Capillarity of Red, White and Intermediate Muscle Fibers in Trained and Untrained Guinea-Pigs

Previous studies have shown that quantitative changes in skeletal muscle capillarity occur in response to exercise (CARROW et al.[1]), cross innervation and thyrotoxicosis (ROMANUL and POLLOCK[2]). Muscle fiber capillarity is directly related to the level of oxidative metabolism (ROMANUL[3]), and both of these parameters are responsive to chronic exercise programs (CARROW, HOLLOSZY, BARNARD et al.[1,4,5]). Since red fibers (high oxidative metabolism) appear to be preferentially used in treadmill running and swimming (EDGERTON et al.[6]), changes in capillarity as it relates to the level of oxidative enzyme activity was studied in trained guinea-pigs.

Materials and methods. 12 adult male guinea-pigs were used in the experiment. 6 control and 6 experimental animals were used to study the soleus muscles, while the medial gastrocnemius (MG) muscles were studied from 4 controls and 4 experimentals. The experimental animals were exercized on a motor-driven treadmill 5 days per week for 18 weeks. The maximum work load lasted 50 min/day and included 20 min of sprints in addition to endurance running at 40 m/min, 2% grade. The animals were killed at the end of the 18th week by a sharp blow to the head.

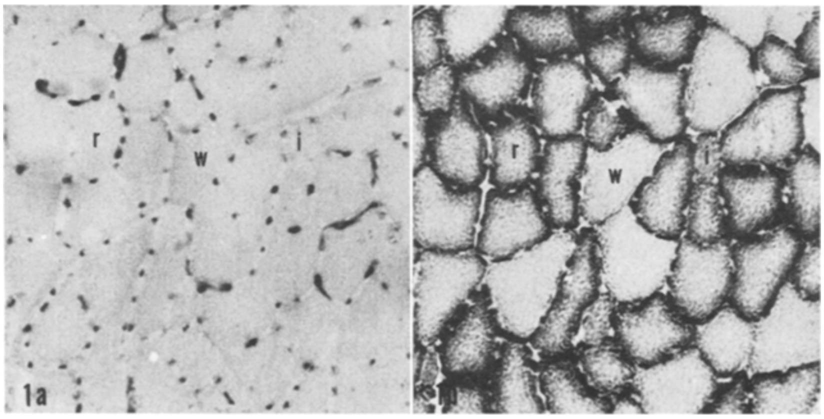
The belly of the soleus and MG was removed and frozen with liquid nitrogen. Sections 8 μ thick were cut and stained for reduced nicotinamide adenine dinucleotide diaphorase (NADH-D) activity, as described by NOVIKOFF et al.[7], to determine fiber type and the Gomori procedure for demonstrating alkaline phosphatase (GOMORI[8]) in capillaries. Photographs of serial sections of the soleus and of the white region of the MG were examined with respect to fiber type (see Figure 1, A) and capillary quantity (Figure 1, B). The number of vessels in contact with each fiber along with the classification of fiber type was recorded.

NADH-D presumably reflects the amount of oxidative metabolism in fibers; those fibers with the greater NADH-D activity being red and those lowest in NADH-D activity being white. Red fibers are further identified by their large subsarcolemmal aggregations of mitochondria. The usually larger white fibers stain lightly throughout their diameter. Fibers with a moderate amount of staining localized in the periphery, but not clearly red or white, are classified as moderates (EDGERTON et al.[9]). Intermediate fibers have minimal subsarcolemmal aggregates of NADH-D activity and smaller diformazan granules than are usually seen in the red, moderate or white fibers. The intermediate fiber has been postulated as being the slow-twitch fiber of skeletal muscle while the other 'types' are fast-twitch (EDGERTON and SIMPSON[10]).

Results and discussion. The capillarity associated with white, moderate and soleus intermediate fibers in the control animals did not differ significantly from the capillarity of those same fiber types in the experimental animals. A significant difference in the capillarity of the red fibers between the control and trained (4.50 to 5.84 capillaries/fiber) animals was demonstrated (Table). These figures do not represent an absolute mean capillary: fiber ratio as would be if simply the total number of capillaries in a given area were divided by the total number of muscle fibers because in many cases capillaries were counted more than once if they were associated with more than one fiber. For a similar reason, statistically valid comparisons (lack of independence of data for each fiber type) between either control or experimental values for individual fiber types cannot be made.

Inasmuch as an increase in red fibers occurs with exercise (EDGERTON et al.[9]), it might be argued that in this study white fibers did not show increased capillarity because by taking on the characteristics of red fibers, they were no longer considered white but red and would be counted as red fibers. However, if this alone was what occurred, there would have been no increase in the number of capillaries associated with red fibers.

In treadmill running the red fibers have been shown to be used preferentially (EDGERTON et al.[6]). The increase in capillarity associated with the red fibers after repeated



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[1] R. E. CARROW, R. E. BROWN and W. D. VAN HUSS, Anat. Rec. *159*, 33 (1967).

[2] C. A. F. ROMANUL and M. POLLOCK, in *Modern Neurology* (Little, Brown and Co., Inc., Boston 1969), p. 203.

[3] C. A. F. ROMANUL, Arch. Neurol. *12*, 497 (1965).

[4] J. O. HOLLOSZY, J. biol. Chem. *242*, 2278 (1967).

[5] R. J. BARNARD, V. R. EDGERTON and J. B. PETER, J. appl. Physiol. *28*, 762 (1970).

[6] V. R. EDGERTON, D. R. SIMPSON, R. J. BARNARD and J. B. PETER, Nature *225*, 866 (1970).

[7] A. B. NOVIKOFF, W. SHIN and J. DRUCKER, J. biophys. biochem. Cytol. *9*, 47 (1961).

[8] G. GOMORI, Proc. Soc. exp. Biol. Med. *42*, 23 (1939).

[9] V. R. EDGERTON, R. J. BARNARD, J. B. PETER, D. R. SIMPSON and C. A. GILLESPIE, Expl Neurol. *27*, 46 (1970).

[10] V. R. EDGERTON and D. R. SIMPSON, J. Histochem. Cytochem. *17*, 828 (1969).

endurance-type demands of treadmill running is compatible with the apparent selective use of red fibers as shown with glycogen depletion.

CARROW et al.[1] reported greater capillarity increases in white regions of the gastrocnemius than in the red regions of the same muscle. His method did not include establishing the type of each fiber such as was done with NADH-D in this study; rather, zones were selected for examination. We found in our sections that even in white areas significant quantities of red fibers were present. Thus an increase in capillarity in the white zone could have been due simply to an increase in capillarity of the red fibers.

No studies heretofore considered nor showed any change in capillarity with respect to fiber type following an exercise training program. This study shows a selective effect on red fibers and is compatible with previous data showing a greater activity of red than white fibers in endurance type exercise[11].

Mean number of capillaries in contact with muscle fibers of various histochemical classifications

		White	Moderate	Red	Intermediate[b]
Control (4)		3.9 ± 0.6	4.6 ± 0.3	4.5 ± 0.2	5.3 ± 0.3
	n[c]	74	26	22	265
Exper. (4)		3.8 ± 0.4	4.9 ± 0.4	5.8 ± 0.3[a]	5.2 ± 0.1
	n	69	42	50	275

[a] Significant difference between control and experimental ($P < 0.05$). [b] Intermediate fibers taken from 6 soleus muscles. [c] n, number of fibers.

Résumé. Le dressage des cochons d'Inde sur un moulin de discipline augmente la capillarité des fibres musculaires rouges, tandis que les fibres blanches et intermédiaires ne changent pas. Ces résultats sont compatibles avec les données précédentes qui conseillent d'employer de préférence les fibres rouges dans les exercices de résistance.

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Divergence of the in vitro and in vivo Extracellular Space Measurements in Heart Muscle

As has already been published, the extracellular space indicator inulin-carboxyl-C^{14} did not distribute uniformly in the heart muscle in vitro, while in vivo a nearly homogenous distribution could be demonstrated[1]. In a succeeding experimental series, however, we failed also to find a fairly uniform indicator distribution in the myocardium in vivo (Table). The experiments were performed in the same tissue, namely the left ventricular wall of the hearts of female Syrian golden hamsters (London School of Hygiene-strain), and under rather identical conditions as previously described[1]. As the reason of this apparent discrepancy, we claim the deviation in the tissue slicing technique. Formerly, the myocardial piece was sectioned into a nearly cube-like central part and into a total of 6 peripheral parts (one for each face of the cube), while now we preferred to get a rectangular central part, paying more attention to the middle layer of the ventricular wall (Figure 1). Nevertheless, we referred to the previous weight proportion of about 1 to 3 between central and peripheral parts.

Statistically exact evaluation of the in vivo results showed significantly less indicator in the center of the myocardial wall than in its periphery similar to the in vitro results gained by an identical slicing technique ($p < 0.01$, sign-test) (Table). Yet a comparison of both methods demonstrated in the center a significantly higher ($p < 0.05$, Wilcoxon-test) and in the periphery a significantly lower ($p < 0.01$, Wilcoxon-test) content of indicator in vivo than in vitro.

From the results described above it is concluded that the extracellular space in the ventricular wall is variable. This reasoning may be explained by morphology. In mammalian hearts coronary arteries and veins are located in the epicardium, while in the inner layer there are intertrabecular sinusoids. In addition there are also 2 networks of lymphatics in the heart, one in the epicardium and the other in the endocardium[2]. These extracellular compartments are missing in the middle layer of the ventricular wall. In addition, the inner and outer layer does not only consist of myocardium but also of fatty and connective tissue, which might have quite another interstitial space than the pure myocardium of the middle layer. In order to reflect those morphological characteristics, the in vivo and in vitro methods seem to be equivalent, and it may solely depend on the way the tissue is sectioned. But it is worthwhile to point out that in our previous in vitro experiments a similar indicator distribution pattern could be discovered, though cutting out a cube-like central part, while under in vivo conditions this could not be revealed[1]. Hence it is very doubtful whether the in vitro data do really represent morphological characteristics of the ventricular wall or whether they are only an indicator of pecularities due to the in vitro technique. That the latter is more likely may be inferred from a comparison of the in vitro and in vivo results (Table). The striking discrepancy of the indicator distribution between both methods seems to be a consequence of tissue damage partly by cutting the myocardium prior to incubation and also by swelling, vacuolisation and myolysis during the incubation. These

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[1] K. LOSSNITZER and T. F. KELLEY, Experientia *24*, 126 (1968).

[2] A. HARRY and B. M. PATTEN, in *Pathology of the Heart*, 2nd edn (Ed. S. E. Gould; Charles C. Thomas Publisher, Springfield 1960), p. 93.