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Capillary Density of Skeletal Muscle in Dogs Exposed to Simulated Altitude[1] (38556)

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A number of anatomical and physiological changes have been reported in man and in animals living in chronic hypoxia. It is generally believed that these changes are adaptative in nature, and they help the animal tolerate a low Po_2 environment. Possibly these features or mechanisms are optimally developed in man and in animals native to high altitude. However, the study of the acute situation, when a lowland animal is exposed to a low oxygen environment, offers the opportunity to ascertain how fast these changes occur, and, on occasion, provides insight into the underlying mechanisms.

Valdivia (1) has found that high altitude guinea pigs (at 4540 m) have a greater number of open capillaries in skeletal muscle than sea level controls. He regarded this as an important adaptative mechanism to chronic hypoxia even though muscle fiber size was approximately the same in all animals. More recently Cassin *et al.* (2) showed that the number of capillaries per unit surface area in skeletal muscle of rats exposed to 6,100 m for 5 wk increased significantly. However, the evidence of an increased capillarity in hypoxic conditions and its role in facilitating O_2 delivery is not conclusive. Furthermore, there is no experimental evidence that exposure of larger mammals to hypoxia results in structural alterations of peripheral tissues.

Materials and Methods. Three adult mongrel dogs (two males and one female) native to Denver (1610 m PB 635 mmHg) were studied before and after a 3-wk exposure to a simulated altitude of about 4880 m (ambient pressure 435 mmHg) during which time they received food and water *ad libitum*. Because the dogs were trained to run on a treadmill, the sternothyroideous muscle was chosen for study in order to minimize the possible effects of exercise on the skeletal muscle. Before and after exposure to high altitude, a portion of this muscle was removed under general anesthesia (sodium pentobarbital 30 mg/kg). Immediately after removal, the muscle was placed in 10% buffered formalin for approximately one month, embedded in paraffin and cut transversely in 7 μm sections which were then stained using the periodic acid-Schiff (PAS) reaction as modified by Hecht (3). All samples were treated identically and often simultaneously to keep shrinkage constant. An average of 75 sections were obtained from each muscle sample.

Thirty fields selected at random from each muscle sample were measured. The number of capillaries per field was counted. Diameters of capillaries and muscle fibers were measured using a calibrated eyepiece micrometer. Because of their circular shape, only one measurement was made per capillary, whereas in muscle fibers, in which shape varies, the shortest and longest diameters were measured and an arithmetic average was then calculated.

The following morphometric technics described by Weibel and coworkers (4) were used to quantitate some parameters of tissue structure.

(A) Relative volumes of capillaries and muscle fibers were calculated using a special eyepiece graticule[2] with 21 lines of equal length arranged in an equilateral triangular network placed at random on the microscopic section (4). The number of end-points of these lines falling on capillaries and muscle fibers over the total number of end-points, 42, provides an estimate of the relative volume density, i.e., the relative volume of that structure over the total tissue volume. This tech-

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[2] Manufactured by Wild Heerburgg Inst., Inc. CH-9435, Heerburgg, Switzerland, No. 5678.

TABLE I. HISTOLOGICAL DATA IN SKELETAL MUSCLE OF DOGS BEFORE AND AFTER EXPOSURE TO SIMULATED ALTITUDE (435 mmHg) FOR 3 WKS.

Dog bw (kg)	Altitude (mmHg)		Capillary density (cap/mm²)	Capillary diameter (μm)	Capillary volume (%)	Relative capillary surface area (μm²/1000 μm³)	Fiber diameter (μm)	Fiber volume (%)	Relative fiber surface area (μm²/1000 μm³)
	635	m̄	728	5.1	1.3	9.6	62.3	93.6	97.3
		SD	133	1.1	1.7	4.0	7.6	2.6	14.2
25.9	435	m̄	1372	4.6	2.6	18.6	45.8	91.9	118.7
		SD	139	0.5	2.0	6.3	5.3	2.8	17.1
	635	m̄	562	4.8	1.4	9.8	68.3	94.0	77.3
		SD	142	0.3	1.9	4.2	7.3	3.8	18.1
26.2	435	m̄	1150	4.7	2.9	20.8	44.1	91.4	110.2
		SD	200	0.4	1.6	5.9	5.5	3.4	12.0
	635	m̄	582	4.6	1.9	7.4	64.2	91.4	82.5
		SD	99	0.8	1.8	4.0	8.7	9.7	15.6
22.5	435	m̄	1267	4.5	4.2	17.0	47.1	90.0	104.2
		SD	187	1.4	2.8	7.6	5.5	3.2	11.5
	635	m̄	624	4.8	1.5	8.9	65.0	93.0	85.7
		SD	145	0.8	1.8	5.2	8.2	6.2	18.0
	435	m̄	1263	4.6	3.2	18.8	45.7	91.1	111.0
		SD	198	0.9	2.3	6.7	5.5	3.2	14.9
			P <0.005	N.S.	N.S.	<0.005	<0.025	N.S.	<0.025

nic is based on Delesse's principle as modified by Glagoleff (4).

(B) Surface area of capillaries and muscle fibers in a unit volume of tissue (relative surface density) was calculated by counting the number of intercepts of straight lines of a graticule[3] with capillaries or muscle fibers and then dividing this number by the length of the line as originally suggested by Tomkeieff and Henning (4).

The significance of the differences in these data were analyzed by the randomized block design, using a Nova 12-k computer. The level of significance was considered to be less than 0.025.

Results. Table I shows the average values and SD of data obtained in this study and Fig. 1 depicts typical microscopic sections. In Denver, at 1610 m above sea level, the average capillary density in cross sections of skeletal muscle was 624 cap/mm². Approximately a twofold increase in the number of capillaries (mean 1263 cap/mm²) was found after 3 wk at 435 mmHg. As a consequence, and because capillary diameter remained unchanged, the capillary volume density, i.e. the relative volume occupied by capillaries in a unit volume of tissue, and the relative surface area of capillaries per unit volume of tissue also doubled.

A significant decrease in the average diameter of the muscle fibers from 65 to 46 μm was found after three weeks at 435 mmHg, but the relative muscle fiber volume per unit volume of tissue remained the same. The relative surface area density for muscle fibers increased from 85.7 μm²/1000 μm³ at 635 mmHg to 111.0 μm²/1000 μm³ at 435 mmHg. The number of capillaries per muscle fiber did not change upon exposure to hypoxia.

Discussion. Since, in mammals, oxygen must reach cells by diffusion from capillaries, the anatomical arrangement of these vessels is of importance in the process of oxygen delivery, especially in conditions in which the arterial O_2 tension is low. Krogh's equa-

[3] Manufactured by Olympus Corp. of America, New York, No. 2-C320.

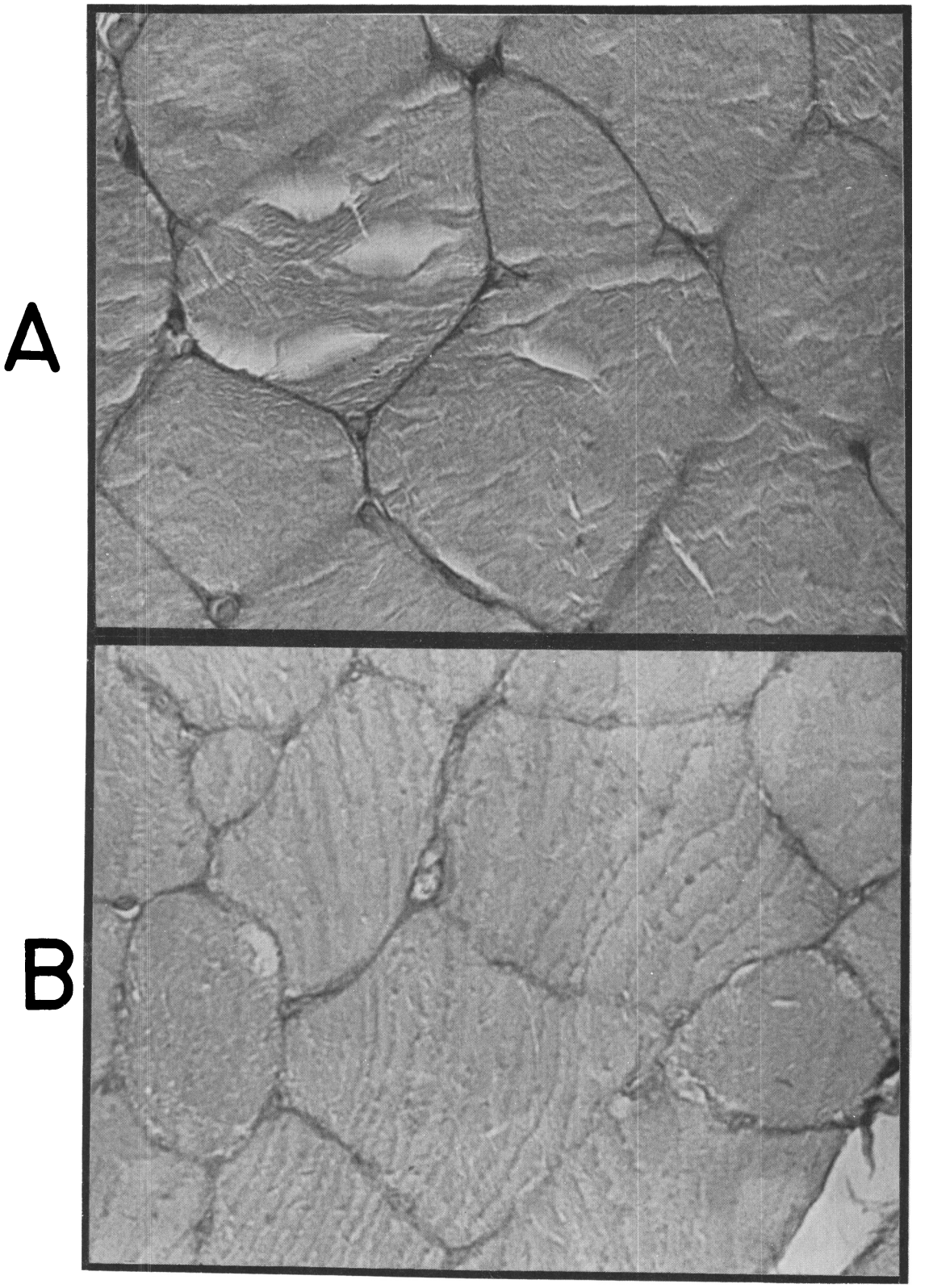

FIG. 1. Photomicrographs of dog's skeletal muscle at 635 mmHg, A, and after a 3-wk exposure to 435 mmHg, B. The size of the muscle fibers is smaller after exposure to hypoxia. The capillaries, which are red stained with PAS, can be counted without difficulty. Periodic acid-Schiff and Van Gieson ×400.

tion for cylindrical diffusion shows that the partial pressure at any point in the muscle fiber is a function of the square of the distance between the capillary and that point and therefore shorter diffusion distances are a very effective way to facilitate oxygenation of cells. It is important to realize that the absolute number of capillaries per unit surface area and/or the fiber/capillary ratio do not necessarily provide a clear picture of the diffusion distances involved in O_2 transport at the tissue level if the size of the individual fibers and the intercapillary distances are ignored.

The absolute number of capillaries per unit area in skeletal muscle in sea level animals appears to be related to body size and metabolic rate, so small animals have rich capillary networks while large animals have relatively fewer capillaries (5, 9). The number of capillaries around each muscle fiber is also related to the capacity of the fibers for oxidative metabolism (6). Species variability, animal muscular activity and differences in white and red muscle fiber composition may change this general relationship (5, 6). It is also known that tissues with high metabolic rates, such as the myocardium, have a high capillary density, as well as a short maximum diffusion distance. These factors allow efficient utilization of the available oxygen (arterial O_2 content).

Rats exposed to altitudes of 8000 m for 3 mo had a greater number of capillaries in the cerebral cortex with smaller intercapillary distances than control rats kept at low levels (7). Increased vascularization of the retina has been observed ophthalmoscopically in rabbits rendered severely hypoxic (6000–9000 m) (8). This increased capillarity has also been reported in animals in their normal hypoxic habitat. High altitude (4540 m) guinea pigs have a greater number of open capillaries in skeletal muscle than sea level guinea pigs, but no changes have been observed in the muscle fiber size (1). There is evidence that this increased capillary density develops within weeks. Rats exposed to 6100 m for 5 wk also show a greater number of capillaries in skeletal muscle than controls (2). Our findings provide strong experimental evidence indicating that, in dogs, the adaptation to an environment of low Po_2, (arterial Po_2 of about 50 mmHg), causes anatomical changes that develop within weeks and would tend to facilitate the oxygenation of skeletal muscle cells.

In this report, the most striking change observed following a 3-wk exposure to hypoxia was a 30% reduction in average fiber diameter associated with maintenance of the number of capillaries per fiber. The reduction in fiber diameter, from 65 to 46 μm, represents a reduction in cross sectional area of about 50% if a circular or hexagonal fiber shape is assumed. As a consequence of the change in cross-sectional area of the muscle fiber, the number of capillaries per unit area and the relative surface area density doubled. The relative volume of fibers per unit volume of tissue and the body weight of the animals remained unchanged with no apparent reduction in total muscular mass as a result of changes in the size of the individual fibers. We believe that the muscle samples studied were primarily of the same muscle fiber type composition because the capillary/fiber ratios were the same at both altitudes. In two of these dogs, an increase in mitochondrial protein content was measured after 3 wk at 435 mmHg.

It is well known that not all capillaries are open at the same time. Technics that make use of dye injections measure the number of capillaries that are open and so higher capillary counts do not necessarily indicate the development of new vessels but could be the result of capillary recruitment. Histochemical methods, such as PAS, by reacting with the capillary basement membrane, provide a total capillary count regardless of their functional state at the time of sampling. Therefore an increase in capillary density is definitive evidence of the development of new vessels if the muscle mass remains constant.

The maximal diffusion distance for oxygen is probably close to the radius when a circular cross section is assumed for the muscle fiber (9) or the side of a hexagon when hexagonal geometry is considered (10). Therefore the reduction in fiber size that occurs upon exposure to simulated altitude is advantageous because in these conditions, the capillary blood has a low Po_2, but the metabolic rate is essentially the same.

The arterial Po_2 in these dogs decreased

from an average of 72 mmHg in Denver to 47 mmHg at 4880 m. However, due to the compensatory hemodynamic and hematologic changes the $P_{\bar{v}}o_2$ fell only from about 37 to about 32 mmHg so that the average capillary Po_2, calculated using Barcroft's formula, fell from 54 to 38 mmHg. Under these conditions, and if the diffusion constant and the metabolic activity of the muscle do not change, it is estimated that the Po_2 in the center of the muscle fiber in hypoxia is even higher than that found at sea level, due to the decreased size of the muscle fiber.

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