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Article *in* The American journal of physiology · July 1977

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Capillary supply of skeletal muscle fibers in untrained and endurance-trained men

PER BRODAL, FRANK INGJER, AND LARS HERMANSEN
Anatomical Institute, University of Oslo; Institute of Work Physiology; Norwegian College of Physical Education and Sport; and Joint Medical Services, Norwegian Armed Forces, Oslo, Norway

BRODAL, PER, FRANK INGJER, AND LARS HERMANSEN. *Capillary supply of skeletal muscle fibers in untrained and endurance-trained men.* Am. J. Physiol. 232(6): H705–H712, 1977 or Am. J. Physiol.: Heart Circ. Physiol. 1(6): H705–H712, 1977. – Muscle fiber diameters and numbers of capillaries per fiber, per square millimeter, and around each fiber were determined in needle biopsies from the lateral part of the quadriceps muscle of 23 young men. Twelve subjects were untrained (UT) and eleven were endurance-trained (ET) athletes. Average values for maximal oxygen uptake were 51.3 (UT) and 72.0 ml/kg·min (ET). Mean fiber diameters were not significantly different in the two groups (48.8 and 49.1 μm). The capillaries per fiber ratios were 1.77 ± 0.10 and 2.49 ± 0.08 (mean ± SE) in the UT and ET groups, respectively. The numbers of capillaries around each fiber were 4.43 ± 0.19 (UT) and 5.87 ± 0.18 (ET). The numbers of capillaries per mm^2 were 585 ± 40 (UT) and 821 ± 28 (ET). Fiber diameters were 28% smaller in ultrathin than in fresh-frozen sections from the same biopsies. After correction for this difference, the numbers of capillaries per mm^2 were 305 and 425 in the UT and ET, respectively. The capillaries per fiber ratio increased with increasing fiber diameter, but not sufficiently to maintain the number of capillaries per mm^2. Fibers containing many mitochondria are surrounded by more capillaries than fibers with few mitochondria.

quantitative electron microscopy

DURING PHYSICAL CONDITIONING the capacity to transport oxygen during maximal work is increased 15–30% (24). Approximately half of this is attributable to an increase in cardiac output (24, 25). The remaining 50% is explained by an increased oxygen extraction by the working muscle. Several mechanisms for this adjustment in oxygen extraction has been suggested (24), one possibility being an increase in the capillary density of skeletal muscle.

A classical approach (15) to the study of oxygen transport in peripheral tissues has been to calculate the Po_2 gradients between capillaries and cells. To do this, dimensions of the capillary bed, the diffusion coefficient, and the metabolic rate of the tissue are determined. The accuracy of these calculations depends critically upon precision of the determination of capillary density (capillaries per mm^2).

Despite numerous attempts to determine the density of capillaries in skeletal muscle of various mammals, no general agreement has been reached with respect to normal values and changes during physical conditioning (9, 16, 21). The different results can only partly be explained by the fact that different animal species and different muscles have been used (20). All previous studies, except one (25), have used the light microscope, with which a definitive identification of a capillary might be difficult (1, 11, 16, 17, 19, 20, 21, 23). The objective of the present study was to employ the electron microscope to identify positively all capillaries. Values for the capillaries per fiber ratio and capillary density in normal young men and endurance-trained athletes were obtained, and the influence of diameter and mitochondrial content of muscle fibers was also examined.

MATERIALS AND METHODS

The subjects were 23 men between 18 and 34 years of age. Twelve of these had not been engaged in regular physical training during the last 5 yr, and had never taken part in any competitive sports. Eleven subjects had been engaged regularly (4–7 times a week) in hard endurance training and competition (cross-country skiing or long-distance running) (Table 1).

After being thoroughly familiarized with the procedures, all subjects gave their consent for the determination of maximal oxygen uptake and taking of muscle biopsies. Maximal oxygen uptake was measured according to the procedure of Hermansen (10). All expired gas samples were analyzed using the Scholander apparatus (26). After local anesthesia of the skin, needle biopsies (2) were taken from the lateral part of the quadriceps muscle, 10–15 cm above the proximal part of the patella. In most cases one biopsy was taken from each leg. The biopsies were then usually divided longitudinally into two or more blocks. Those selected for electron-microscopic examination were immediately immersed in a cooled mixture of 2.5% glutaraldehyde and 2–4% paraformaldehyde in a 0.1 M cacodylate buffer at pH 7.4. The remaining blocks were frozen in Freon (Virginia Chemicals) cooled by liquid nitrogen for late ATP-ase staining (see below). The blocks for electron microscopy were fixed for 2 h in the aldehyde solutions, then postfixed for 1 h in 1% buffered osmic acid, dehydrated in graded solutions of acetone, and embedded in TAAB embedding resin. Semithin (1 μm) and ultrathin sections were cut using an LKB Ultrotome. The semithin

sections were stained with *p*-phenylendiamine. Ultrathin sections were stained with lead citrate and examined in a Siemens Elmiskop 1. Semithin sections were examined in the light microscope to ensure the necessary true transverse sectioning. Ultrathin sections were examined using a primary magnification of 10,000. The outlines of the muscle fibers were simultaneously drawn with 500 times magnification (Fig. 1) using an X-Y writer connected to the specimen stage (28). Only fibers with the whole circumference within the section and capillaries close to these fibers were used for later calculations (Fig. 1). Vessels without a continuous cellular lining external to the basal lamina of the endothelial cells were counted as capillaries (Fig. 2*A*).

Fibers in cross section were classified on the basis of number of subsarcolemmal mitochondrial aggregates observed (Fig. 2, *B* and *C*). Each fiber was classified into one of the following categories: M1, no subsarcolemmal aggregates present; M2, one or two aggregates present; and M3, three or more aggregates present. Usually the whole circumference of 50 (20–120) fibers could be drawn from one section. Sections from two or more blocks from one person were often examined in order to obtain a larger number of fibers (range 78–132 fibers from each subject). The fibers were counted and their "lesser diameters" (6) were measured.

Different methods were used for the determination of the capillaries per fiber ratio and capillaries per mm^2. For the latter, only a rectangular part of the drawing with fairly closely packed fibers was used (indicated with broken line, Fig. 1). Within the area fibers and capillaries were counted (counting only half the number of fibers and capillaries crossing the edges of the rectangle), and after determining the size of the rectangle, the number of fibers and capillaries per mm^2 could be calculated for each person.

To determine the capillaries per fiber ratio, we used the total number of capillaries and fibers in the whole drawing (Fig. 1). However, the number of capillaries needs correction, since the capillaries in the periphery of the drawing (indicated with a circle and cross in Fig. 1) "belong" not only to the drawn fibers, but also to the adjoining fibers not included in the drawing. An approximate correction was obtained by subtracting half the number of capillaries in the periphery from the total number of capillaries. The capillaries per fiber ratio could then be calculated (corrected number of capillaries/number of fibers). This correction is important, because in seven sections containing an average number of 77 fibers (53–104), capillaries per fiber ratios based on uncorrected numbers of capillaries were 16 (13–21)% higher than when using corrected numbers.

For each fiber the number of capillaries in close contact was counted and the average number was calculated for each person and group (*capillaries around each fiber, 4th col,* Table 2). The sharing factor (that is, the average number of fibers sharing one capillary) was determined by dividing the average number of capillaries around each fiber with the capillaries per fiber ratio.

To determine shrinkage in the material, fiber diameters were measured in sections stained for myosin ATPase activity (6) from fresh-frozen blocks from the same biopsies used for EM study (see above). In addition, measurements in enlarged photomicrographs of semithin sections, cut just before or after the ultrathin sections, were used to determine any reduction in fiber

TABLE 1. *Age, weight, and maximal oxygen uptake of experimental subjects*

Groups	Age, yr	Wt, kg	Maximal O_2 Uptake, ml/kg·min
Untrained	24	74.4	51.3
n = 12	(22–31)	(65.5–88)	(44.4–57.7)
Endurance trained	23	68.5	72.0
n = 11	(18–34)	(58.7–75.1)	(58.7–77.3)

Values are means; ranges are given in parentheses.

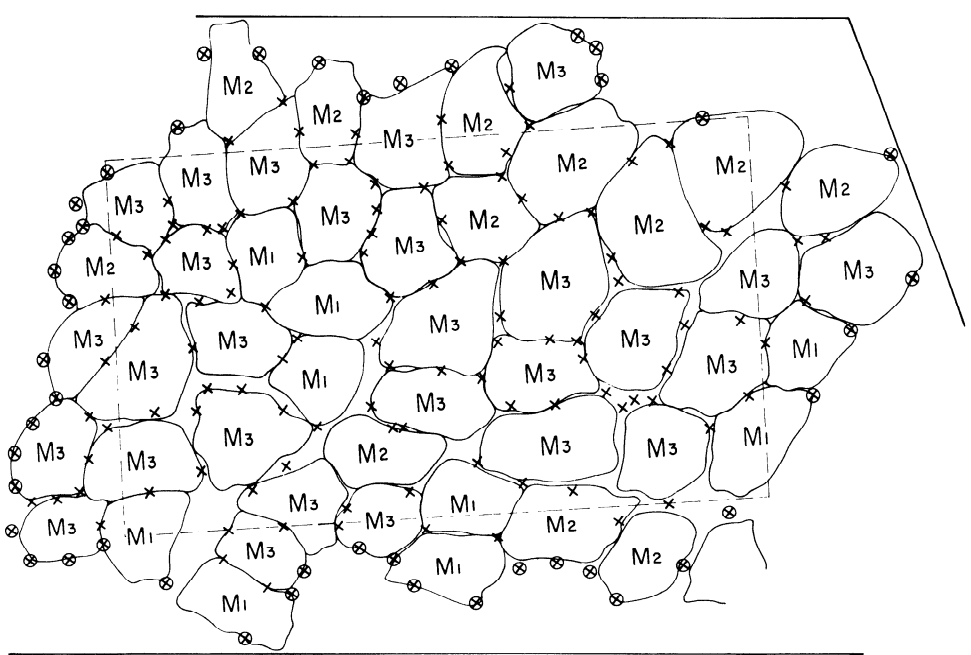


FIG. 1. Slightly schematized example of drawing of an ultrathin section made with X-Y writer connected to an electron microscope (see text). This section is from an endurance-trained athlete with maximal oxygen uptake of 70 ml/kg·min. A total of 49 fibers are drawn in outline and used for capillaries per fiber ratio determination. All fibers are classified as M_1, M_2, or M_3 (see text). All capillaries are indicated by a cross, ×; those in periphery are indicated by a cross within a circle, ⊗. Half the number of the latter capillaries are subtracted from the total number of capillaries before calculating the capillaries per fiber ratio. Broken line indicates the area (0.10 mm^2) used for calculating the number of capillaries and fibers per mm^2.

TABLE 2. *Diameter of muscle fibers and number of capillaries per fiber and per square millimeter in untrained and endurance-trained subjects*

Groups	n	Diameter, μm	Capillaries per Fiber	Capillaries Around Each Fiber	Capillaries per mm²	Fibers per mm²
Untrained	1,124	48.8 ± 2.5	1.77 ± 0.10	4.43 ± 0.19	585 ± 40	321 ± 31
(12)		8.6	0.33	0.67	140	108
Endurance trained	1,067	49.1 ± 1.1	2.49 ± 0.08	5.87 ± 0.18	821 ± 28	313 ± 16
(11)		3.8	0.26	0.59	97	55

Values are means ± SE and SD. The number of subjects is given in parentheses. *n*, number of fibers.

diameter caused by compression during ultrathin sectioning (Table 3). Identical fibers were measured before and after ultrathin sectioning.

The Student *t* test for independent samples was used for testing the significance of differences between the two groups of subjects.

TABLE 3. *Linear shrinkage of ultrathin sections as compared to frozen and semithin sections*

Subjects n	Frozen Sections		Semithin Sections		Ultrathin Sections		% Reduction of Mean Fiber Diam†
	n	Diam, μm*	n	Diam, μm	n	Diam, μm	
9	1,557	70±2.5			770	50±1.9	28±2.1 (20–37)
7			181	56±2.9	181	49±2.2	13±0.9 (10–17)

n, number of fibers. * Mean value ± SE of the mean diameter for each person † Mean of individual values; range is given in parentheses.

RESULTS

Size of capillaries and fibers. Most capillaries were 3–5 μm in diameter, and none was less than 1 nor more than 7 μm.

Mean fiber diameters are practically identical in the untrained and the endurance-trained subjects (Table 2). The distribution of fiber diameters collected in groups of 10 μm is illustrated in Fig. 3, and corresponds well to previous data from human quadriceps muscle (3).

Capillaries per muscle fiber. The average number of capillaries per fiber is 41% greater in the endurance-trained than in the untrained subjects, i.e., 2.49 and 1.77, respectively ($P < 0.001$; Table 2). When the average number of capillaries around each fiber (Table 2) is compared in the two groups, the difference is 33%, i.e., 5.87 and 4.43 in the endurance-trained and untrained group, respectively ($P < 0.001$). Thus, each capillary is, on the average, shared by fewer fibers in the trained (2.35 ± 0.03 SE) than in the untrained (2.50 ± 0.03) subjects ($P < 0.005$).

Capillaries and fibers per square millimeter. The average number of capillaries per mm² was 820 and 585 in the trained and untrained group, respectively. The difference (40%, $P < 0.001$; Table 2) corresponds well with the average difference in the number of capillaries per fiber in the two groups. The difference in the number of capillaries is not due to different mean fiber diameters (Table 2). Nor are the average number of fibers per mm² significantly different, i.e., 313 and 321 for the trained and untrained groups, respectively. In Fig. 4 it is shown that with a greater fiber diameter (fewer fibers per mm²), the number of capillaries per mm² is lower. The maximal average fiber diameter for all subjects is 64 μm and the minimal is 37 μm. From Fig. 4 it can be estimated that a 10-μm increase in the lesser diameter corresponds to approximately 120 fewer fibers per mm², and about 150 fewer capillaries per mm².

Correction for shrinkage. The shrinkage of the material during processing for electron microscopy caused an average of 28% reduction of the lesser diameter when compared to frozen sections in nine subjects. Consequently, the fiber area was reduced by 48%. During the ultrathin sectioning, the sections were compressed, reducing the fiber diameter by 13% as compared to the semithin sections (Table 3). When corrected for 48% shrinkage the number of capillaries per mm² was 305 (untrained) and 425 (endurance trained). The numbers of fibers per mm² was 167 (untrained) and 163 (endurance trained).

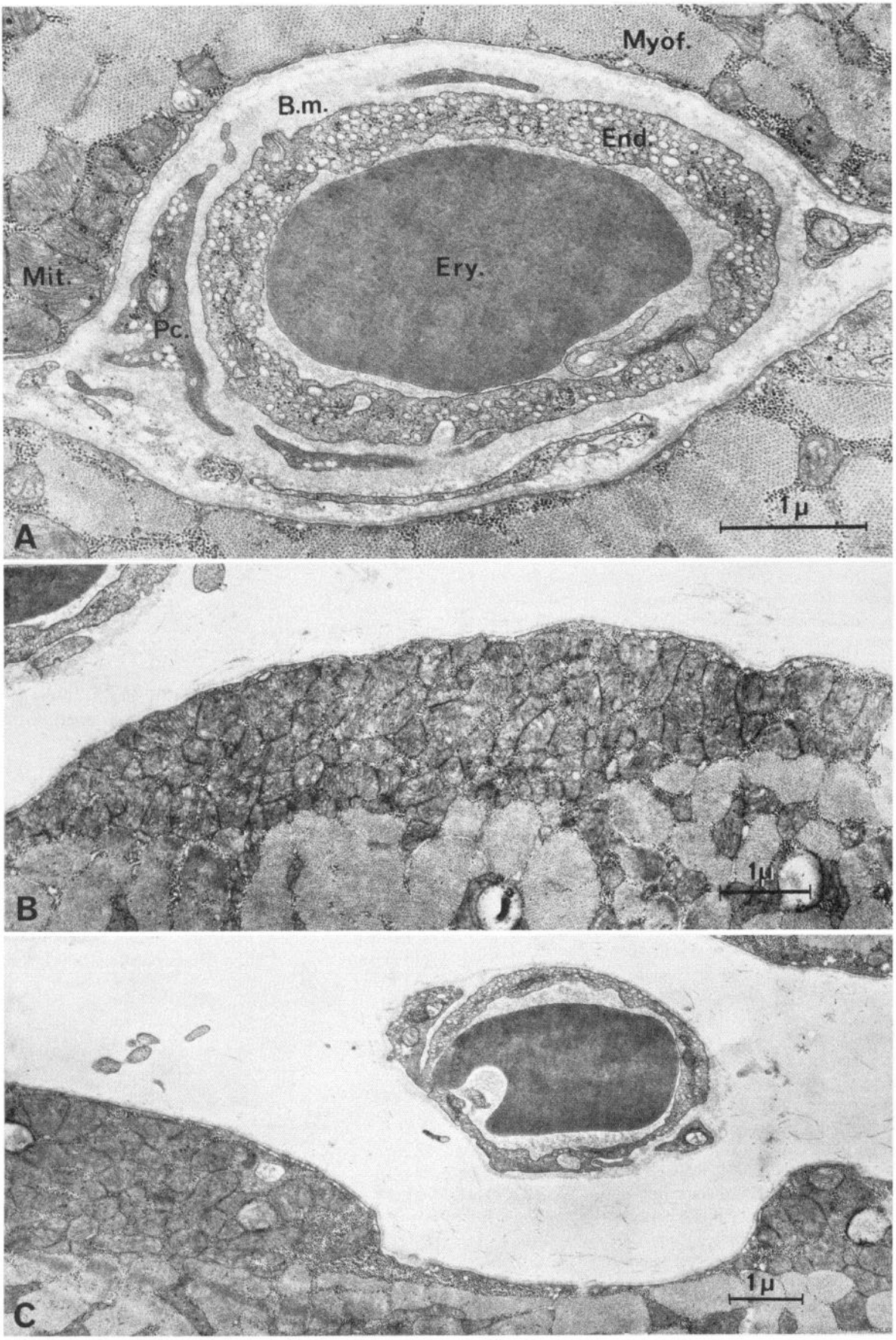
Fiber diameter and number of capillaries around each fiber. Figure 5 shows that an increase in fiber diameter is accompanied by a linear increase in the number of capillaries around each fiber if values based on less than 100 fibers are excluded. In both the untrained and trained groups, a 20-μm increase in fiber diameter is associated with one additional capillary around each fiber. This is, however, out of proportion to the increase in fiber area. An increase of fiber diameter from 40 to 60 μm (i.e., 50%) increases the fiber area by 125%, while the increase in the number of capillaries is only 25%.

Mitochondrial content and number of capillaries around each fiber. Figure 6 shows that the number of capillaries around each fiber increases with the number of mitochondria within the fiber in both groups. The difference can not be ascribed to differences with regard to fiber diameters, since mean fiber diameters differ only slightly between the groups.

DISCUSSION

Quantitative determinations of the capillary supply of skeletal muscle are beset with several methodological and theoretical problems.

First, it has been difficult to find methods visualizing all capillaries. A number of investigators have counted small blood vessels packed with erythrocytes or filled with ink or other media, perfused under high pressure through an artery into the vascular bed of muscle. However, as pointed out by several investigators (9, 21, 27), all capillaries are apparently not packed with red blood cells nor filled with the perfusion medium. Histochemical staining is another approach that has been



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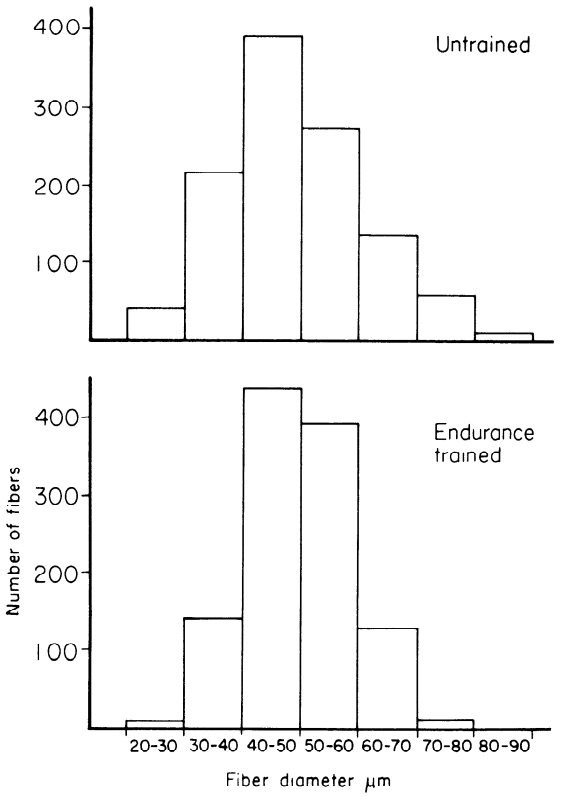

FIG. 3. Histogram showing distribution of fiber diameters in untrained and endurance-trained groups.

used to visualize capillaries. To our knowledge, alkaline phosphatase and the periodic-acid-Schiff (PAS) reaction are the only two methods used on human skeletal muscle. The latter of these was used in three quantitative studies (1, 11, 19), while the former was used by Romanul (23). In this study (23) no quantitative data were given. The combination of the above-mentioned methods to visualize capillaries and the light microscope to identify the capillaries may be one of the factors contributing to the wide range of values for capillary density found in the literature. In the present investigation we have employed the electron microscope to identify positively all capillaries. The identification is simple and based on the following objective criteria: diameter less than 6 μm, continuous basal lamina, and no continuous cellular layer external to the endothelium. Lymphatic capillaries are mainly confined to the perimysial tissue, are larger than 6 μm, and lack a continuous basal lamina (14, 31). Consequently, they do not constitute a significant part of the vessels counted in the present study.

Second, in addition to the problems associated with the identification of capillaries, other problems arise in the calculations of the spatial distribution of capillaries in skeletal muscle. Many investigators have expressed the distribution in capillaries per mm² (i.e., capillary density). However, the measurements of capillary density are influenced by several factors. For instance,

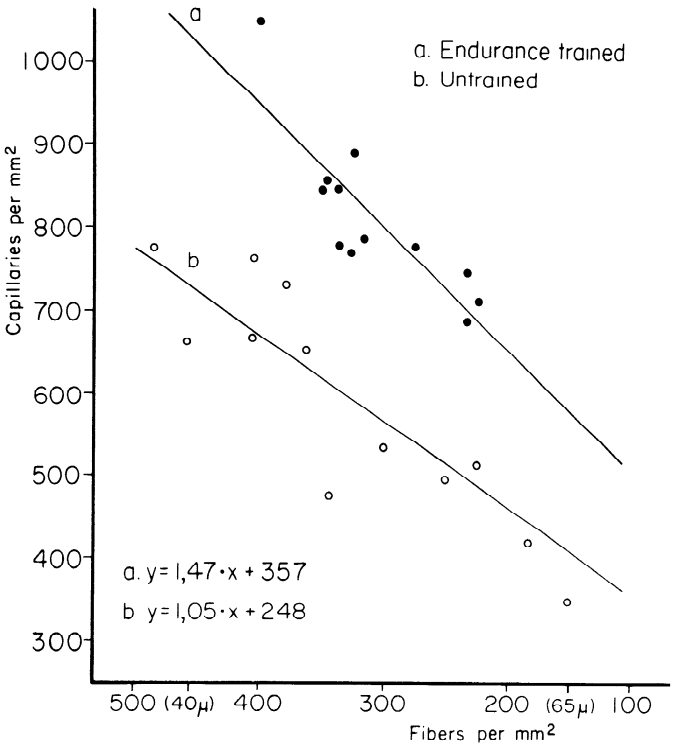

FIG. 4. Diagram showing relation between capillary density (capillaries per mm²) and fiber area (fibers per mm²). Each point represents values from one subject. Two diameter values are indicated to show relationship between number of fibers per mm² and lesser fiber diameter.

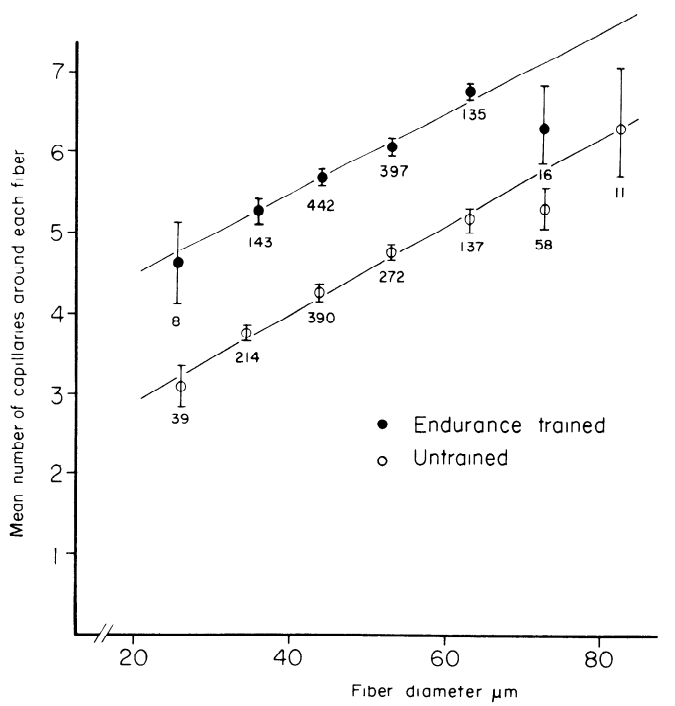

FIG. 5. Diagram showing relation between number of capillaries around a fiber and fiber diameter. Each point represents the mean value of all fibers having diameters within a 10-μm interval. Small bars represent standard errors. Number of fibers within each group is indicated below each point.

FIG. 2. *A*: electron micrograph showing example of typical capillary as seen in present material (×25,000). B.m., basement membrane; End., endothelial cell; Ery., erythrocyte; Mit., mitochondria; Myof., myofibril; Pc., pericyte. *B*: electron micrograph of a typical subsarcolemmal aggregate of mitochondria (×16,500). *C*: electron micrograph showing typical relation of capillaries to mitochondrial aggregates (×15,000).

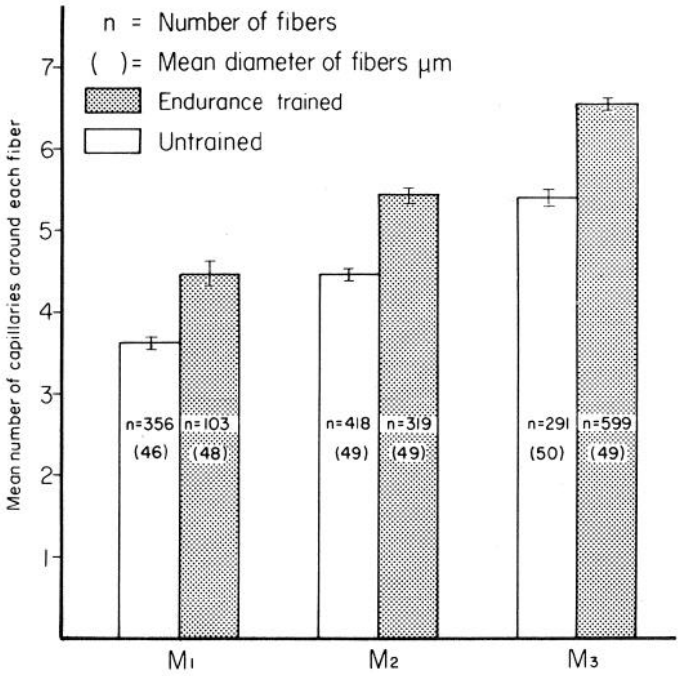

FIG. 6. Histogram showing relation between number of capillaries around a fiber and mitochondrial content of fiber. Small bars at tops of columns indicate standard errors. M_3 is fiber type with highest content of mitochondria (see text).

shrinkage or swelling of the tissue during the histological procedures will markedly affect the results. In the present investigation we tried to determine the degree of shrinkage in our material and corrected for it. However, these corrected values for capillary density are accurate only if the fiber diameters in fresh-frozen sections are unaltered from the native state, a question which has not been solved. For example, a 10% linear shrinkage of the native fibers during preparation of the frozen sections would alter our corrected number of capillaries per mm² from 305 to 250 in the untrained, and from 425 to 350 in the endurance-trained subjects. When there is a 10% swelling, the corrected number is 360 (untrained) and 510 (endurance trained). Thus, a direct comparison of the results for capillary density between our results and those of previous studies may not be fruitful.

Capillaries per fiber. With the above-mentioned problems in mind, the results of the present investigation will be discussed in relation to other quantitative studies performed on the lateral part of the quadriceps muscle in man. The capillaries per fiber ratio is not affected by shrinkage or swelling, and should therefore be suited to a comparison of results obtained by different methods. The capillaries per fiber ratio for the untrained subjects of Hermansen and Wachtlova (11), Pařizková et al. (19), and Andersen (1) were 1.08, 0.81, and 1.30, respectively. In the present investigation we found a ratio of 1.77, i.e., 36% higher than the highest value obtained previously in human skeletal muscle (1). For trained subjects the corresponding values were 1.5 (11), 2.15 (1), and 2.49 (present study). The present investigation offers no definite clues that could explain the differences between our results and those of previous studies. However, even the capillaries per fiber ratio is influenced by factors such as distribution of fiber types and the degree of training of the muscle groups investigated. It is known from earlier studies (7) that the distribution of "red" and "white" fibers in samples from the quadriceps muscle varies considerably from one untrained subject to another. With regard to the degree of training, it should be noted that the values for maximal oxygen uptake were much lower in the untrained young men studied by Pařizková et al. (19), and somewhat lower in the group of Andersen (1), than in the present study. The untrained group of Hermansen and Wachtlova (11) had about the same value (50.2 ml/kg · min) as found in the present study. However, it seems unlikely that differences in fiber distribution and degree of training can account for more than a fraction of the differences observed in the capillaries per fiber ratio between the present and earlier studies (1, 10, 18). To what extent other factors, such as methodology, have contributed cannot be answered at present.

Fiber diameter and number of capillaries. The present investigation showed that regardless of the degree of training, large fibers are surrounded by more capillaries than are the small fibers (Fig. 5). However, the increase in the number of capillaries around each fiber, due to increase in fiber diameter, is not large enough to keep the number of capillaries per mm² constant (Fig. 4). This is an important point in the evaluation of the effect of training on capillary supply to skeletal muscle, since training may affect also the diameter of the fibers (17, 20, 30). Cotter et al. (5), for instance, found a substantial increase in the number of capillaries per mm² in rats after chronic electrical stimulation of muscle nerves. The fiber diameter was found to decrease, but no data were given. If no capillaries were lost, the number of capillaries per mm² had to increase, and new capillaries may not have been produced by training in their experiments.

Capillary density and subsarcolemmal aggregates of mitochondria. The determination of absolute number of mitochondria (12) was not possible in the present investigation. We therefore chose a semiquantitative method, i.e., a fiber classification based on the number of subsarcolemmal aggregates of mitochondria (Fig. 2, *B* and *C*). This classification is used only to study the relationship between mitochondrial content and the number of capillaries around the fibers. It is not possible to make a direct comparison between our method of classification and those based on histochemical techniques (6). However, in the untrained subjects it seems likely that the M_3 group contains mainly "red" or type-I fibers, while the M_1 group consists mainly of "white" or type-IIB fibers (6). Our finding that the number of capillaries around each fiber is dependent upon the mitochondrial content of the fiber (Fig. 6) is in general agreement with previous light-microscope studies (1, 4, 23, 27, 29). Moreover, the large number of mitochondria observed in the fibers of endurance-trained athletes are in agreement with previous electron-microscope studies in man (7, 12, 13). In our endurance-trained subjects 59% of the fibers were of the M_3 type, and 10% of the M_1

type. The corresponding figures for the untrained subjects were 27% (M_3) and 34% (M_1). These differences in the mitochondrial content in the untrained and endurance-trained subjects are probably due to differences in both the degree of training and in distribution of "red" versus "white" fibers (7).

Capillary density in untrained and endurance-trained subjects. The numbers of capillaries per mm^2 and per fiber were approximately 40% higher in the untrained subjects. The difference in maximal oxygen uptake between the two groups was also 40%. Theoretically, the differences in the capillary supply of skeletal muscle may not be an effect of training but of heritage. However, in experimental animals (4, 5, 17, 20, 30) and in a longitudinal study of three male subjects (1), physical training led to an increase in capillary density. Thus, it seems likely that the difference in capillaries per mm^2 between the untrained and endurance-trained subjects in the present study is at least partly due to the effect of physical training.

With regard to oxygen diffusion in muscle, the number of capillaries around each fiber and the size (i.e., diameter) of the fiber are two important factors. Physical training may lead to an increase in the number of capillaries around each fiber (Fig. 5) and also to an increase in fiber diameter (hypertrophy). However, the number of capillaries per mm^2 may not increase at all if the hypertrophy is pronounced (Fig. 4). This appears to be the case in the study of Hermansen and Wachtlova (11), in which the well-trained subjects had about 30% larger fibers than the untrained subjects. The capillaries per fiber ratio was 44% and the maximal oxygen uptake 42% greater in their well-trained than in their untrained subjects (11).

We conclude that the number of capillaries per fiber is most probably increased by long-term physical training. Whether the capillary density is increased or not depends on the degree of fiber hypertrophy induced by training. There is a close relationship between the number of capillaries around a fiber and the number of subsarcolemmal aggregates of mitochondria. Moreover, the increased extraction of oxygen observed after physical conditioning might well be explained by an increase in capillary density.

Received for publication 8 June 1976.

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