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Capillary Density of Skeletal Muscle in Andean Dogs[1] (39309)

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We have previously reported that the capillary density of canine skeletal muscle increases twofold after only 3 weeks of exposure to a simulated altitude of approximately 4880 m (P_B = 435 mm Hg) (1). This appears to be the consequence of a decrease in the average diameter of the muscle fibers, from 65 to 45 μm, which represents a 50% decrease in the cross-sectional area of the fibers. Calculations indicate that these changes help to maintain adequate levels of P_{O_2} in this tissue when arterial P_{O_2} is low. Therefore, it seemed important to investigate the histological characteristics of skeletal muscle in dogs indigenous to high altitude, in whom the adaptive mechanisms to hypoxia should be optimally developed. This paper reports a histological study in skeletal muscle of dogs native to the Andes.

Materials and methods. The sternothyroid muscle from five mongrel dogs (7.1-16.4 kg) born and living in Cerro de Pasco, Peru (elevation 4350 m, P_B = 458 mm Hg) was removed immediately after the animal was sacrificed. This muscle was selected because it should be minimally affected by the degree of activity of the animal and because we had previously studied this muscle in normal dogs and in dogs subjected to simulated altitude for 3 weeks (1).

These muscles were fixed in 10% buffered formalin and embedded in paraffin (mp 56-57°C). With an American Optical rotary microtome, serial 7-μm transverse slices were cut and stained with the periodic acid-Schiff (PAS) reaction and Van Gieson's. Each muscle yielded 75 slices from which a total of 30 microscopic fields were chosen at random for examination. It is known that considerable shrinkage occurs as a result of these methods for processing the muscle tissues. The absolute dimensions measured are, therefore, different from those existing in the living animal. These muscle samples, however, were treated in a fashion similar to that used on muscles previously studied by us. The relative shrinkage and, thus, the data obtained should be comparable.

On each field, measurements were made to determine the total number of capillaries per square millimeter and the diameters, relative volume, and relative surface area for both capillaries and muscle fibers. The methods used to quantitate these parameters have been previously described in detail (1). In summary, diameters were measured using a calibrated micrometer eyepiece. In the case of fibers where shape varies, two diameters per fiber were taken 90° apart and then averaged. Relative volume was calculated using the random point counting method and a specially designed graticule eyepiece (2). In this stereological technic the fractional area of a component in a given area, for example capillaries or fibers, provides a quantitative estimate of the fractional volume of that component in the total mass, which in this instance is the muscle tissue. Fiber and capillary relative surface area were calculated by a method described by Weibel *et al.* (2). In this method the relative surface area is obtained by measuring the number of intersections of a line of known length through a component. The capillary:fiber number ratio was calculated from the capillary number density and the fiber number density. The latter was computed assuming a hexagonal shape for the muscle fibers, with the fiber diameter being equal to the distance between two opposite corners of the hexagon.

Results. Table I shows average values ± 1 SD of histological parameters measured in the muscle samples of five Andean dogs. High capillary densities and small muscle fiber diameters were found in the muscles of the Andean dogs. The number of capillaries per square millimeter was about three times

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TABLE I. HISTOLOGICAL DATA IN SKELETAL MUSCLE OF FIVE DOGS NATIVE TO 4350 m (458 mm Hg).

	Capillary density (capillaries/mm²)	Capillary diameter (μm)	Capillary volume (%)	Relative capillary surface area (μm/1000 μm³)	Fiber diameter (μm)	Fiber volume (%)	Relative fiber surface area (μm²/1000 μm³)
$\bar{M}$	2016	3.8	4.1	38.7	27.2	88.4	174.0
SD	189	1.1	2.6	7.8	4.9	7.8	25.8

that found in muscles from dogs native to 1610 m (Denver) (1). Because of the high capillary densities, the relative volumes and relative surface areas for the capillaries were high. Mean muscle fiber diameter was 27.2 μm in these Andean dogs, which is less than half the diameter found in muscle from Denver dogs (1). The capillary:fiber number ratio calculated from these values was 1.1.

Figure 1 shows the frequency distribution for fiber size in these animals. The distribution followed a bell-shaped curve with most of the fiber diameters falling between 20 and 30 μm.

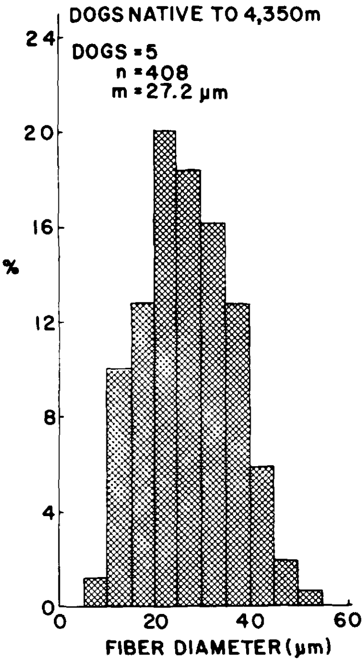

FIG. 1. Relationship between fiber diameter (μm) and percentage of fibers in five Andean dogs.

Discussion. The adaptation of mammalian species to a low P_{O_2} environment is accompanied by a variety of anatomical, biochemical, and physiological changes. Apparently most of these changes contribute to maintain an adequate O_2 tension in the tissue cells and are, therefore, beneficial to the animal. Several adaptive mechanisms have been described in the pulmonary and circulatory systems and in circulating blood, while considerably less is known about the tissue adaptation to hypoxia.

Valdivia (3) found greater capillary density in the skeletal muscle of guinea pigs native to the Andes (4540 m) than in sea level controls. Because India ink was used, no indication was given as to whether the increased number of capillaries was due to the development of new vessels and/or the recruitment of previously closed ones. Since filling of all capillary vessels with India ink is not assured, this technique has been criticized (4, 5). The size and number of muscle fibers per square millimeter were the same in these two groups of guinea pigs, resulting in a 20% higher capillary:fiber number ratio in the high altitude animals. It also has been demonstrated histochemically that the capillary density increases in skeletal muscle of rats rendered severely hypoxic (6). We found a twofold increase in capillary density, accompanied by a 30% reduction in average fiber diameter, in dogs exposed to simulated altitude for 3 weeks, while the capillary:fiber number ratio remained unchanged (1).

Increases in capillary density do not necessarily mean more efficient tissue oxygenation; the geometrical distribution is critical. According to Krogh's model, the availability of O_2 to muscle tissue is dependent on the number of capillaries and their location in respect to the fibers (4). Krogh's formulation assumes a cylinder of tissue dependent on a central capillary, so the predominant factor determining the amount of O_2 reaching the innermost portions of the muscle fiber, if metabolic rate and diffusion rate are constant, is the distance from the open cap-

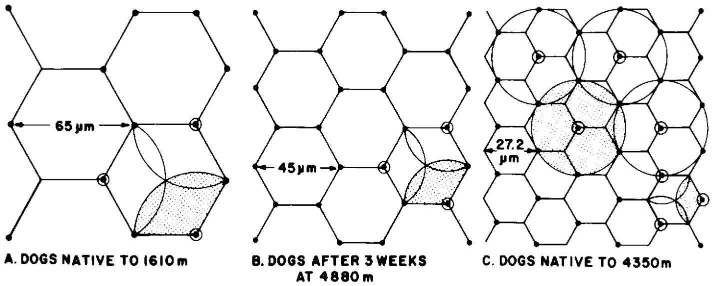

FIG. 2. Models of distribution for capillaries and fibers in dog skeletal muscle at different altitudes (A, B, C). Also shown are the values for the average muscle fiber diameter as measured directly. In A and B the C:F ratio is 2.0, with six capillaries (closed circles) around each fiber. Note that in this situation only three open capillaries (double circles) are sufficient to cover the entire cross section of a muscle fiber. The area of influence of one capillary is shown by stippled area. In C the C:F ratio is 1.0 with only three capillaries per fiber, located in alternate corners. In this situation proper oxygenation of the fiber could be reached if all of the three capillaries are open. Note that if only a few capillaries are open, the cylinder of tissue for each of these capillaries would be about 45 μm in diameter, which is similar to the condition depicted in B.

illary. Under conditions of increased O_2 demands, such as exercise, when the capillary P_{O_2} is low, as in hypoxia, simply increasing the number of capillaries around fibers of fairly large size would not be an efficient means of maintaining the tissue P_{O_2}. However, if accompanied by a concomitant decrease in the diffusion distance for O_2, which could be achieved by a reduction in muscle fiber diameter, the oxygenation of the innermost part of the cell would be assured. The sternothyroid muscle of chronically hypoxic dogs has smaller muscle fiber diameters than the muscle of the low level animals (Fig. 2). The spatial relationship between capillaries and fibers in the skeletal muscle of Andean dogs appears to be more favorable for O_2 exchange, primarily because of substantial decrease in intercapillary distance, due to smaller fiber diameters (Fig. 2C).

Using Krogh's formula (4), we have estimated the P_{O_2} that theoretically exists at the "lethal corner" of a tissue cylinder, near the venous end of the capillary in the muscle of these Andean dogs. Data on P_{O_2} measured in the blood of these same dogs, in the resting state, was used in these calculations (7). An average P_{O_2} value of 31 mm Hg was obtained for the "lethal corner" in the Andean dogs. When compared to the average value of 25 mm Hg, which was obtained using identical calculations for dogs native to 1610 m studied in our laboratory, it appears that the tissue-adaptive mechanisms operating in the Andean dog are very effective. If no differences in muscle fiber diameter occurred as a result of hypoxia, the P_{O_2} at the "lethal corner" of the Andean dog muscle would be only 16 mm Hg. Therefore, the contribution of tissular changes in the process of adaptation to hypoxia is significant and should be taken into account, not only in environmental hypoxia, but also in pathologic conditions associated with hypoxia.

Summary. High capillary density and small muscle fiber diameters were found in the sternothyroid muscle of dogs native to 4350 m. The number of capillaries per square millimeter was three times greater while the diameter was less than half of those obtained in the same muscle of normoxic dogs. These findings suggest that tissular adaptative mechanisms are important in the process of acclimatization to hypoxia, contributing to the maintenance of adequate levels of P_{O_2} in the tissue in the presence of hypoxemia.

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