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Histochemical studies on the red, white and intermediate muscle fibers of some skeletal mescles. II. The capillary distribution on three types of fibers of some skeletal muscles*

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Abstract

From the histochemical study on the vascular distribution of limb muscles of cats and rats, the following results were obtained. 1. The red, white and intermediate fibers of the soleus and gastrocnemius individually present dissimilar patterns of the vascular distribution. In the transverse section of the muscle, most of the capillaries are found preferentially assembled around the small red fiber. Usually the red fibers are supplied with 4-6 capillaries, while the intermediate fibers 3-4 capillaries and the white 1-2 capillaries. 2. In the longitudinal section, the capillaries run parallel with the muscle fiber. Occasionally, these capillaries branching out for anastomoses cross the fiber almost at right angle. These anastomoses are more frequently observed on the red fibers. 3. The soleus muscle is better supplied with capillaries than is the gastrocnemius, because this muscle is composed only of two types of capillary rich fibers, namely, the red and intermediate fibers. 4. The mean capillary-fiber ratio of the cat gastrocnemius proves to be 0.6 and that of the soleus 1.4. 5. From these observations, it is considered that there is an intimate relationship between the capillary distribution pattern and the type of individual muscle fibers. 6. The red fibers being with a high oxidative enzyme activity and rich in mitochondria may require an abundant supply of oxygen and have a greater number of capillaries. In contrast to this, the white fibers with a lower enzyme activity and a fewer mitochondria need not to consume so much oxygen and are supplied with a fewer capillaries, because they participate only in phasic contraction. The intermediate fibers, with a moderate enzyme activity, may be considered to have a moderate number of capillaries.

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HISTOCHEMICAL STUDIES ON THE RED, WHITE AND INTERMEDIATE MUSCLE FIBERS OF SOME SKELETAL MUSCLES

II. THE CAPILLARY DISTRIBUTION ON THREE TYPES OF FIBERS OF SOME SKELETAL MUSCLES

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Since the classic observation by RANVIER[11,12], it has been well established that the striated muscles of mammals consist of two different kinds of muscles, namely, red and white muscles. The former is colored deep red and the latter is white by naked eye. And it was further shown that the apparent color differences in the muscle are not due to the circulating blood. DENNY-BROWN[1] revealed that both of these two muscles are composed in different proportion of red and white muscle fibers, that is, the red muscle contains more red fibers than white fibers and the white one more white fibers. However, OGATA[9,10] of our laboratory recently reported that there are more types of fibers than merely the red and the white in mammalian skeletal muscles as judged from their different activities of histochemically demonstrable oxidative enzymes, namely, red, white and intermediate muscle fibers.

Since ancient times the difference between the vascular supply of the red and white muscles has been demonstrated by several workers[3,5,13,15,16,17], but precise observation on the capillary supply of each muscle fiber is scarce. In this paper the author describes that in the limb muscles of cat and rat there are striking differences in the capillary distribution pattern among three types of fibers.

MATERIALS AND METHODS

M. soleus and M. gastrocnemius of healthy adult cats and rats served as materials. After anesthetizing the animals with pentobarbital sodium, limb muscles were exsanguinated by injection of warm physiologic saline solution into the femoral artery. For the observation of capillary supply, an adequate volume of india ink was immediately injected into the same artery. The cross and longitudinal sections were cut into 10—15 μ thick in the cryostat at $-20°C$ and mounted on a slide glass. In some cases very thin strips containing a few fibers

from those muscle receiving the injection were dissected with two needles under the light microscope.

For discrimination of the red, white and intermediate fibers histochemical demonstration of succinic dehydrogenase was employed. That is, these thin sections were incubated for about 15 minutes at 37°C in the substrate solution consisted of M/5 sodium succinate 5 ml, M/10 phosphate buffer, pH 7.4, 5 ml, Nitro-BT, 5 mg/3 ml, 6 ml and distilled water 10 ml. Then the sections were fixed in 10% formalin and covered with glycerin. By this method the muscle specimens are stained lightly with succinic dehydrogenase and three types of fibers can be distinguished by the differences in their enzyme activities. Furthermore, the intramuscular capillaries are clearly observed with injected india ink.

RESULTS

The soleus muscles of cat and rat were dark red, while the gastrocnemius were white in color by naked eye. In the exsanguinated animals by the injection of physiologic saline solution the soleus was still reddish. Gastrocnemius: In transverse section of the gastrocnemius three types of fibers were clearly distinguished by the difference in the succinic dehydrogenase activity. The small red fibers were stained deep blue showing a higher dehydrogenase activity, the large white fibers stained faintly with a low enzyme activity and the third type of fibers, intermediate in size, stained purple, a moderate enzyme activity (Fig. 1). Interfibrillar capillaries were discriminated by the particles of the injected india ink as small colored or short linear dots which were distributed in an orderly manner among the muscle fibers. These particles were observed constantly around the muscle fiber, close to the sarcolemma and never seen intrafibrillarly, showing no transition through the capillary walls.

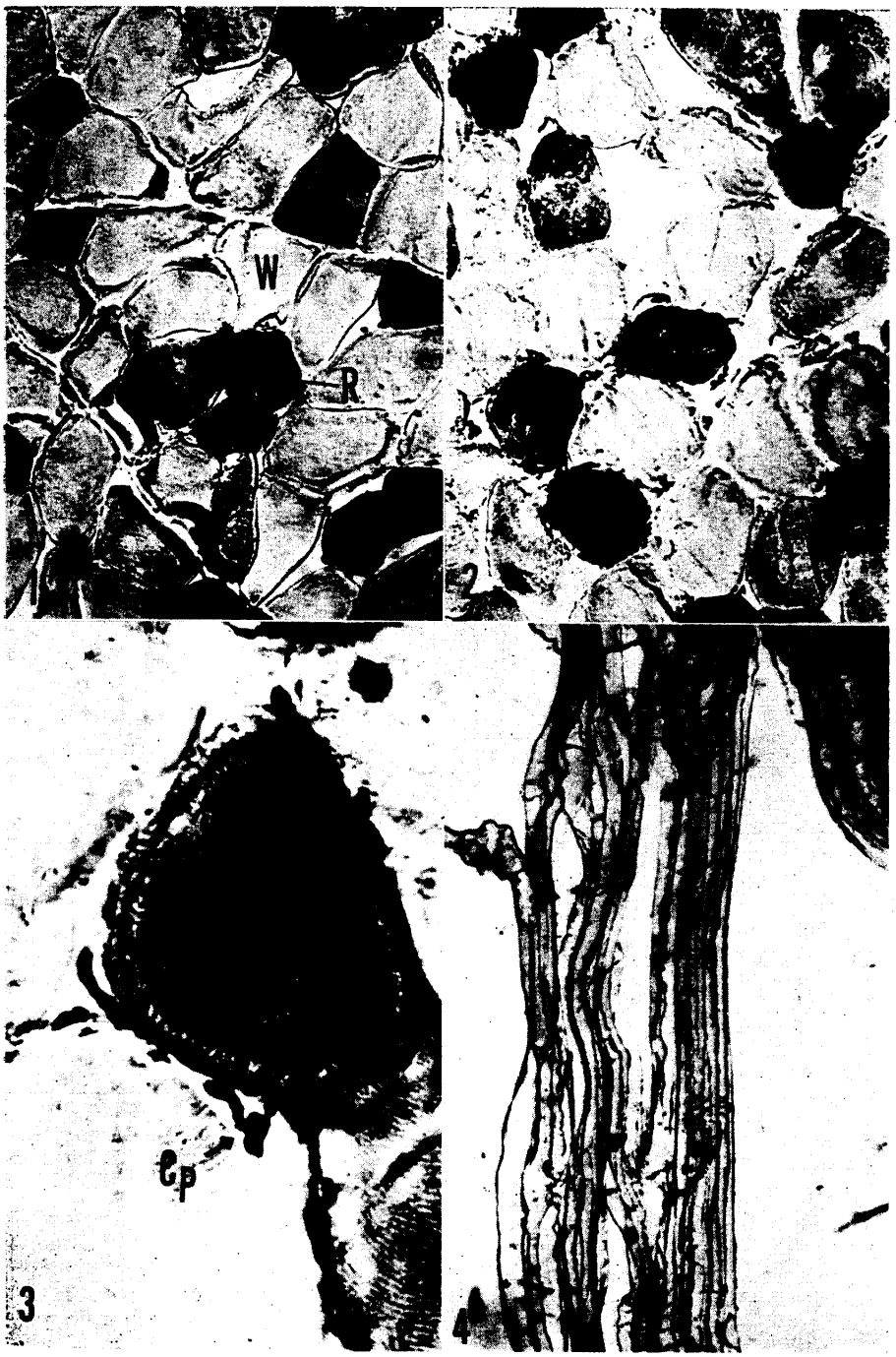
In the cross-section most of the capillaries assembled around the small fibers. Usually the red fiber was supplied with 4—6 capillaries, while the intermediate fiber with 3—4 capillaries and the white fiber with 1—2 capillaries (Figs. 1, 2, 3). Some of the red fibers were surrounded by the capillaries on

Fig. 1 Gastrocnemius of the cat, cross-section, india ink injection and succinic dehydrogenase. Note three types of muscle fibers, namely, the small red fibers (R) which show a high enzyme activity, while the large white fibers (W) a low activity. The intermediate fibers (M), intermediate in size, show a moderate enzyme activity. The capillaries are seen assembled around the red fibers. ×315

Fig. 2 Gastrocnemius of the cat, cross-section, india ink injection and succinic dehydrogenase. The capillaries are assembled around the red fibers. ×315

Fig. 3 Gastrocnemius of the cat, cross-section, india ink injection and succinic dehydrogenase. Note the capillaries (Cp) that assemble around the red fibes. ×1, 300

Fig. 4 Soleus of the cat, longitudinal section, india ink injection, unstained. The capillaries run parallel with each other. ×122


W
R
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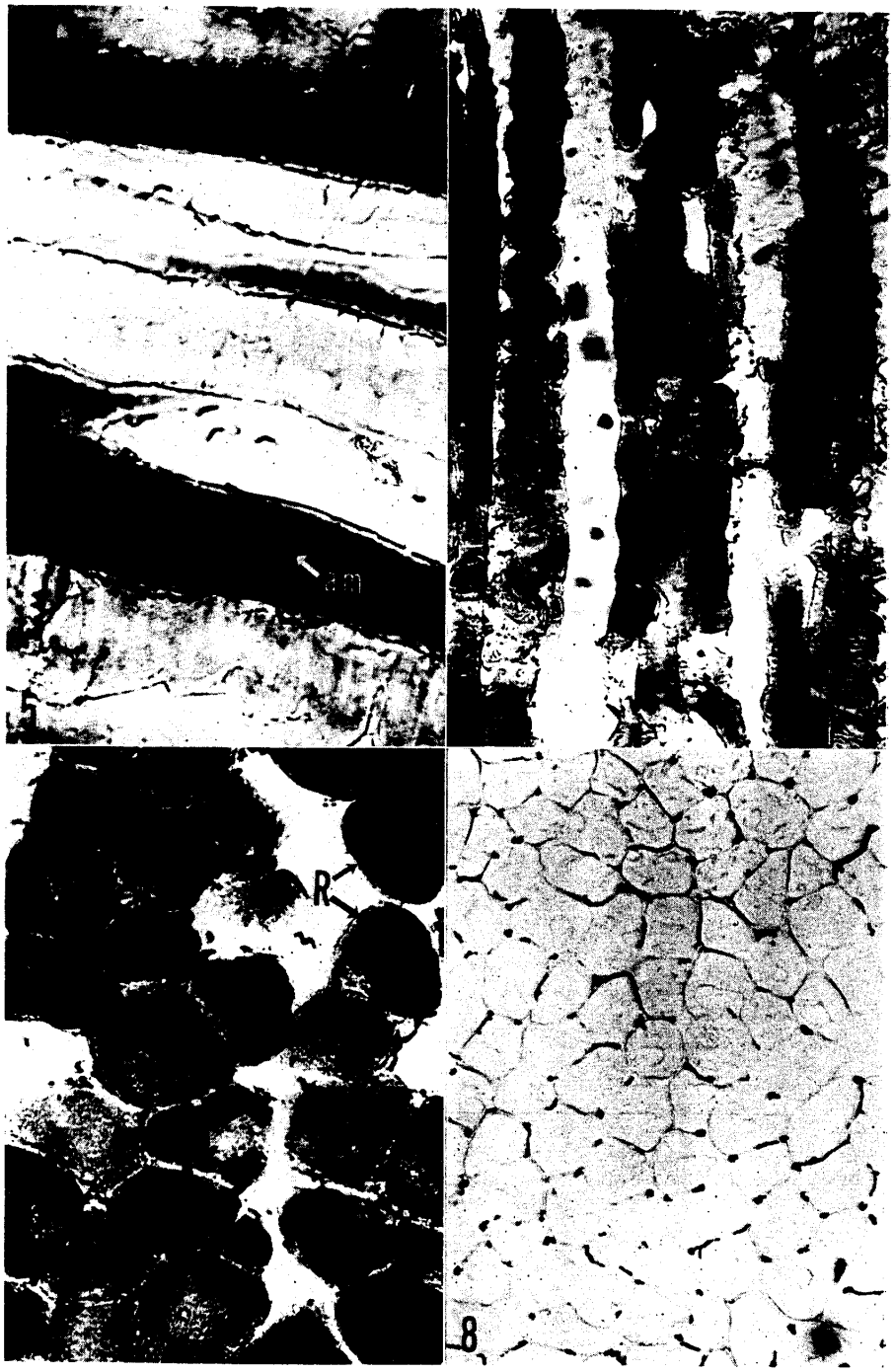
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almost whole circumference of the fiber. This shows perhaps the branches of capillary anastomoses. The white muscle fiber usually had only 1—2 capillaries which were preferentially distributed to the side close to the red or the intermediate fiber.

In the longitudinal section of this muscle the capillaries ran in parallel with the fibers (Fig. 4). As is observed in the transverse section, a number of capillaries are supplied to the red fiber and they run parallel with each other along the muscle fiber. Occasionally the capillaries branching out for anastomoses crossed the fiber almost at right angle like a knob of bamboo (Fig. 5). These anastomoses were more often observed on the red fiber than on the white and the intermediate. In the muscle streched to its normal length during rest the capillaries were straight, but in the contracted muscle these capillares were very sinuous.

The mean capillary-fiber ratio (i. e., number of capillaries/number of muscle fibers in a unit area) of the cat gastrocnemius is 0.6. Soleus: This muscle is composed of only two kinds of muscle fibers except a few which show a lower enzyme activity. In transverse section the red fibers with a higher dehydrogenase activity are stained deep blue, while the intermediate fibers with a moderate activity are stained purple. Both of them are almost the same in size (Fig. 7).

The vascular pattern in the soleus is in a strong contrast to that of the gastrocnemius. It seems that far more capillaries are distributed in the soleus, because this muscle is composed of only two types of capillary rich fibers (Figs. 7, 8). The capillary distribution of each of the red and intermediate fibers in the soleus is similar to that in the gastrocnemius. That is, the red fibers are supplied with a number of capillaries, while the intermediate with a moderate number of capillaries.

The mean capillary-fiber ratio of the cat soleus is 1.4.

DISCUSSION

Since ancient times many investigators have revealed striking differences in the pattern of vascular supplies between the red and white muscles. KROGH[5]

Fig. 5 Gastrocnemius of the cat, longitudinal section, india ink injection and succinic dehydrogenase. Note the capillary anastomoses. ×315

Fig. 6 Soleus of cat, longitudinal section, india ink iniection and succinic dehydrogenase. Note the capillary distribution. ×122

Fig. 7 Soleus of the cat, cross-section, india ink injection and succinic dehydrogenase. Note two types of fibers, namely, the red fibers (R) which show a high enzyme activity and the intermediate fibers (M) with a moderate enzyme activity. ×315

Fig. 8 Soleus of the rat, cross-section, india ink injection, unstained. Note the capillaries among the muscle fibers. ×260

was one of the first to attempt to correlate the number of capillaries to the area of the muscle supplied. He stated that the number of capillaries per square mm of the muscle appeared to be a function of the intensity of the metabolism, being higher in small mammals than in large forms. STOEL[16] reported for the rabbit a fewer capillaries than fibers per square mm in the white muscle, and more capillaries than fibers in the red. WATZKA[17] also described similar observations. DUYFF and BAUMAN[3] showed in the same animal, 2—3 capillaries per fiber for the red muscle and 1.3—1.5 capillaries per fiber for the white. However, they concluded that a clear-cut relationship between the number of capillaries and fiber size, muscle color, function, could not be established.

Recently, SMITH and GIOVACCHINI[15] demonstrated two dissimilar patterns of the vascular supply between the red and white muscles of the rabbit. They pointed that one of the striking differences was the manner in which the vessels approached the muscle, that is, the vessels to the red muscles branched frequently before entering and these muscles were observed to have 11—16 separate small arteries, while those to the white muscles seldom branched before entering and these muscle were observed to have only two or three larger vessels. And it was further shown that the mean capillary fiber ratio for the former was found to be 1.7 while the latter had a ratio of 0.5.

Thus, all of these authors have revealed the dominant vascular supply to the red muscles, but they assumed that in a muscle each fiber is surrounded by an equal number of capillaries. In the present study on cat and rat limb muscles, similar results were obtained, namely, the capillary fiber ratio of the cat soleus as a red muscle specimen was 1.4, while that of the gastrocnemius as a white muscle specimen was 0.6. Furthermore, most of the capillaries preferentially assembled around the small red fibers. While the large white fibers were observed to keep only 1—2 capillaries and the intermediate ones to keep a moderate number of capillaries between the red and white.

According to OGATA's work[9,10] and previous paper[8], these three types of fibers show histochemically different reactions from the degree of the oxidative enzyme activities, and further indicate that the red muscle fibers participate in contractile tonus, the intermediate ones in plastic tonus and the white ones in phasic contraction. Therefore, from these observations it may be considered that there are intimate relationships between the capillary distribution and the enzyme activity, function, size of individual striated muscle fibers. In other words, the red fibers with a high oxidative enzyme activity and abundant mitochondria may be considered to require an ample supply of oxygen. While, the white fibers with higher glycogen and phosphorylase contents and a fewer mitochondria may not need so much oxygen, because they participate only in phasic contraction. The intermediate fibers with a moderate enzyme activity may be supplied with

a moderate number of capillaries. Most recently, ROMANUL[18] has described the capillary supply in muscle fibers using the histochemical technique for alkaline phosphatase and some oxidative enzymes. He also pointed out that the oxidative metabolic activity is directly proportional to the number of capillaries surrounding every muscle fibers.

According to MILLIKAN's study[7], myoglobin, which is contained more abundantly in the red fiber, does act as an oxygen reservoir. LAURIN[6] in his biochemical studies on the red and white muscle varieties states that these varieties may be fundamentally different in their manner of managing and utilizing oxygen, and points out that there is an inverse ratio between the myoglobin content of muscle and its power to carry out glycolytic processes.

All these observations lead us to postulate that these three types of fibers in the striated muscles have individually different basic mechanism and different manner of utilization of oxygen. The red muscle fibers may be considered to have many capillaries and much myoglobin for their tonic contraction and a high requirement of oxygen supply.

SUMMARY

From the histochemical study on the vascular distribution of limb muscles of cats and rats, the following results were obtained.

1. The red, white and intermediate fibers of the soleus and gastrocnemius individually present dissimilar patterns of the vascular distribution. In the transverse section of the muscle, most of the capillaries are found preferentially assembled around the small red fiber. Usually the red fibers are supplied with 4—6 capillaries, while the intermediate fibers 3—4 capillaries and the white 1—2 capillaries.

2. In the longitudinal section, the capillaries run parallel with the muscle fiber. Occasionally, these capillaries branching out for anastomoses cross the fiber almost at right angle. These anastomoses are more frequently observed on the red fibers.

3. The soleus muscle is better supplied with capillaries than is the gastrocnemius, because this muscle is composed only of two types of capillary rich fibers, namely, the red and intermediate fibers.

4. The mean capillary-fiber ratio of the cat gastrocnemius proves to be 0.6 and that of the soleus 1.4.

5. From these observations, it is considered that there is an intimate relationship between the capillary distribution pattern and the type of individual muscle fibers.

6. The red fibers being with a high oxidative enzyme activity and rich in mitochondria may require an abundant supply of oxygen and have a greater

number of capillaries. In contrast to this, the white fibers with a lower enzyme activity and a fewer mitochondria need not to consume so much oxygen and are supplied with a fewer capillaries, because they participate only in phasic contraction. The intermediate fibers, with a moderate enzyme activity, may be considered to have a moderate number of capillaries.

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