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# Effect of Hypoxia on the Capillarity of Guinea Pig Skeletal Muscle[1] (40452)

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Under conditions of sustained hypoxia the oxygenation of the tissues of mammals is helped by a variety of adaptive changes that occur in the respiratory and cardiovascular systems (1–3). These changes reduce the fall in partial pressure of $O_2$ between the ambient air and the capillary vessels. In spite of this, $P\acute{c}O_2$ and $P\bar{v}O_2$ are lower in animals at high altitude than at sea level, apparently reducing the tissue $PO_2$ (1–4). This has prompted investigators to look for histological and biochemical adaptations which could compensate for the reduced capillary blood $PO_2$.

Increased capillarity in tissues has been reported to occur in response to hypoxia (5–8). Valdivia (8) using India ink injections found that guinea pigs native to the Peruvian mountains had a higher muscle capillary density than sea level controls. This resulted from more capillaries around muscle fibers of similar cross sectional area. In the dog, the increased capillary density was associated with a smaller fiber cross sectional area at high altitude (5, 6). We found recently that rats exposed to moderately severe hypoxia for 3–6 weeks had the same capillary density and muscle fiber geometry as normoxic rats of similar body weight (9). In the light of these findings the possibility of species variability was considered and a reevaluation of the work on the Peruvian guinea pig was undertaken.

Using a histochemical technique that stains capillaries independent of their functional state (10) we have studied the capillarity of skeletal muscle of guinea pigs from the Peruvian Andes and from sea level. It was felt that animals native to the Andes would show the full effects of adaptation to hypoxia. We have also studied, using the myosin ATPase reaction, fiber composition in the muscles in these same guinea pigs. Capillary counts in areas of known fiber composition were compared because capillary to fiber ratio and capillary density change with fiber composition (11, 12) and, in some species, with normal maturation (13, 14).

*Material and methods.* Forty-four mongrel guinea pigs were used in these experiments. Twenty-four males from sea level, weighing between 200 and 637 g, were studied in Lima (150 m). Twelve males and eight females, weighing between 353 and 795 g, obtained in the Peruvian Andes at elevations between 3800 and 4000 m above sea level, were studied immediately after arrival in a laboratory located at 3300 m. Histochemical studies were carried out in only 13 of the 20 high altitude guinea pigs. Of these, nine were male and four were female.

The animals were sacrificed with an overdose of sodium pentobarbital (ip). The soleus and the gastrocnemius–plantaris muscles were dissected out and weighed while moist to the nearest mg. The muscles were cut transversely at the widest point of the belly and frozen in isopentane cooled (−130°) with liquid nitrogen. The samples were then stored in liquid nitrogen until sectioning at 20 $\mu$m thickness in a cryostat. In the gastrocnemius, only the central portion of the medial head was studied.

The myofibrillar ATPase method, after preincubation at pH's of 4.5 and 9.4, was used for the identification of fibers (15). In a few guinea pigs, the oxidative capacity of the fibers was studied by the DPNH tetrazolium reductase technique and correlated with the ATPase method (15).

Three fiber types were identified with these techniques: Fast twitch glycolytic (FTG), fast twitch oxidative–glycolytic (FTOG) and slow twitch oxidative (STO). This classification proposed by Peter *et al.* (16) corresponds to the IIB, IIA and I offered by Dubowitz and

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Brooke (15) and to the white, red, intermediate used by us in previous publications (9, 13). FTOG fibers had the highest oxidative capacity and the lowest ATPase activity as judged by the DPNH tetrazolium reductase and the myofibrillar ATPase after preincubation at pH of 4.5. FTG fibers had the lowest oxidative capacity and intermediate ATPase activity. STO fibers had a very high ATPase activity and a high oxidative capacity. As shown by Dubowitz and Brooke (15) the myofibrillar ATPase reaction after acid preincubation permits the visualization of three fiber types. Because the medial head of the gastrocnemius shows some heterogeneity (Fig. 1) the area of study should be clearly identified. In the medial head of the gastrocnemius, photomicrographs were taken of

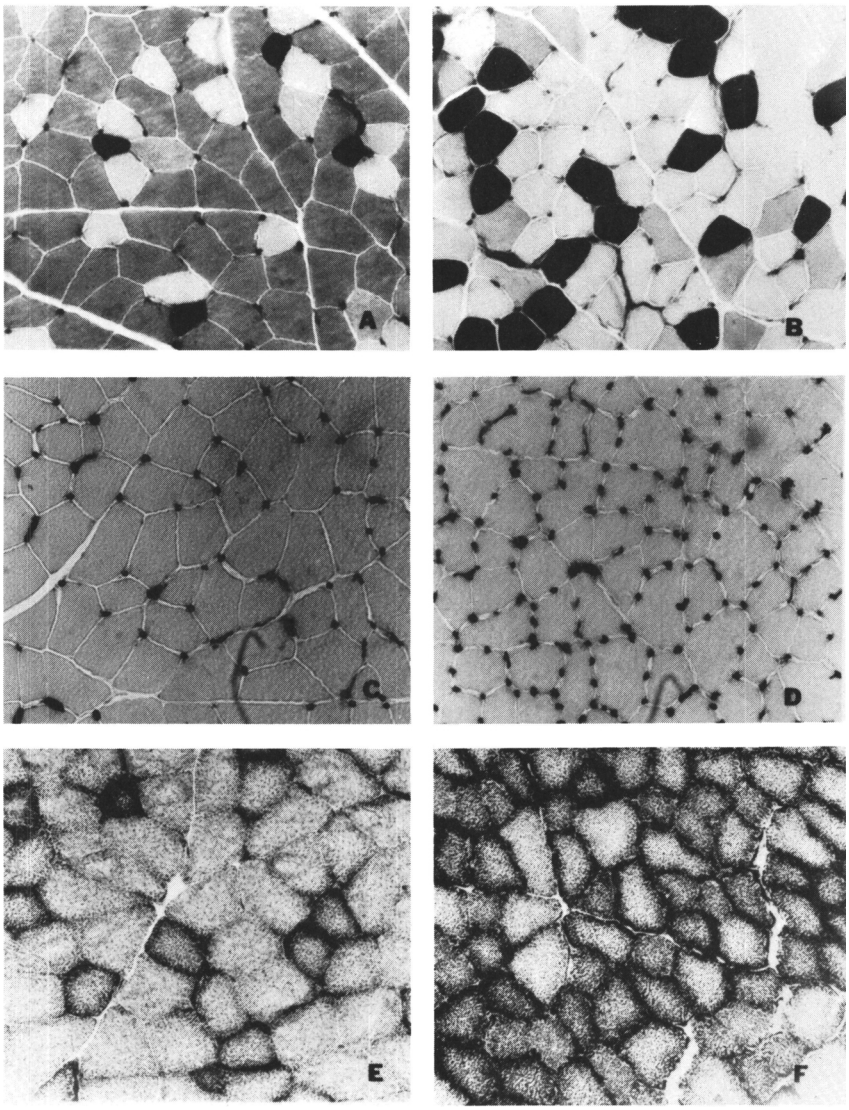


FIG. 1. Photomicrographs of cross sections of the medial head of the gastrocnemius of a guinea pig. Panels A through D were processed for the myosin ATPase reaction. A and B after preincubation at a pH of 4.5, C and D after preincubation at a pH of 3.8. Panels E and F were processed for the DPNH tetrazolium reductase reaction. The left hand panels are photomicrographs from the lateral area of the medial head while the right hand panels are from the middle portion of the same cross section. Note the difference in fiber composition, capillary density, C:F and in oxidative activity.

three to four areas in the middle of the cross section where FTOG fibers are abundant. In the soleus, in which only STO fibers are present, two photomicrographs were obtained. The photomicrographs were then projected on a screen at a known magnification to estimate by stereology, using the differential point-count method, the area occupied by each fiber type (17). The absolute numbers of fibers were also obtained. With this information the fiber density and the average muscle fiber cross sectional area were calculated. The average number of fibers counted in each gastrocnemius was 641. In the soleus, where fiber population is homogeneous, a smaller number was counted.

The same ATPase reaction after preincubation at a pH between 3.8 and 4.0 was used to stain capillaries (10). This technique demonstrates ATPase-like activity in the capillary endothelium and permits visualization of capillaries, open and closed. We have compared the quality of this technique against accepted ones and prefer it mainly because the color differences (black and white) greatly facilitate the identification of capillaries (10). In each muscle sample 30 different microscopic fields were used to count capillaries directly under oil immersion (1000×). These fields covered the same areas where fiber composition was determined. Each field had an actual area equal to 0.0181 $mm^2$. These direct counts were then averaged and values for capillary density were expressed as capillaries/$mm^2$. Capillary to fiber ratios (C:F) were calculated by dividing capillary density by fiber density.

*Results.* The weight of the gastrocnemius-plantaris and the soleus muscles increased linearly with increasing body weight. For each muscle, this relation was similar for both sea level and high altitude guinea pigs (Fig. 2). No differences between male and female animals were observed. On the average, the weight of the gastrocnemius-plantaris increased 584% and the soleus increased 387% when body weight increased from 200 to 800 g.

Mean fiber cross sectional area was positively related to body weight; however, these relationships were different for the sea level and for the high altitude guinea pigs (Figs. 3 and 4). The slopes of the regression lines for each muscle were statistically the same, while adjusted means were higher for the muscles of the high altitude animals. This means that at a given body weight the average fiber cross sectional area of both gastrocnemius and soleus was greater in the high altitude guinea

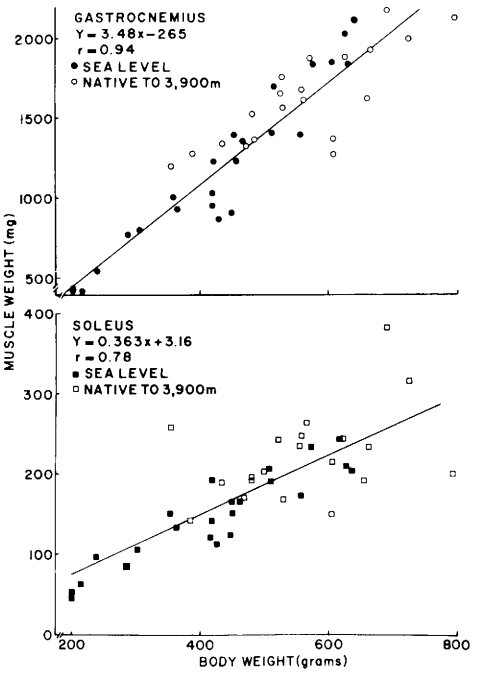


FIG. 2. Relationships between body weight and the weight of the gastrocnemius-plantaris and soleus of sea level and high altitude guinea pigs.

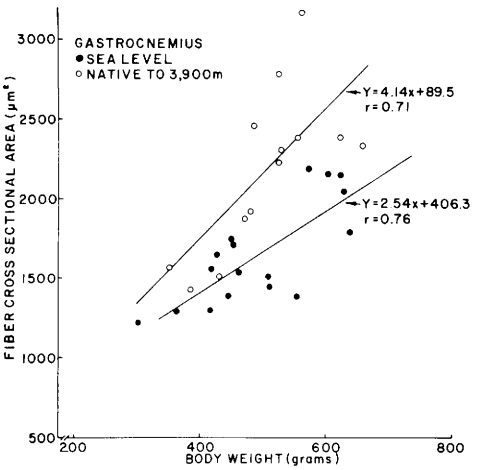


FIG. 3. Relationship between body weight and cross sectional area of the fibers in the gastrocnemius of sea level and high altitude guinea pigs.

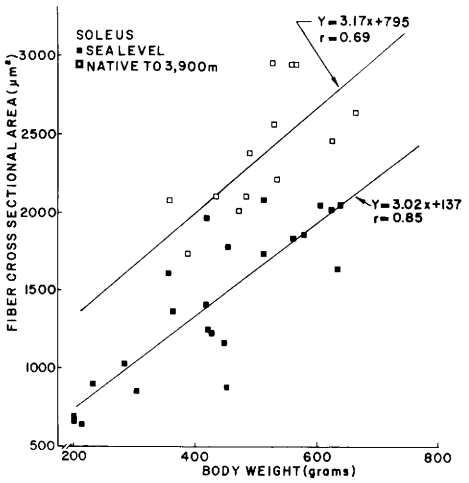


FIG. 4. Relationship between body weight and cross sectional area of the fibers in the soleus of sea level and high altitude guinea pigs.

TABLE I. CAPILLARY DENSITY IN SKELETAL MUSCLE OF SEA LEVEL AND HIGH ALTITUDE GUINEA PIGS.

| Muscle | Origin | $n$ | Capillary density (cap/mm$^2$) | $t$ | $P$ |
|---|---|---|---|---|---|
| Soleus | Sea level | 23 | 600 ± 66* | 1.65 | >0.05 |
| | 3900 m | 13 | 567 ± 37 | | |
| Gastrocnemius | Sea level | 20 | 718 ± 68 | 1.63 | >0.05 |
| | 3900 m | 13 | 757 ± 63 | | |

* Values are means ± SD.

pigs. No differences between male and female guinea pigs were observed.

No significant correlation between capillary density and body weight was found for sea level and high altitude guinea pigs; however, the capillary density tended to be less in the muscles of the larger animals. For a given muscle there was no difference in capillary density between sea level and high altitude animals (Table I). Capillary density was, however, higher in the gastrocnemius than in the soleus ($P < .01$) apparently as a consequence of differences in fiber composition. Because at a given body weight the fiber cross sectional areas of the gastrocnemius and soleus were approximately the same, a greater C:F was measured in the gastrocnemius than in the soleus. The fiber composition for sea level and hypoxic animals was the same. The soleus muscle had only STO fibers, while the medial head of the gastrocnemius showed three distinct fiber types (Fig. 1, Table II). We found no changes in fiber composition with increasing body weight.

The C:F increased as the cross sectional area of the muscle fibers increased. The relationship between C:F and fiber cross sectional area was linear and unique for a given muscle. Hence, only one regression line represents data from sea level and high altitude animals (Fig. 5).

*Discussion.* The guinea pigs used were mongrel animals raised domestically by Peruvian farmers and were readily available at sea level as well as at high altitude. Guinea pigs are indigenous to the Peruvian Andes and are believed to have been domesticated in the "agricultural era" about 1000 BC (18). Adaptive mechanisms are thought to be maximally developed in this species. Histochemically the muscles of these mongrel guinea pigs are similar to those obtained from reputable laboratory suppliers in the U.S.A.

In mammals, postnatal growth of skeletal

TABLE II. FIBER COMPOSITION IN SKELETAL MUSCLE (GASTROCNEMIUS, MEDIAL HEAD) OF SEA LEVEL AND HIGH ALTITUDE GUINEA PIGS.

| Fiber type | Origin | Fiber population % | $t$ | $P$ |
|---|---|---|---|---|
| FTOG | Sea level | 45.5 ± 5.0* | 0.78 | >0.05 |
| | 3900 m | 44.0 ± 4.8 | | |
| FTG | Sea level | 31.2 ± 6.7 | 1.24 | >0.05 |
| | 3900 m | 30.5 ± 5.3 | | |
| STO | Sea level | 22.6 ± 4.7 | 0.28 | >0.05 |
| | 3900 m | 25.0 ± 5.0 | | |

* Values are means ± SD.

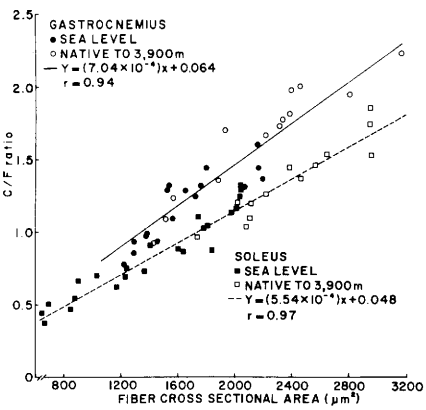


FIG. 5. Relationship between fiber cross sectional area and C:F in the gastrocnemius and soleus of sea level and high altitude guinea pigs.

muscle is mainly due to hypertrophy of fibers rather than to hyperplasia (19). Faulkner *et al.* (20, 21) have found a linear increase in the fiber cross sectional area of the plantaris with increasing body weight. In our work the relationships between body weight and muscle weight were similar in sea level and high altitude guinea pigs. At a given body weight, however, the mean fiber cross sectional area was larger in the hypoxic guinea pigs, suggesting that the total number of fibers in a muscle cross section may be smaller.

In contrast with previous reports on the Peruvian guinea pig, we did not find a higher capillary density in the gastrocnemius or the soleus of guinea pigs living at high altitude. This lack of differences in skeletal muscle capillarity between sea level and high altitude guinea pigs agrees with our results in rats exposed to simulated altitude equivalent to 5100 m for 6 weeks (9). Valdivia (8) who had previously studied the Peruvian high altitude guinea pig, found that these animals had a higher capillary density with a greater number of capillaries around fibers of the vastus lateralis, rectus femoris and in the lateral and medial heads of the gastrocnemius than sea level guinea pigs. Miller and Hale (7) found that polycythemia alone can cause increased capillary density. Unfortunately, the India ink injection used by Valdivia and by Miller and Hale to visualize capillaries does not permit one to conclude whether the increased capillary density was due to development of new vessels or more complete filling of the existing ones.

A word of caution about the techniques used in the assessment of the changes in capillarity is required. The intravascular injection of liquids containing stains or small inert particles to visualize capillaries has been criticized since it was first used. Krogh (22) was beset by incomplete filling of skeletal muscle capillaries when using India ink in gelatin and he unmistakably pointed out his difficulty with such a method. More recently, Plyley and Groom (23), using Microfil, a silicon elastomer, as the injectate, found considerable variability (between 50–99%, median 87%) in the percentage of filled capillaries. Willner and Groom (24) have recently reported a linear increase in the percentage of capillaries filled with Microfil when perfusion pressure was raised from 80 to 250 cm $H_2O$. Even at 450 cm $H_2O$ pressure, only 85% of the capillaries were filled. We have also encountered difficulties in achieving a homogeneous filling using India ink in gelatin. In our best experiments we were able to fill about 80% of the capillaries visualized with the ATPase method. It is known that at any given time a considerable proportion of capillaries in resting skeletal muscle are not perfused, a situation that does not occur during exercise, when most of them appear to be open. This means that blood flows through capillaries intermittently because of closing and opening of the precapillary sphincters (25). This fact and the different viscosities of the injectates, in relation to blood itself, explain in part the difficulties with the injection methods. The ATPase technique to visualize capillaries does not depend on filling. It involves a histochemical reaction at the capillary endothelium and is, thus, independent of the functional state of the microcirculation.

Capillary density in these guinea pigs, either normoxic or hypoxic, was not related to body weight. However, as muscle fibers increase in cross sectional area there was a linear increase in C:F. That C:F is dependent on fiber cross sectional area has been reported previously (14, 26, 27) despite differences in the methodology, in the muscle studied and in the animal species under investigation.

The slopes of the lines relating fiber cross sectional area and C:F were different in the soleus and the gastrocnemius. This apparently is a function of differences in fiber composition since FTOG fibers have more capillaries around them than FTG and STO fibers (12). Increases in the area of the FTOG fibers (highly oxidative) that depend preponderantly on aerobic metabolism seem to be accompanied by a greater increase in the number of capillaries around them than around the FTG and STO fibers.

Our values of C:F ranged between 0.37 and 1.86 in the soleus and from 0.78 to 2.23 in the gastrocnemius. These changes in C:F with increases in the girth of the fibers appear to be compensatory since the area of muscle cross section served by a capillary is approximately the same in each muscle. We have recently reported that in the solei of three different animal species, the percentage area

of muscle tissue cross section served by a capillary located at a certain distance is maintained within narrow limits by the increases in C:F as the fiber cross sectional area increases (14). Despite eightfold differences in fiber cross sectional area, the difference in diffusion distances was never more than 10 μm.

*Summary.* Capillarity and fiber composition were studied by the ATPase technique in frozen muscle samples of mongrel guinea pigs from sea level (SL)(body weight: 200–637 g) and from the Peruvian Andes at 3900 m (HA)(body weight: 350–795 g). Capillary density (CD), capillary to fiber ratio (C:F), fiber cross sectional area (FCSA) and fiber composition were measured in the soleus and the central portion of the medial head of the gastrocnemius. CD was always higher in the gastrocnemius (718/mm$^2$ SL, 757/mm$^2$ HA) than in the soleus (600/mm$^2$ SL, 657/mm$^2$ HA). No correlation between CD and body weight (BW) was found. Mean FCSA was linearly related to BW. At a given BW, FCSA was greater in the HA than in the SL animals. C:F increased linearly with FCSA in the SL and the HA groups, but the relationships were different in the two muscles: C:F = 7.04 $\times$ 10$^{-4}$ FCSA + 0.064, $r$ = .94 in the gastrocnemius, C:F = 5.54 $\times$ 10$^{-4}$ FCSA + 0.048, $r$ = .97, in the soleus. The soleus had only slow twitch oxidative fibers, the gastrocnemius showed three fiber types. No differences in fiber composition in either muscle were observed with hypoxia. It appears that this degree of hypoxia was not enough to produce changes in capillarity or in fiber composition. The increase in C:F as FCSA increases apparently maintains constant the mass of tissue perfused by one capillary.

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