CHANGES IN THE CAPILLARY BED OF SKELETAL MUSCLE AT VARIOUS TIMES AFTER NERVE SECTION

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Quantitative changes in the capillary bed of the gastrocnemius muscle of rats were studied after division of the sciatic nerve. The total (functioning) capillary bed was identified by intravenous injection of black ink. Functioning capillaries were detected by the discovery of erythrocytes in their lumen, using a histochemical method for demonstrating peroxidases with the aid of benzidine and hydrogen peroxide. The number of capillaries and fibers was counted in transverse sections through the muscle and the "coefficient of capillarization" was calculated. The investigation showed that the functioning capillary bed falls by 36% during the first 10 days, and the total capillary bed falls by 28.6% in 20 days. In the period of reinnervation (30th day) an increase was observed both in the total number of capillaries and in the number of functioning capillaries.

KEY WORDS: capillary bed; gastrocnemius muscle; denervation.

Although a few papers on the problem have been published [2, 11-14, 16-18], the state of the microcirculation in a denervated muscle, notably the capillary bed, has received little study. In denervation atrophy the number of capillaries per square millimeter section of a muscle has been shown [16] not to change during the first month, and not until the end of the second month was a very small decrease in the number of capillaries found. These results were interpreted by their discoverers as evidence of the absence of serious changes in the capillary network after denervation. No other references to a quantitative study of this problem could be found in the literature.

The object of this investigation was to study the state of the capillary bed of the gastrocnemius muscle in rats during the period of denervation and reinervation.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 180-200 g. The right sciatic nerve was divided and sutured [7]. The middle part of the medial head of the right gastrocnemius muscle was taken for investigation on the 10th, 20th, and 30th days after the operation. The corresponding muscles of intact rats served as the control. The muscles were fixed in 25% glutaraldehyde [15]. Transverse sections 50 μ in thickness were cut on a freezing microtome. The different parts of the sections were photographed under standard conditions on the MBI-6 microscope, negatives were examined on the Mikrofoto-MPO-1 screen, and the number of muscle fibers and capillaries was counted; the values obtained were expressed per square millimeter. On the basis of these results the coefficient of capillarization was determined by the equation: $K = (\text{number of capillaries per mm}^2/\text{number of fibers per mm}^2) \times 100$.

Functioning capillaries were identified from the discovery of erythrocytes in their lumen, using a histochemical method to detect peroxidases, with the aid of benzidine and hydrogen peroxide (Fig. 1a). The

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TABLE 1. Functioning Capillary Bed of Gastrocnemius Muscle of Rats at Various Times after Denervation (benzidine method); M ± m

Series No.	Time after denervation (days)	Number of capillaries in 1 mm²	Number of fibers in 1 mm²	Coefficient of capillarization (K)
1	Control (14)	898±26,7	580±29,5	155±4,3
2	10 (8)	720±22,3 (P_1<0,001)	721±33,0 (P_1=0,005)	100±5,5 (P_1<0,001)
3	20 (8)	789±41,6 (P_1=0,04; P_2=0,15)	907±22,2 (P_1<0,001; P_2<0,001)	87±4,9 (P_1<0,001; P_2=0,09)
4	30 (10)	1032±53,8 (P_1=0,03; P_3=0,003)	972±59,0 (P_1<0,001; P_3=0,3)	106±6,6 (P_1<0,001; P_3=0,04)

Legend. Here and in Table 2, number of animals in group is given in parentheses.

TABLE 2. Total (perfusable) Capillary Bed of Gastrocnemius Muscle of Rats at Various Times after Denervation (ink method); M ± m

Series No.	Time after denervation (days)	Number of capillaries in 1 mm²	Number of fibers in 1 mm²	Coefficient of capillarization (K)
5	Control (6)	1146±22,0 (P_1<0,001)	655±17,5	175±3,7 (P_1=0,002)
6	20 (7)	1188±28,7 (P_5=0,3)	950±34,3 (P_5<0,001)	125±2,9 (P_5<0,001)
7	30 (8)	1394±43,3 (P_5<0,001; P_6=0,002)	981±32,9 (P_5<0,001; P_6=0,5)	142±4,7 (P_5<0,001; P_6=0,01)

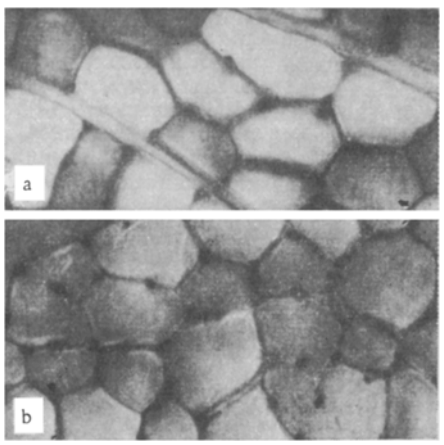

Fig. 1. Transverse section through normal gastrocnemius muscle: a) capillaries demonstrated by benzidine method; b) capillaries demonstrated by the use of ink. 200×.

whole capillary bed ("functioning" or perfusable) was demonstrated by means of black ink (Fig. 1b). For this purpose the ink, purified by centrifugation (40 min at 8000 rpm) and warmed to 37°C, was injected intravenously in a dose of 5 ml/200 g body weight. The liver and spleen were first ligated so as to prevent the absorption of ink by these organs. Statistical analysis of the data was carried out by Peters' method, using Mollenhauer's factor [4].

EXPERIMENTAL RESULTS

In the 20 days that elapsed after denervation the number of muscle fibers per square millimeter transverse section increased by 1.5 times (Tables 1 and 2), on account of a corresponding decrease in their diameter. From the 20th until the 30th day the decrease in diameter of the muscle fibers stopped. This was evidently due to reinnervation, for according to earlier observations [6, 7], it is at this time that the regenerating nerve can be seen to grow into the gastrocnemius muscle; indeed, reinnervation affects the metabolism of the muscle at this period [3, 5]. However, motor activity had not yet been restored by the 30th day.

The experiments to demonstrate the state of the capillary bed in the denervated muscle by the benzidine method showed that the number of capillaries per square millimeter was reduced by 20% on the 10th day, remained substantially unchanged during the next 10 days, and increased by 13% from the 20th to the 30th day (Table 1). The coefficient of capillarization fell by 36% during the first 10 days, fell lower still during the next 10 days, and rose by 22% during the period of reinnervation. These results show that changes in the functioning capillary bed during muscle atrophy are revealed more clearly by comparing the coefficients of capillarization than by determining the number of capillaries per square millimeter of transverse section through the muscle. In the latter case no account is taken of the degree of muscle atrophy and the consequent unequal "shift" of the field of vision, i.e., the intrusion of muscle fibers and associated capillaries into the standard field of vision.

The number of capillaries detected by the ink method in the control animals (Table 2) was greater than the number obtained by the benzidine method. Comparison of these values shows that the functioning

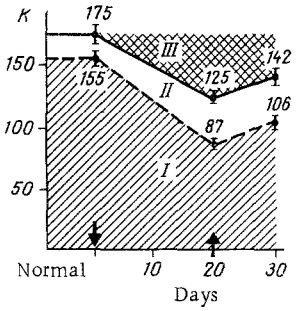

Fig. 2

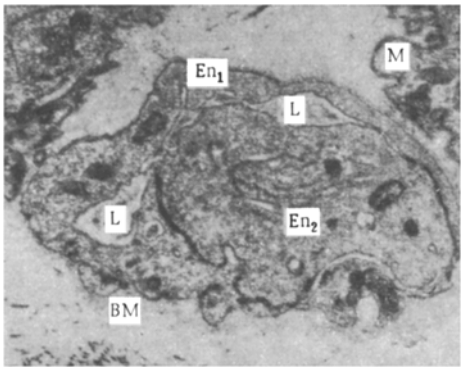

Fig. 3

Fig. 2. Dynamics of changes in capillary bed of gastrocnemius muscle after denervation. Continuous line – ink method; broken line – benzidine method; ↓ denervation; ↑ reinnervation; I) functioning capillary bed; II) reserve capillary bed; III) undetectable capillary bed.

Fig. 3. Transverse section through blood capillary. Gastrocnemius muscle, 20 days after nerve section. BM) Capillary basement membrane; M) border of muscle fiber; L) slit-like lumen of capillary; En_1 and En_2) endothelial cells. Fixation: 2% paraformaldehyde + 2% OsO_4; staining: uranyl acetate + lead citrate. 10,000 ×.

(open) capillaries in the gastrocnemius muscle of rats in a resting state accounted for 88.6% of the total number of capillaries, the remaining 11.4% being in reserve. These results agree with those obtained by other workers [8, 10].

On the 20th day after division of the nerve the number of capillaries per square millimeter section was not significantly changed in this series (Table 2). However, the decrease in the coefficient of capillarization in this case pointed clearly to a substantial decrease in the total number of perfusable capillaries (functioning and reserve). Consequently, the total capillary bed in the period of denervation also was significantly reduced (by 28.6%). An increase both in the total number of capillaries per square millimeter and in the coefficient of capillarization was found in the period of reinnervation.

The dynamics of changes in the capillary bed in the period of denervation and reinnervation is illustrated in the graph (Fig. 2), which shows that a fraction of capillaries not demonstrated by the methods used becomes apparent after denervation. In the period of reinnervation it is on account of this fraction that the number of reserve and functioning capillaries rises.

The electron-microscopic investigation showed that the decrease in the number of capillaries observed after denervation was due to a reduction in their lumen (Fig. 3). The results of these experiments do not agree with those obtained by Mrazkova and Puzanova [16], who found no significant decrease in the number of capillaries in a muscle in the early periods after denervation. The reason is evidently differences in the methods used. First, they studied different muscles (semitendinosus, caudofemoralis), and second, they estimated the state of the capillary bed from the number of capillaries per square millimeter of section. However, it follows from the results now described that this index is inadequate for assessing the state of the capillary bed in a skeletal muscle during the development of atrophy in the muscle. Third, as their control, Mrazkova and Puzanova used the contralateral muscle; it is known, however, that after division of a nerve on one side, atrophic changes, although less severe, also develop in the muscles of the contralateral limb.

The present experiment thus showed that after denervation of a skeletal muscle the perfusability of the capillary bed as a whole is reduced, and so also is the number of functioning capillaries. In the reinnervation period opening of capillaries is observed, thus improving the nutrition of the muscle and, consequently, facilitating its recovery. The capillary bed responds to denervation and reinnervation more rapidly and intensively than muscle tissue.

Previous work in the writers' laboratory showed significant changes in the ultrastructure and permeability of capillaries and, in particular, of venules in the period of denervation, from which it was con-

cluded that significant changes take place in the microcirculation in neuroregulatory disorders and that microcirculatory disturbances play an important role in the development of neurogenic dystrophies [1, 9]. The results of the present investigations confirm these conclusions.

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