Total Capillary Bed in Striated Muscle of Guinea Pigs Native to the Peruvian Mountains

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ABSTRACT

VALDÍVIA, ENRIQUE. Total capillary bed in striated muscle of guinea pigs native to the Peruvian mountains. Am. J. Physiol. 194(3): 585-589. 1958.—The total capillary bed in skeletal muscle of guinea pigs has been determined by perfusion with India ink and counting capillaries in cross sections of gelatin embedded muscle. Comparative studies have indicated the importance of age, the muscle studied and the site where counts were performed. Capillaries were more evenly distributed in the red than the white areas of the muscle. Free exercise or restriction in cages during the life span did not materially affect the results, although in cross sections some gross enlargement of the red areas was observed in the free exercise group. A significantly greater number of capillaries per square millimeter of muscle tissue were observed in the red area of muscles from animals native to the Peruvian mountains. The red area of these muscles was also more extensive than in the sea level born controls. The possible adaptive significance of these characteristics has been indicated.

The relation between capillary bed and functional demands are well known from the classical studies of Krogh (1), who also pointed out the significant role of the capillary bed in the interchange of oxygen and carbon dioxide at different levels of metabolic activity. Hurtado and his group (2) have observed a high degree of muscle efficiency during exercise in the native residents at high altitude. They perform muscular work with some economy in the oxygen consumption and with a lower production of lactate, as compared with men living at sea level. One of the mechanisms that may contribute to this higher efficiency can be an increased total capillary bed in the striated muscle that will permit more surface contact between the muscle fiber and the circulation, especially with increased muscular activity. Determinations of functional capillary bed, or the number of open capillaries at different levels of muscular activity cannot be made as accurately or be as easily compared as the determinations of the total capillary bed, which represents the maximum capillary supply of a tissue. Different methods have been used to determine the total capillary bed in voluntary striated muscle or myocardium, Krogh (1), Paff (3), Martin (4), Smith (5) and Hakkila (6). The results appear to depend upon the method and the animal studied. The object of the present investigation is to determine the total capillary bed of representative striated muscles of guinea pigs and to compare the results obtained in animals born at sea level with those of guinea pigs native to the Peruvian mountains (altitude, 13,800 ft.).

METHODS

Adult male guinea pigs were used in these experiments. The animals were not fed, but given water, during the 12 hours prior to anesthesia. Nembutal, 30 mg/kg, was used intraperitoneally as the anesthetic. The abdominal aorta was cannulated 1 cm cephalad to the left renal artery, and isotonic saline solution at 38°C was perfused at a pressure of 85-90 mm of Hg until the blood was replaced.
India ink diluted 1:9 in isotonic saline solution was then perfused for an interval of at least 10 minutes.

The posterior extremities were fixed in neutral 10% formalin. The muscles were dissected and embedded in 10% gelatin for 24 hours and in 20% gelatin for an additional 24 hours. Commercial gelatin sheets were satisfactory, but granular gelatin was more convenient. Transverse sections 30-40 microns in thickness were obtained from the middle third of each muscle. Gelatin balsam was used for mounting and collodion was used for sealing the sections. All counts were made with a compound binocular microscope using a 10X ocular and 43X objective. The capillaries and fibers in a field of known area were counted. In the same field the capillaries surrounding each single fiber were also counted. The values for each muscle were based on counts of at least three fields from 10 to 20 slides; so each figure, representing one muscle, was the average of 30 or more counts. Representative longitudinal sections were also studied.

Four mixed red and white muscles were used in this study, two from the thigh (rectus femoris and vastus lateralis) and two from the leg (internal and external gastrocnemius). One white muscle located anteriorly in the thigh was also studied; it is designated as the anterior white muscle of the thigh (fig. 1).

Three different groups of animals were studied: group A consisted of guinea pigs born at sea level (Peru). They were kept in large...
pens where free running was permitted during the entire life span. Group B was formed by guinea pigs born in Wisconsin and maintained during their entire life span in small cages (3–4 animals in 3000 cm² of cage surface). Group C guinea pigs were born in the Peruvian mountains at an altitude of 13,800 feet and kept at 14,900 feet. These guinea pigs had been maintained free in open spaces on the small farms of the owners and in our laboratory.

RESULTS

The muscles present in cross section a darker or red area and a lighter or white area. After perfusion with India ink the red and white areas can be more readily distinguished, because the red area appears darker due to the greater capillarity and better injection of the dye.

The relation of white and red portions of vastus lateralis muscle of the thigh is illustrated in figure 2. These muscles were previously perfused with India ink solution. The red area was slightly more extensive in group A than in group B animals. In group C guinea pigs the red portion was more extensive than in group A or B animals.

Red and white muscle fibers of guinea pigs were compared by examination of cross sections. In the areas of red muscle the fibers were fairly uniform in size and shape (fig. 3) with an average surface of 1800 square micra per fiber. The capillaries were evenly distributed around most of the muscle fibers and the number per square millimeter as well as the number surrounding a single fiber are shown in table 3.

The fibers in the areas of white muscle on cross section were somewhat more variable in size and shape (fig. 4). The largest white fibers were consistently larger than the red fibers. The capillary counts in white muscle (table 1) were always less than those of red muscle (table 3) and never exceeded 800 capillaries/mm². In white muscle they were always more irregularly distributed so that there was considerably more variation in the counts.

The importance of age can be seen in table 2, in which counts of capillaries from the red portion of vastus lateralis of guinea pigs weighing between 500 and 550 gm are shown. These animals came from the same source as group A. The younger guinea pigs had a greater number of capillaries and fibers than the older ones weighing between 650 and 800 gm. The ratio of capillaries to muscles was slightly decreased.

Table 3 represents the capillary counts from the three groups of animals, all the counts being obtained from the red portion of each muscle. Four muscles were represented: vastus lateralis and rectus femoris from the thigh and external and internal gastrocnemius from the leg. No significant difference was found between groups A and B; however, the high altitude animals, although presenting the same number of muscle fibers per square millimeter, had an average increase in the number of capillaries (approximately 42%). The ratio of the number of capillaries to the number of fibers was also greater in the high altitude guinea pigs (group C).

The number of capillaries counted around one single muscle fiber also showed a significant increase in the high altitude muscles. At high altitudes the average was 1.4 capillaries more than at sea level, or an increase of approximately 20%.
Longitudinal sections from the perfused muscles of the guinea pigs born at high altitude (fig. 5) had relatively more capillaries and more anastomoses.

**Table 1. Counts on the white muscle from 10 guinea pigs raised in pens, free running (Group A)**

<table>
<thead>
<tr>
<th>Muscles</th>
<th>No. of Capillaries per mm²</th>
<th>No. of Fibers per mm²</th>
<th>Ratio: Capillaries to Fibers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior white (thigh)</td>
<td>0.86 ± 0.072</td>
<td>0.46 ± 0.048</td>
<td>1.48 ± 0.072</td>
</tr>
<tr>
<td>Vastus lateralis</td>
<td>0.74 ± 0.040</td>
<td>0.54 ± 0.039</td>
<td>1.45 ± 0.080</td>
</tr>
</tbody>
</table>

Values that follow ± are standard errors of the mean.

**Table 2. Importance of age. Counts from red portion of vastus lateralis (Group A)**

<table>
<thead>
<tr>
<th>Animal Weight, g</th>
<th>No. of Capillaries per mm²</th>
<th>No. of Fibers per mm²</th>
<th>Ratio: Capillaries to Fibers</th>
</tr>
</thead>
<tbody>
<tr>
<td>500-550</td>
<td>1.09 ± 0.052</td>
<td>0.89 ± 0.055</td>
<td>2.24 ± 0.069</td>
</tr>
<tr>
<td>650-800</td>
<td>1.85 ± 0.057</td>
<td>1.35 ± 0.049</td>
<td>3.45 ± 0.066</td>
</tr>
</tbody>
</table>

Values that follow ± are standard errors of the mean.

**Table 3. Total capillary bed of striated muscle. Comparative counts on the most vascularized area (red) of 4 muscles**

<table>
<thead>
<tr>
<th>Muscle</th>
<th>No. of Capillaries per mm²</th>
<th>No. of Fibers per mm²</th>
<th>Ratio: Capillaries to Fibers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vastus lateralis (thigh)</td>
<td>2.10 ± 0.160</td>
<td>0.92 ± 0.092</td>
<td>2.28 ± 0.157</td>
</tr>
<tr>
<td>Rectus femoris (thigh)</td>
<td>2.10 ± 0.160</td>
<td>0.92 ± 0.092</td>
<td>2.28 ± 0.157</td>
</tr>
<tr>
<td>Internal gastrocnemius (leg)</td>
<td>1.85 ± 0.057</td>
<td>1.35 ± 0.049</td>
<td>3.45 ± 0.066</td>
</tr>
<tr>
<td>External gastrocnemius (leg)</td>
<td>1.45 ± 0.035</td>
<td>1.15 ± 0.029</td>
<td>2.47 ± 0.071</td>
</tr>
</tbody>
</table>

**Group A—to guinea pigs raised in pens, free running**

**Group B—to guinea pigs raised in cages**

**Group C—to guinea pigs, native to the Peruvian mountains**

Values that follow ± are standard errors of the mean.

**Fig. 5. Longitudinal section of vastus lateralis muscle, red portion. Guinea pig 78 native to the Peruvian mountains, group C.**

**DISCUSSION**

Striated muscle contains capillaries with a regular and narrow lumen, situated mainly parallel to the fibers. Therefore, the total capillary bed closely represents the maximum blood supply for this tissue when the capillary diameter is not altered. Cross section of striated muscle permits an evaluation of the number of capillaries as well as an accurate comparison in relation to the number of fibers from different kinds of muscle. In an effort to enhance the perfusion, various fluids were used (dextran, Ringer's solution) with or without vasodilator drugs, such as nitrates or histamine. However, histamine produced vasoconstriction in guinea pigs, and other perfusion mixtures did not improve our results. The color of the striated muscle is due to several factors. The amount of sarcoplasm is the most important, but the amount of myohemoglobin or other pigments in the sarcoplasm and the amount of blood in the capillaries are also of importance. These factors may vary with the specie, strain, age of animal etc. According to Krause (7) the capillary bed is the most important factor influencing the muscle color of cats and rabbits. Lawrie (8) has shown differences in myohemoglobin content of white and red muscle in different mammals and birds. Our results have demonstrated the contribution of the capillary bed to the color of the muscle.
The most important single factor in comparing results of one study with another is the technique used in obtaining sections. Embedding in gelatin has proven to be most effective because shrinkage caused by paraffin embedding is avoided. The studies on guinea pig muscle reported by Paff (3) and Krogh (1) presented more capillaries and more fibers per square millimeter than we obtained by our method; such a difference is due to shrinkage caused by paraffin embedding. Results obtained with celloidin embedded muscle of rabbits reported by Stoel (9) are similar to our data on gelatin embedded tissue.

The group of younger guinea pigs had a greater number of both fibers and capillaries than did the older ones, and the ratio of capillaries to muscle fibers was decreased. Animals of the same age and weight were used for the comparative studies. Animals allowed to exercise freely showed only some increase in the extension of the red, more vascularized area, but no significant increase in the capillary bed of this area.

The different methods used for the determination of the number of capillaries in a given area of tissue have been subjected to various criticisms, but it is evident that the results obtained in fixed preparations do not necessarily represent the condition existing during life, either at rest or during muscular activity. However, within the limitation imposed by these considerations and on the basis of the comparative value of our results observed with identical technic, the guinea pigs born and raised at high altitudes appear to have a greater number of blood capillaries in a square millimeter of muscle tissue and a higher ratio of capillaries to fibers in the same area than do those born and raised at sea level. As already mentioned, the high altitude guinea pigs represent an animal strain well acclimatized for centuries to the low pressure environment. The presence of a greater absolute and relative number of capillaries in the muscular tissue may be regarded as an important adaptative mechanism at high altitudes. The increase represents a more satisfactory mechanism for the diffusion of oxygen from the capillary blood to the active tissue, compensating in some degree for the disadvantages of the low tension of this gas in the circulating blood. It has been mentioned that Hurtado and his collaborators (2) have observed that acclimatized native residents at high altitudes are able to tolerate a higher degree of physical activity than sea level residents and that they accomplish this task with a lower production of lactate and some economy in oxygen consumption. This may be facilitated by the increase in capillary bed demonstrated in this work, but must ultimately depend on intracellular mechanisms.

Studies are being conducted to establish whether these observations of the Peruvian animals can be reproduced after long term exposure to simulated high altitude in low pressure chambers. This investigation may contribute to an elucidation of the participation of the capillary bed in the general mechanisms of adaptation or acclimatization to chronic hypoxia.

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REFERENCES