Capillary development during exposure to chronic hypoxia

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CASSIN, S., R. D. GILBERT, C. E. BUNNELL, AND E. M. JOHNSON. Capillary development during exposure to chronic hypoxia. Am. J. Physiol. 220(2): 448–451. 1971.—Thirty male rats were exposed to an altitude of 6,150 m for 36 days in a decompression chamber while 26 similar animals were maintained at sea level for the same time. Capillary counts in gracialis and plantaris muscles in some experimental and control animals were compared by utilizing a specific stain for endothelial cells. A significant increase in muscle capillary counts was found in experimental animals if the counts were expressed per unit area. However, the number of muscle fibers per square millimeter of tissue also increased. Because there were probably no new muscle fibers in these animals, the relative increase in fibers per unit area is thought to be due to the smaller size of the muscle fibers in the experimental animals. When the counts were compared in experimental and control animals on the basis of capillaries per muscle fiber, a difference between the two groups was not observed. On the basis of these data it appears to us that exposure to hypoxia results in an opening of preexisting capillaries rather than an increase in vascularity.

Valdivia (10, 11, 12) demonstrated that the muscles of guinea pigs living at high altitudes contained more open capillaries than those at sea level. It was not clear, however, whether the increased number of capillaries represented the development of new capillaries or the opening of pre-existing ones.

The aim of this investigation was to compare tissues from animals exposed to 6,150 m for 5 weeks with sea-level controls. A histochemical technic specific for endothelial cells was used to determine whether the chronic hypoxia promotes new capillary development.

MATERIALS AND METHODS

Two groups of male Carworth Farm strain N rats were used in this study. Group A consisted of 12 experimental and 8 control animals. Group B consisted of 18 experimental and 18 control animals. The animals are separated into two groups because of slight differences in technic applied to each group. Both gracialis and plantaris muscles were examined in Group A, while only the gracialis muscle was examined in Group B. In addition, capillary counts were expressed per muscle fiber for Group A. In Group B capillary counts were expressed per unit area. The control animals for both groups were housed at sea level in a room kept between 22 and 23° C for the duration of the experiment. The experimental animals for both groups were exposed to simulated altitude at the same ambient temperature in a low-pressure chamber. The altitude animals were taken from sea level to 6,150 m (307 m/min) in 4 days at daily increments in altitude of 1,538 m. They were maintained at 6,150 m for 36 days, and then returned to sea level. Animals were, however, returned to sea level for approximately 1 hr daily to permit us to clean their cages and replenish their food and water. Both groups of animals received tap water and Purina Laboratory Chow ad libitum.

On the 36th day of the experiment, control and experimental animals were sacrificed by a quick blow to the head. Organ-to-body weight ratios for heart, testes, adrenals, and kidneys were determined on Group B. Also transverse sections of fresh gracialis muscles were cut and fixed in absolute alcohol at 4° C for 24 hr. Gracialis and plantaris muscles in Group A were cut and fixed in a similar fashion to those of Group B. After two changes in acetone, the tissues were cleared in benzene at 4° C for 15 min, and embedded in paraffin at 51° C under vacuum within 45 min. Cross sections of all muscles were cut 15 μ in thickness, mounted without adhesive, and stained by a method specific for alkaline phosphatase. To stain tissues, slides were deparaffinized in petroleum ether, hydrated in a series of graded acetone solutions, and placed in a medium (at pH 9) containing substrate (naphthylphosphate) for 1 hr at 22° C. The medium consisted of: a) 10.0 ml of 0.1% Na-naphthylphosphate, b) 3.0 ml of 5% Na2B4O70 10 H2O, c) 1.0 ml of 10% MgCl2, and d) 10.0 ml of 0.08% fast red salt (5 chloro-a-anisidine). All reagents were prepared in triple-distilled water. After histochemical staining, the sections were rinsed in water, counterstained in Mayer’s hematoxylin for 4 min, and mounted in warm glycerin jelly.

Capillary counts of gracialis muscle in Group B were made by noting the number of stained endothelial cells per unit area of section. For Group A the counts in gracialis and plantaris muscle were made by noting the number of stained endothelial cells per muscle fiber. With the technic described, endothelial cells are stained red. A calibrated binocular compound microscope was used for counting endothelial cells at magnification of ×100 (photographs were made ×125). Three to five sections of muscle tissue per animal were studied. Capillary counts for animals in Group B are expressed as number per square millimeter of tissue, whereas
capillary counts in Group A are expressed as number per muscle fiber.

RESULTS

The effects of chronic exposure to simulated altitude (6,150 m) upon body weight in Group B animals are shown in Fig. 1. All of the animals were apparently in good health at the start of the experiment, but during exposure to altitude one-third of all experimental animals died. The deaths that ensued in the 5-week period were attributed to respiratory tract infection although the pathology in many ways resembled the state that Sundstroem (9) considered adrenal insufficiency. Although both groups of animals started out at the same body weight, the experimental animals, in contrast to the control animals, gained very little weight for the first 21 days. They did gain weight from 21 days on, but were lighter than the controls at the termination of the experiment.

Organ-to-body weight ratios, as well as hematocrit ratios, determined at termination of the experiment, are shown in Table 1 for 10 control and 11 experimental animals from Group B. The organ-to-body weight ratios for heart, testes, kidneys, and adrenal glands are significantly greater in the altitude group than in the control group. Comparison of absolute organ weight changes shows that hearts and adrenals of hypoxic animals weigh more than those of control animals. In contrast, the kidneys and testes are smaller than those of control animals. Hematocrit ratios are significantly higher for the hypoxic animals than for controls.

Table 2 shows the mean capillary counts in gracilis muscles of eight control animals and seven experimental animals.

### Table 2. Capillary count in gracilis muscle

<table>
<thead>
<tr>
<th>Type of Animal</th>
<th>No. of Animals</th>
<th>Mean Wt, g</th>
<th>No. of Capillaries/mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>287</td>
<td>108.9 ± 3.54*</td>
</tr>
<tr>
<td>Experimental</td>
<td>7</td>
<td>154</td>
<td>154.2 ± 5.12†</td>
</tr>
</tbody>
</table>

* Values are means ± se. †Significantly different from control (P < .001).

![FIG. 1A: mortality rate of rats exposed to chronic hypoxia (360 mm Hg). B: mean body weights of rats shown in A during course of experiments.](http://ajplegacy.physiology.org/)

![FIG. 2. Cross section from gracilis muscle of control rat. Stained for alkaline phosphatase. Heavy dark-stained areas are endothelial nuclei (stained red). Smaller dark-staining units are muscle nuclei (stained blue).](http://ajplegacy.physiology.org/)
animals from Group B. Sections were taken from similar areas of muscle in both groups, and counts were made without prior knowledge of treatment. The data were then separated into control and experimental groups and photographs were taken of representative sections. These are presented in Figs. 2 and 3. There appear to be more muscle fibers present in comparable sections of gracilis muscle from experimental animals than in the control animals. Also the number of dark-appearing (stain-red) endothelial cells is greater in the experimental group. Table 3 shows the mean capillary counts made on plantaris and gracilis muscles from Group A animals. From the table it is apparent that the number of capillaries per muscle fiber is the same for experimental and control animals for both muscle groups.

**DISCUSSION**

Hurtado (4, 5) has described mean PO\textsubscript{2} gradients from tracheal air to mixed venous blood in individuals at sea level and those at altitude. Natives acclimatized to altitude show a total drop in PO\textsubscript{2} from tracheal air to mixed venous blood of about 48 mm Hg in contrast to a PO\textsubscript{2} drop of 105 mm Hg in individuals at sea level. The smaller PO\textsubscript{2} gradient in the chronically hypoxic individuals is due to a series of adaptive mechanisms. It seems probable that an increased vascularity (4, 5) and an increase in myoglobin (6) content of muscle may occur. These changes should be useful in providing for the constancy of oxygen tension in the tissues which is compatible with diffusion and utilization of oxygen. Evidence has been presented (10, 11, 12) that guinea pigs born and raised at Morococha (4,385 m) have a greater number of capillaries than controls at sea level. A question that frequently arises is: Are new functional capillaries developed in muscles that are under hypoxic stress or do already existing capillaries open up? Previous studies (8, 10, 11, 12) were concerned with counts of capillaries as determined by India ink or the presence of red blood cells. Unfortunately, one can only determine an increase in vascularity by these methods. It occurred to us that one way to resolve the problem would be to stain endothelial cells of capillaries and count them.

One of us (E.M.J.) devised a modification of a histochemical (7) technic that is used to stain alkaline phosphatase. The staining technic depends on alkaline phosphatase (phosphomonoesterase 1) splitting the substrate naphthylphosphate and the resultant combination of the naphthyl group with the diazo dye (5-chloro-o-anisidine) to give a red-brown color at a pH of 9.0 to 9.6 (1, 2, 3). Muscle nuclei are counterstained with hematoxylin. There is no evidence of the presence of alkaline phosphatase in normal muscle from a variety of animals and man (2). Although skeletal muscle fibers are negative for alkaline phosphatase, the walls of capillaries and their endothelial lining stain well for the enzyme. Thus, it was a relatively easy matter to stain and count the capillaries. Just as with other histological technics, there are disadvantages to the one used in this study. Of particular disadvantage to the histochemical technic is the extreme care that must be exerted in dealing with the tissues so as not to destroy enzyme and the lability of the stain. From the data in Table 2, there seems to be an apparent increase in number of capillaries in gracilis muscle of animals exposed to chronic hypoxia. It was noted, however, but not quantitated that the number of muscle fibers per square millimeter was also increased in these experimental animals. Therefore, the relative increase in fibers per unit area is thought to be due to the smaller size of muscle fibers in the experimental animals. When the capillary counts were made on the basis of number per muscle fiber as in Table 3 there appeared to be no significant difference in the number of capillaries between experimental and control animals.

On the basis of these data it is concluded that the increase in vascularity noted in our experiments and in previous

<table>
<thead>
<tr>
<th>Type of Animal</th>
<th>Muscle</th>
<th>No. of Animals</th>
<th>No. of Capillaries/Muscle Fiber</th>
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<tbody>
<tr>
<td>Control</td>
<td>gracilis</td>
<td>9</td>
<td>0.91 ± 0.03*</td>
</tr>
<tr>
<td>Experimental</td>
<td>gracilis</td>
<td>8</td>
<td>0.89 ± 0.03</td>
</tr>
<tr>
<td>Control</td>
<td>plantaris</td>
<td>8</td>
<td>1.90 ± 0.02</td>
</tr>
<tr>
<td>Experimental</td>
<td>plantaris</td>
<td>8</td>
<td>1.78 ± 0.07</td>
</tr>
</tbody>
</table>

Values are means ± SE.
experiments resulted from an opening of preexisting capillaries rather than from the formation of new ones.

REFERENCES


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