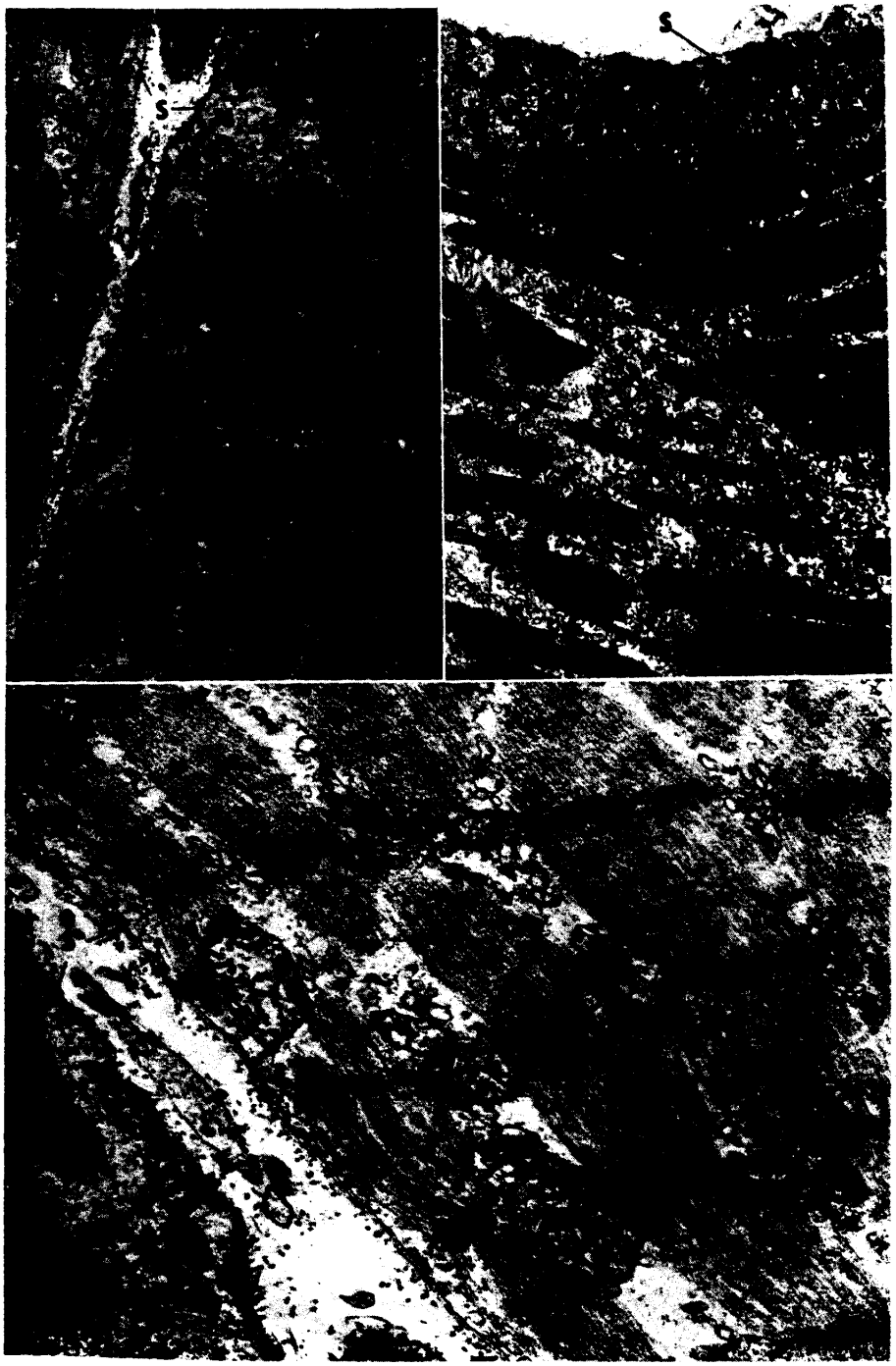
The sarcoplasmic reticulum was well developed (Figs. 5 and 6). The number of glycogen particles was variable, although usually more numerous than that in red or intermediate fiber.

In the succinoxidase specimen, no heavy subsarcolemmal formazan deposi-

Fig. 4 White muscle fiber. In the subsarcolemmal and interfibrillar spaces, a few mitochondria are dispersed. Osmium fixation. × 8,000, $M_1$, $M_2$, R, S: See Figs. 1, 2, 3.

Fig. 5 White muscle fiber. Osmium fixation. Sarcoplasmic reticulum is very well developed in the interfibrillar space. × 13,500, N, S, R, $M_2$: Refer to Figs. 1, 2, 3.

Fig. 6 White muscle fiber. Osmium fixation. Mitochondria ($M_1$, $M_2$) have a less dense matrix than that of the red fiber. Cristae are poorly developed and run separately in different directions. Sarcoplasmic reticulum (R) is very well developed and the number of glycogen particles (G) is large. × 22,400, S, Z: See Fig. 2


S
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S

tion was observed. In the interior of the fiber a few fine formazan particles were dispersed, however, large formazan particles could not be seen.

In the intermediate fiber, usually mitochondria were sporadically dispersed in the subsarcolemmal space; however, in some parts of subsarcolemmal space, a moderate number of mitochondria could be observed grouping together (Fig. 7). Accumulations of mitochondria in the interfibrillar space were sacrcely seen. In each interfibrillar space, a pair of mitochondria was usually seen, one found on each side of the Z-line; however, at the level of A-band few mitochondria were observed (Figs. 8 and 9). The size of mitochondria was usually small or medium. The shape was oval, elongated oval or irregular. The cristae of mitochondria were moderately developed and most of them extended rather parallel to the Z-line, however, some of them ran in different directions.

The sarcoplasmic reticulum was generally a little more developed than that of the red fiber and less developed than that of white fiber. The number of glycogen particles was variable but usually intermediate between that of the red and the white fibers.

In the succinoxidase specimen, subsarcolemmal deposition of formazan was sporadically demonstrated and its amount was consistently less than that of the red fiber. In the interior of the fiber, a few large formazan particles, possibly smaller than those in the red fiber, were occasionally demonstrated at the peripheral zone of muscle fiber. Moderate amounts of fine formazan particles were distributed in a net-like manner in the interior of fiber.

No marked differences were observed among the structure of myofibril in the red, white and intermediate fibers.

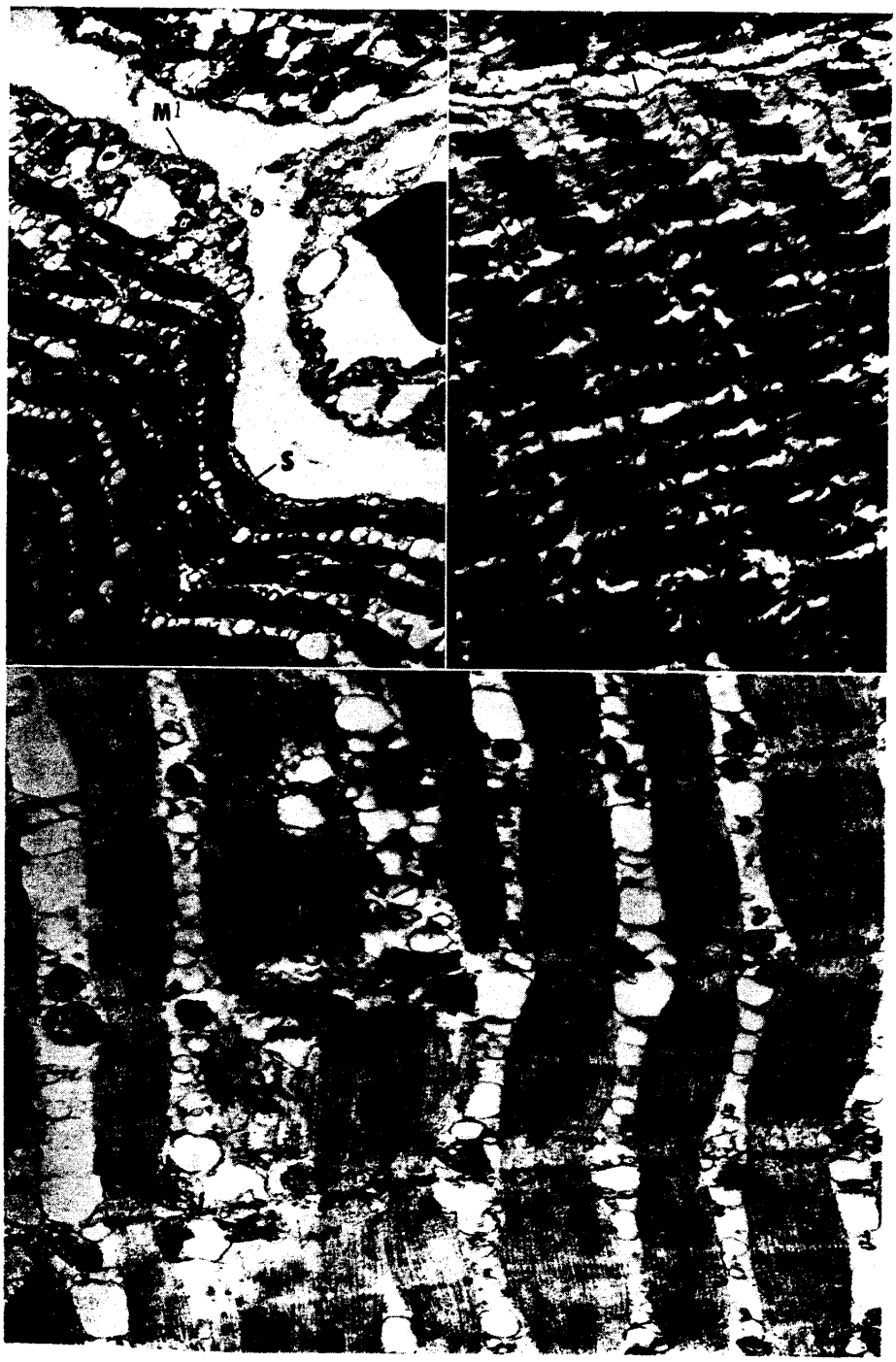
## DISCUSSION

Since the well-known work of GRUTZNER[7], it has been established that the striated muscle of mammals is composed of two kinds of muscle fibers, red and white ones. OGATA[1] and OGATA and MORI[2], distinguished the third type of muscle fiber which shows an intermediate activity of oxidative enzymes between

Fig. 7 Intermediate muscle fiber. Formalin-osmium fixation. In some part of subsarcolemmal space, especially in the region near nucleus, accumulation of a moderate number of mitochondria ($M_1$) is seen. × 8,000 N, S, $M_2$: See Figs. 1, 3

Fig. 8 Intermediate muscle fiber. Formalin-osmium fixation. In the subsarcolemmal and interfibrillar spaces, (S) no accumulation of mitochondria is seen. A pair of small mitochondria is usually observed on each side of Z-line in interfibrillar space ($M_2$). × 8,000, A: A-band, Z: Z-line.

Fig. 9 Intermediate muscle fiber. Formalin-osmium fixation. The mitochondria is of an oval or elongated oval shape ($M_2$). The cristae are moderately developed and usually extend parallel to Z-line (Z). Sarcoplasmic reticulum (R) is not so well develoded as that in the white fiber. × 28,500


M
S

those of the red and the white fibers. This fiber was named as the "medium fiber" or "intermediate fiber" by the author. From the present study, it has been elucidated from the electron microscope observation that the main differences among these three types of fibers are in the contents of mitochondria, sarcoplasmic reticulum and glycogen particles.

The red fiber is very rich in mitochondria lying in the subsarcolemmal space and interfibrillar space, which form the accumulations at many parts of the subsacrolemmal space and interfibrillar space. The white fiber has only a few mitochondria, being dispersed in the subsarcolemmal and interfibrillar spaces. The intermediate fiber has a moderate number of mitochondria. Usually in the subsacrolemmal space it has noly a few mitochondria, but in some parts of subsarcolemmal space especially near the nucleus the accumulation of moderate number of mitochondria is seen. In each interfibrillar space a pair of mitochondria is seen one on each side of Z-line. Mitochondrial accumulations as seen in the red fiber, are rarely met with in the white and the intermediate fibers.

The structure of mitochondria was different in these three types of fibers. The cristae of red fiber mitochondria were well developed, but those of the white fiber poorly developed, and those of the intermediate fiber moderately. The differences in the content and structure of mitochondria in these fibers paralleled well to the histochemical results of oxidative enzyme reaction in muscle fibers, i. e., the red fiber had a strong activity of oxidative enzymes, the white fiber a weak activity and the intermediate fiber a moderate activity. This difference in oxidative enzyme activity in these fibers seems to arise, because most of the oxidative enzymes were contained in mitochondria.

The difference in the content of glycogen particle was also observed in these fibers, i. e., the white fiber had more glycogen particles than that of the red one. This result correlated well with the biochemical study by OGATA[8] who showed that the white fiber of rabbit contained 3.7 times more glycogen than the red fiber.

The development of sarcoplasmic reticulum was different in these fibers, i. e., it was very well developed in the white fiber, but less in the red and intermediate fibers. Nowadays it is thought that the sarcoplasmic reticulum may serve to transmit the excitatory impulses to the interior of the muscle fiber. If this is true, it correlates well with the present results in that the rapidly contracting white fiber had a well developed sarcoplasmic reticulum while the slow contracting red fiber had a poorly developed sarcoplasmic reticulum. The function of the intermediate fiber is unknown. However, it may be assumed from the degree of development of sarcoplasmic reticulum that this fiber is a slow contracting fiber and has a rather similar function to the red fiber (for example, the red fiber participates in contractile tonus and the intermediate fiber in plastic

tonus). However, further study is necessary to substantiate this assumption.

In the physiological study, BULLER *et al.*[9,10] reported the existence of two types of muscle fibers and two types of motor units in cat. In previous histochemical studies[1,2], it was observed that most of vertebrate striated muscles were composed of three types of muscle fibers. From the present study the three different types of muscle fibers could be more clearly demonstrated by electron microscopic observation. Consequently, it may be reasonably assumed that there will be three different types of motor units in vertebrates and a smooth contraction of the muscle as a whole will be due to a harmonized cooperation of three motor units.

## SUMMARY

An electon microscopic study on the structural differences among the red, white and intermediate muscle fibers of mice was made and the following results were obtained.

1. The red fiber contained very numerous mitochondria, the white fiber a few and the intermediate fiber a moderate number. The distribution of mitochondria was different in each type of muscle fiber. The cristae of mitochondria of the red fiber was quite well developed, that of the white fiber poorly and that of the intermedtate fiber moderately.

2. Sarcoplasmic reticulum of the white fiber was considerably well developed but that of the red and intermediate fibers poorly developed.

3. Glycogen particles were abundant in the white fiber, less in the intermediate fiber and least in the red fiber.

## ACKNOWLEDGEMENT

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