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From the Laboratory of Experimental Biology (Head: Professor P-I Brånemark), Department of Anatomy, and the Department of Orthopaedic Surgery II (Head: Professor B. Stener), University of Göteborg, Sweden

MICROVASCULAR SUPPLY OF SKELETAL MUSCLE FIBRES

A microangiographic, histochemical and intravital microscopic study of hind limb muscles in the rat, rabbit and cat.

By

RHAGNAR MYRHAGE

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R. M.

The present thesis is based on the following publications:

I. Myrhage, R., and Eriksson, E. Vascular arrangement in hind limb muscles of the cat. Submitted for publication in J. Anat., 1977.

II. Myrhage, R. Capillary supply of the muscle fibre population in hind limb muscles of the cat. Submitted for publication in Acta physiol. scand., 1977.

III. Myrhage, R., and Hudlická, O. The microvascular bed and capillary surface area in rat extensor hallucis proprius muscle (EHP). Microvasc. Res. 11, 315-323 (1976).

IV. Myrhage, R., and Hudlická, O. Capillary growth in chronically stimulated adult skeletal muscle. - As studied by intravital microscopy and histological methods in rabbits and rats. Accepted for publication by Microvasc. Res., 1977.

The papers will be referred to in the text by their Roman numerals.

CONTENTS

INTRODUCTION

Skeletal muscles are composed of muscle fibres with differing speed of contraction, fibre diameter, and metabolism. *Slow* contracting fibres have a higher activity of oxidative enzymes but usually a smaller diameter than *fast* contracting fibres (Eccles *et al.*, 1958; Dubowitz & Pearse, 1960). Most muscles contain both slow and fast fibres, and it has been suggested that the amount of each type of fibres is related to the anatomical position and the functional utilization of each muscle. Anti-gravitation muscles, which are situated deep within a muscle group, have a higher percentage of slow fibres than superficially located muscles. This interrelationship has been found in several laboratory animals (Ariano *et al.*, 1973) as well as in man (Edgerton *et al.*, 1975).

The maximal blood flow capacity is 2-3 times higher in slow muscles (e.g. the soleus) than in fast muscles (e.g. the gastrocnemius) - for a review see Hudlická (1973). While the arrangement of large intramuscular vessels (Spalteholz, 1888;Hammersen, 1964) and the amount of vessel anastomoses (Saunders *et al.*, 1957; Stingl, 1973) seem to be similar in the two types of muscles, the capillary network is denser in slow muscles than in fast muscles (Ranvier, 1874; Romanul, 1965; Cotter, 1975). Differences in blood-flow characteristics and reactive hyperaemia have also been suggested (Gray, 1971; Burton & Johnson, 1972).

Endurance exercise increases the muscle fibre diameter but also the activity of oxidative enzymes in the fibres (Holloszy *et al.*, 1971). The number of capillaries is enhanced parallel to the fibre hypertrophy but there is no change in the number of capillaries per mm^2 of muscle tissue (Hermansen & Wachtlová, 1971).

The motor neurons are assumed to have some "trophic" influence on the muscle fibre metabolism (Guth, 1968). If the motor nerves to a "slow" and to a "fast" muscle are sectioned and then resutured in *reversed* position ("cross,innervation"), a parallel change of the fibre metabolism and the capillary density will follow (Romanul & Pollock, 1969). Similarly, the number of capillaries and the oxidative capacity of "fast" muscles will be increased when the intact motor nerve is intermittently stimulated for more than 4 days, with an impulse frequency naturally occurring in the nerves to "slow" muscles (Pette *et al.*, 1973; Brown *et al.*, 1976). This method of intensifying the muscle activity is usually referred to as"chronic" or "long-term" stimulation. In contrast to muscle exercise, it does not cause fibre hypertrophy, but there is still an increased capillary density. Furthermore, the chronically stimulated "fast" muscle gains several features characteristic of a "slow" muscle (Hudlická *et al.*, 1977).

It has been questioned whether the vascular architecture and the muscle fibre population is different in very thin and extended muscles as compared to thicker, polygonal or fusiformed muscles. Doubts have also been raised concerning the existence of actual capillary growth in muscles subjected to chronic stimulation. These problems will be elucidated in the present investigation, which deals with angiographic, intravital microscopic and histochemical analyses of the relationship between the peripheral vascular bed and the fibre population in hind limb muscles.

REVIEW OF THE LITERATURE

CLASSIFICATION OF MUSCLE FIBRES

Skeletal muscles were first distinguished by their differences in pigmentation and content of lipid droplets, as *white* and *red* (see review by Close, 1972). The white muscles are *fast* contracting while the red are *slow* contracting, as shown e.g. by Denny-Brown (1929). Histochemical analyses of the enzyme activity in muscle fibres have later revealed that most skeletal muscles contain three separate types of fibres.

Table 1

CLASSIFICATION OF MUSCLE FIBRES

	Fast twitching		Slow twitching
Dubowitz & Pearse (1960)	II	(II)	I
Brooke & Kaiser (1970)	IIB	IIA	I
Henneman & Olson (1965)	A	B	C
Burke *et al.* (1971)	FF	FR	S[1]
Peter *et al.* (1972)	FG	FOG	SO[2]
	(white)	(red)	(red/intermediate)

[1] FF = fast-fatigable; FR = fast-resistant to fatigue;
S = slow-very resistant to fatigue.

[2] FG = fast-glycolytic; FOG = fast-oxidative-glycolytic;
SO = slow-oxidative.

Originally, these types of fibres were called white, intermediate and red (e.g. Ogata, 1958). When it was found that only the intermediate fibres have a slow contraction pattern, the terminology was revised to fast-twitch white, slow-twitch intermediate and fast-twitch red (Barnard *et al.*, 1971).

In clinical practice, fast and slow contracting muscle fibres have usually been recognized as type II and type I respectively (Table 1). Subgroups of type

II fibres (II A and II B) were described by Brooke & Kaiser (1970) from studies of the actomyosin ATPase activity at various pH levels (Table 2).

A third system of nomenclature has been used by e.g. Henneman & Olson (1965) and Kugelberg & Edström (1968), by which the white, red and intermediate fibres are called A, B, and C, respectively. These terms might, however, cause confusion since other investigators (e.g. Stein & Padykula, 1962) designate intermediate fibres as B and red fibres as C.

Repetitive stimulation of the muscle fibres innervated by a single motor neuron will result in depletion of the glycogen content in these muscle fibres. The whole motor unit is then accessible for histochem-

Table 2

HISTOCHEMICAL STAINING INTENSITY AND METABOLISM OF MUSCLE FIBRES

Enzymes		FG	FOG	SO
SDH[1] NADH$_2$ - diaphorase[2]				
Mitochondrial α-GPDH[3] PAS[4]				
ATPase[5]	pH 9.4			
	pH 4.7			
Metabolic capacity	aerobic:	low	high	moderate high
	anaerobic:	very high	moderate high	low

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[1] SDH = succinate dehydrogenase
[2] NADH$_2$ - diaphorase is oxidizing the co-enzyme NADH$_2$ (nicotinamide-adenine dinucleotide) to NAD.
[3] GPDH = glycerophosphate dehydrogenase.
[4] PAS = periodic acid - Schiff test (for glycogen content).
[5] ATPase = adenine triphosphatase.

ical identification via PAS-staining in combination with e.g., staining for SDH activity (Table 2). With this technique it has been shown that muscle fibres belonging to the same motor unit are histochemically identical (Edström & Kugelberg, 1968; Burke & Tsairis, 1973). The motor units vary in their speed of contraction and their sensitivity to fatigue, and Burke *et al.* (1971,1973) therefore described the three categories of muscle fibres as: fast contracting - fast fatigable, fast contracting - resistant to fatigue, and slow contracting - very resistant to fatigue (Table 1). These terms were considered to be less confusing as compared to "white and red" fibres (since both of these types of fibres are fast contracting) and to "type II and type I" or "A, B, C" fibres (since this nomenclature is also used for nerve fibres).

Peter *et al.* (1972) quantified the glycogen concentration and the activity of several enzymes in muscles dominated by a single type of muscle fibres. It was suggested that the fast-twitch white fibres (the nomenclature by Barnard *et al.*, 1971) should be called *fast-twitch-glycolytic, FG,* since they were found to have a very high anaerobic capacity (high glycogen concentration and high activity of glycolytic enzymes) and a low aerobic capacity (low cytochrome concentration and SDH activity). The fast-twitch red fibres were found to have a moderate to high anaerobic as well as high aerobic capacity (very high cytochrome concentration and SDH activity) and they were therefore called *fast-twitch-oxidative-glycolytic, FOG.* The slow-twitch intermediate fibres had a low anaerobic and a moderate to high aerobic capacity and the name *slow-twitch-oxidative, SO,* was suggested (see Tables 1, and 2).

Some investigators have also described subgroups of FG and FOG fibres (Romanul, 1964; Schmalbruch & Kamieniecka, 1975) and of SO fibres (Askanas & Engel, 1975). These findings might be due to individual enzyme activities in muscle fibres belonging to the same type of fibres (cf. Nolte & Pette, 1972).

VASCULAR ARCHITECTURE AND CAPILLARY DENSITY IN SKELETAL MUSCLE TISSUE

Qualitative studies of injection specimens have revealed a *primary* and a *secondary* anastomosing vascular network, at the pre-capillary level, in skeletal muscles (Spalteholz, 1888; Krogh, 1922; Hammersen, 1964; Stingl, 1969). It was also stated by Spalteholz (1888) that most muscles contain numerous anastomoses. Campbell & Pennefather (1919) postulated three different groups of muscles in man, according to the number of afferent muscle arteries and the pattern of intramuscular arterial anastomoses. Classification of muscles in man has also been based on the vascular arrangement, together with the anatomical and functional features of the examined muscles (Saunders *et al.*, 1957). From investigations of muscles in various animal species, Stingl (1973) emphasized that the pattern of vascular anastomoses is the optimal criterion for grouping of muscles with different vascular architecture. Neither the review of the literature nor the observations in the study by Stingl revealed any correlation between the pre-capillary vascular arrangement and the fibre content in the examined muscles.

However, several investigators have found that muscles dominated by FG fibres contain few, straight capillaries, while muscles rich in SO fibres have numerous, wide and "sinusoidal" capillaries (Ranvier, 1874; Stoel, 1925; Lee, 1958; Romanul, 1965).

Attempts have been made to quantify *the number of capillaries per* mm^2 *of muscle tissue* (see Hudlická, 1973). The calculations were usually performed on cross-sections from muscles subjected to vascular perfusion of India ink. The numbers of capillaries/mm^2 show a considerable divergence, which is usually interpreted to be a result of species differences but mainly caused by tissue shrinkage artefacts and unequivalent diameter of the FG, FOG, and SO fibres (see Table 2).

The number of capillaries per muscle fibre (also called *capillary/fibre ratio*) is independent of the tissue dimensions and therefore considered to be more representative for the capillary density. Data of this type have also confirmed the difference in capillarization found by Ranvier (1874) and other investigators (see also review by Hudlická, 1973).

From histochemical staining of the capillary endothelium, Romanul (1965) observed that the number of capillaries surrounding each muscle fibre was directly proportionate to the activity of oxidative enzymes in the fibres. Plyley & Groom (1975) found no difference in the capillarization of muscle fibres with respect to the fibre metabolism. In fact, they stated that "any differences in capillary density are primarily a consequence of differences in mean fiber size".

AIMS OF THE PRESENT INVESTIGATION

1) To test the representativeness of the tenuissimus muscle for skeletal muscle tissue in general, concerning the vascular architecture and the capillary supply of the muscle fibres. The thin cat tenuissimus was compared with thicker hind limb muscles of the cat (the dorsal part of the biceps femoris, the gastrocnemius - both heads separately - and the soleus] in the following respects:
   a. muscle anatomy and vascular architecture (I).
   b. evaluation of the muscle fibre population and the capillary supply of fibres with differing diameter and metabolism (II).
2) To develop a method suitable for intravital studies of skeletal muscles subjected to various levels of activity. The rat extensor hallucis proprius (EHP) was selected as experimental muscle (III).
3) To quantify the total capillary surface area and search for direct evidence of growing capillaries in chronically stimulated rabbit tenuissimus and rat EHP muscles (IV).

METHODOLOGICAL CONSIDERATIONS

GENERAL

Combined structural and functional studies have been found necessary for analyses of the regulatory mechanisms involved in the blood flow distribution in skeletal muscle. This type of experimental approach is possible by intravital microscopy, but these *in situ* observations with transmitted light are limited to thin muscles (III).

The vasculature of thicker muscles can be analysed in injection specimens. With radiographic technique it is possible to visualize the whole vascular tree, and even extramuscular vessels (cf. Saunders *et al.*, 1957). The main disadvantage of this method is the poor resolution of the microvascular bed, even if radiopaque injectants with minimal particle size and viscosity are used. The microvessels can, however, be studied satisfactorily after Indian ink perfusion and clarifying of the muscle according to the method of Spalteholz (1888, 1911; see also Romeis, 1948). A certain degree of tissue shrinkage is unavoidable and to reach the deeper parts of the muscle belly, the specimens have to be sectioned. From consecutive serial sections it is possible to reconstruct the whole intramuscular vessel tree with reasonable accuracy.

Measurements of vessel dimensions can be carried out during intravital microscopy. With controlled physiological conditions these figures would also be representative, since the vascular tone is preserved (cf. Baez, 1969; Eriksson, 1972).

For quantification of the capillary density in skeletal muscle tissue, conventional histological technique with formalin fixation should be avoided because of the tissue shrinkage artefacts. Dye perfusion of the vascular bed is also unreliable, since filling of the capillary network is usually incomplete (cf. Plyley and Groom, 1975; see also Hudlická, 1973).

A preferable method is freeze fixation, cryostat sectioning and histochemical staining for alkaline phosphatase activity (Gomori, 1939; Pearse, 1968). This enables specific identification of the endothelium in all capillaries present in the section, with the degree of tissue shrinkage kept to a minimum.

Correlation of the capillary density to the muscle fibre population is important for a complete understanding of the nutritional characteristics of skeletal muscle tissue. Various methods for histochemical staining of the enzyme activities in muscle fibres have been described (see review by Khan, 1976). With this technique it has been possible to evaluate the capacity of glycolytic, oxidative and lipolytic enzymes in different muscle fibres. Quantification of the cross-sectional area or mean diameter of the fibres can also be performed (cf. Edström and Torlegaard, 1968/69; Cotter *et al.*, 1973) since the linear dimensions of the tissue are fairly well preserved with careful freeze fixation.

The fibre mass of skeletal muscles is grouped in fibre bundles (Figs. 1 and 5). As a constant pattern, most of the large glycolytic FG fibres are located close to the border of this bundle. Thus, evaluation

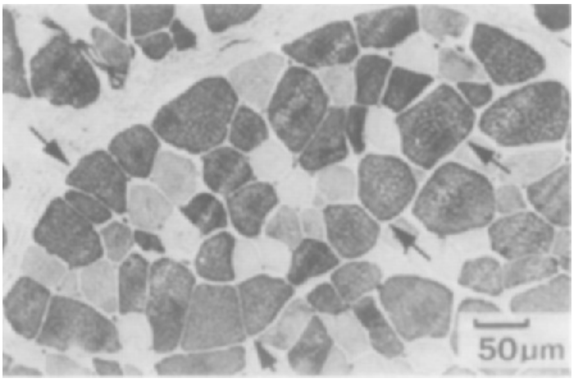

Fig. 1. Muscle fibre bundle (indicated by arrows) in the biceps femoris. Corss-section histochemically stained for the activity of alkaline ATPase, according to the method described by Padykula & Herman (1955).

of the percentage of different types of muscle fibre as well as the number of capillaries per fibre should be based on entire muscle fibre bundles. Randomized fields of the histological cross-section would be difficult to standardize and would most likely be less representative for the total fibre population.

MATERIALS AND ANAESTHESIA

The cats (in I and II) were of both sexes and weighed about 2.5 kg. During the experiments they were anaesthetized with i.v. injections of chloralose (50 mg/kg) after induction with ether.

The analyses of chronically stimulated tenuissimus muscles (IV) were performed on New Zealand Red rabbits of both sexes, weighing 1.7-2.5 kg. For implantation of electrodes they were anaesthetized with thiopentone sodium (100 mg/kg, i.v.) and during intravital microscopy (after terminated stimulation) with pentobarbitone sodium (30 mg/kg, i.v.).

Female Wistar rats, weighing 130-150 g, were used in III and IV. These animals were anaesthetized with ether during the implantation of electrodes. When they were subjected to intravital microscopy the anaesthesia was initiated with 1.5 mg of diazepam and then completed with pentobarbitone sodium (3-4 mg/100 g body weight), intraperitoneally.

ANGIOGRAPHY

Via a cannula in the abdominal aorta, the hind limbs of cats and rabbits were perfused with 500 ml of Indian ink, diluted with saline 1:1, after pre-perfusion with 300 ml of Perfadex^R (Pharmacia, Sweden). Papaverine (0.2 mg/kg) was added to induce maximal vasodilatation and the perfusion pressure was 13.3 kPa (100 mmHg). The purpose of the pre-perfusion was to wash out blood cells and plasma as far as possible. Ink particles have been found to form aggregates in contact with plasma proteins (Brånemark *et al.*, 1968), and any such aggregates would interfere with the filling of the microvessels.

After the perfusion, all animals were divided at the lumbo-sacral level. The skin was stripped off from the hind part of the body, which was then fixed in 10% buffered formalin for 7 days. Serial sections, 500 µm thick, were cut (perpendicularly and longitudinally to the direction of the muscle fibres) from the proximal, the central and the distal part of each examined muscle (I). The tenuissimus muscles (IV) were thin enough to be examined without sectioning.

After preparation of the muscle specimens (ad modum Spalteholz), the distinctly delineated vessel tree could be analysed in a 3-D microscope. Comparative measurements of the vascular segments were carried out with an optical micrometer. By using a phase contrast condenser in a Leitz Orthoplan microscope, it was also possible to study the connective tissue around different parts of the vessel tree (I).

The arrangement of large nutritive vessels to the muscle belly (I) was studied by radiography after intravascular perfusion of 500 ml of a 50% saline suspension of barium sulphate (0.4 g/ml, Mixobar^R, Astra, Sweden).

HISTOCHEMISTRY

Fixation of the muscle specimens was performed in propane-propene (II), and in isopentane which was cooled in liquid N_2 (III and IV). Serial sections, 7-10 µm thick, were cut in a cryostat, perpendicularly to the direction of the muscle fibres. For qualitative fibre differentiation, the sections were stained histochemically for the activity of $NADH_2$-diaphorase (Chayen *et al.*, 1973) and alkaline (pH 9.4) actomyosin ATPase (Padykula and Herman, 1955). In some experiments (III), the sections were stained for the activity of SDH (Nachlas *et al.*, 1957) instead of $NADH_2$-diaphorase. Both of these methods demonstrate the oxidative capacity of the individual muscle fibres (Khan, 1976).

The capillaries were visualized by a modified Gomori (1939) method for alkaline phosphatase (IV) and by the method of unspecific alkaline ATPase, as described by Guth and Samaha (1969,1970) - II, III, and IV.

CHRONIC MUSCLE STIMULATION

Rabbit tenuissimus and rat EHP muscles were chronically stimulated for 8 h/day during 6-14 days (IV).

Stainless steel electrodes were implanted, under aseptic conditions, in the vicinity of the common peroneal nerve in rats. To avoid extensive surgery of the tenuissimus muscles (supplied by a deep branch of the sciatic nerve), the electrodes were implanted onto the distal end of the muscle (about 2 cm distal to the area of intravital microscopy). The wires, led subcutaneously to emerge through the back of the animals, were connected by a flexible lead to conventional stimulators (Grass S8, and SD9). During stimulation (square wave pulses, duration 0.3 msec, frequency 10 Hz)* the animals were allowed to move freely in their cages (cf. Salmons and Vrbová, 1969; Pette *et al.*, 1973; Brown *et al.*, 1976). The stimulation intensity (usually 3-6 V) was adjusted to evoke maximal contraction of the muscles, but it was kept low enough to avoid discomfort to the animals.

For control of the operation trauma, one group of rabbits were sham-operated (electrodes were implanted without subsequent stimulation). In rats the contralateral intact and unstimulated muscle served as control, while in rabbits the control muscles had to be taken from different animals to the stimulated ones.

INTRAVITAL MICROSCOPY

The animals were breathing spontaneously via a tracheal cannula. One of the carotid arteries was cannulated for continuous monitoring of the arterial blood pressure (transducer, Statham; pen recorder, Devices). In

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* Close to the normal discharge frequency for motor neurons innervating slow muscles (Eccles *et al.*, 1958).

rabbits the venous blood pressure was measured via a cannula in the small saphenous vein.

The peripheral vascular bed was studied *in vivo* (III, and IV) using Leitz Intravital microscopes (Biomed and Large model, Leitz-Wetzlar, W. Germany. The Large microscope was modified for high resolution recordings by Lab. Exp. Biology, Göteborg).

Objectives (UO x11, NA 0.25; UO x23, NA 0.55; UO x55, NA 0.84) giving a magnification of up to x660 were used. A heat-absorbing green filter (maximal transmission at 5500 Å) was used to minimize the influence of light on the tissue during the observation period.

Recordings of the terminal arterioles, the capillary bed, and the collecting venules were made with the microscope connected to a closed circuit TV system with a video tape recorder (see IV). Photographic registrations were made on Kodak Monochrome film (SO 410) with a Leitz System camera in the Biomed microscope and a Nikon F camera (Nippon Kogaku, Japan) in the Large microscope. Additional cinematographic documentation was performed in the Large microscope (see IV).

EVALUATION OF FIBRE POPULATIONS AND THE CAPILLARY BED

From each series of muscle sections, 10 entire muscle fibre bundles were analysed (see I). This corresponds to about 400 muscle fibres. By comparing photographs from serial sections, alternatively stained for the activity of $NADH_2$-diaphorase (or SDH) and alkaline actomyosin ATPase, different fibre types (FG,FOG,SO or A,B,C) could be distinguished and quantified. Fibre diameters were calculated by taking the mean of 4 polygonal diameter measurements, on each of the studied fibres, in calibrated photographs (Cotter *et al.*, 1973).

The number of capillaries/muscle fibre in 20 adjacent fibre bundles (approximately 6000 μm^2 in rat EHP) was calculated in each series of sections. The number of capillaries surrounding each individual muscle fibre was also registered in II, while the number of capillaries per mm^2 of muscle tissue was calculated for evaluation of the total capillary surface area (TCSA) in III and IV.

During analyses of the video tape recordings, obtained from intravital microscopy, the monitored image was projected onto the focusing glass of a camera obscura. This gave a final magnification of about 3000 times. The architecture of the microvascular tree was registered and the vessel dimensions were measured with a microgauge.

The length of each capillary was measured from the point of which it branched off from the obliquely running terminal arteriole and downstream to its connection with another capillary to form an obliquely running collecting venule. The internal capillary diameter was measured at both the arteriolar and the venular end of individual capillaries.

Capillary surface area in rat muscles (III, and IV) was calculated using a combination of the data obtained from the *in vivo* observations (the dimensions of individual capillaries) and the histochemical analyses (capillary counts and total muscle dimension).

RESULTS AND COMMENTS

COMPARATIVE ANALYSES OF THE TENUISSIMUS AND THICKER HIND LIMB MUSCLES OF THE CAT

Anatomy of the muscles studied

The tenuissimus (also called abductor cruris caudalis; Crouch, 1969) is 12 - 14 cm long and about 0.4 cm wide. It is a quadrilateral muscle, only 0.1 - 0.6 mm thick (see Eriksson & Myrhage, 1972), and has a wet weight approximately 0.25 g. Originating on the coccygeal vertebra, the muscle runs on the medial side of the biceps femoris, close to its dorsal edge (Fig. 2), and inserts on the flat tendon of the biceps as well as in the crural fascia. The muscle fibres, which are only 2 cm long and therefore interdigitated (Adrian, 1925) are oriented parallel to the longitudinal axis of the muscle.

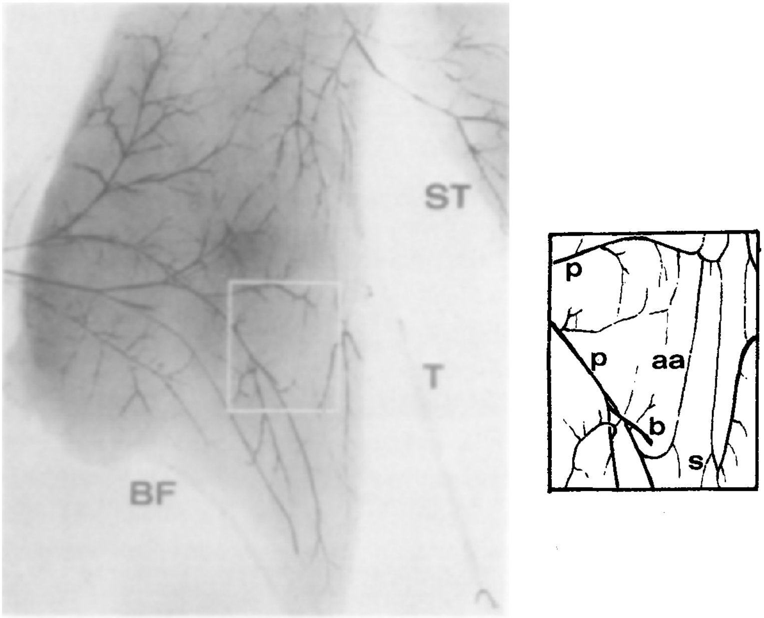

Fig. 2. The biceps femoris muscle (BF). The tenuissimus (T) is slightly dislocated in the dorsal direction. ST is the semitendinosus muscle. Two *primary* arteries (p) are connected by an *arterio-arterial anastomosis* (aa), which is a little wider than the *secondary* artery (s). One of the primary arteries has a branch (b) which during the dissection was found to penetrate the muscle fascia into the subcutaneous tissue and the skin. Full scale radiograph.

The biceps femoris covers most of the lateral surface of the thigh. This muscle is trilateral and considerably thicker than the tenuissimus. The dorsal third of this muscle is distinctly thinner than the rest of the muscle belly. This dorsal part of the muscle might be equal to the small head, but in the literature on cat anatomy the two heads are usually not defined (cf. Crouch, 1969). The dorsal part, which can be separated by blunt dissection, has a wet weight of about 3.2 g. The biceps muscle originates from the tuberosity of the isciadic bone and inserts on the lateral margin of the patella and the proximal third of the tibia. The muscle fibres are oriented parallel to the longitudinal axis of the muscle.

The lateral head of the gastrocnemius (LG) actually consists of three fusiformed beelies converging into a common sheet of the Achilles tendon. Each belly has a separate origin; the small lateral one originates from the deep side of the biceps tendon, the central one from a sesamoid bone (fabella) on the dorsal side of the lateral condyle of the femur and the medial belly originates from the proximal part of the plantaris muscle. Furthermore, the medial belly is fused (deep distally) with the plantaris muscle and (superficially) with the medial head of the gastrocnemius.

The medial head of the gastrocnemius (MG) is a fairly flat muscle with a delicate origin on the medial fabella and the adjacent part of the medial condyle of the femur. The insertion tendon is fused with the LG tendon. These tendons together comprise the superficial part of the Achilles tendon, covering the insertion of the plantaris and the soleus muscles.

The MG has a wet weight of about 7.4 g (the corresponding figure for the LG is 7.6). The muscle fibres of the LG are oriented parallel to the longitudinal axis of the muscle, while the MG fibres are attached to the dorsal muscle fascia and pass obliquely down to the ventral fascia.

The soleus has a long fleshy origin on the lateral side of the proximal third of the fibula and inserts into the deep portion of the Achilles tendon. The wet weight of this muscle is about 1.9 g. The muscle fibres pass from the muscle origin obliquely down to the dorsal muscle fascia.

Arrangement of the vascular tree (I)

The tenuissimus muscle contains one "central" artery (about 70 μm in diameter) and one or two "central" veins (diam., 90 μm). This group of vessels runs parallel to the orientation of the muscle fibres, and usually has an uninterrupted course through the muscle. *Terminal* arterioles (20 μm) and venules (40 μm) branch off from the central vessels in a two-dimensional pattern (Fig. 3a). These vessels run transversely or

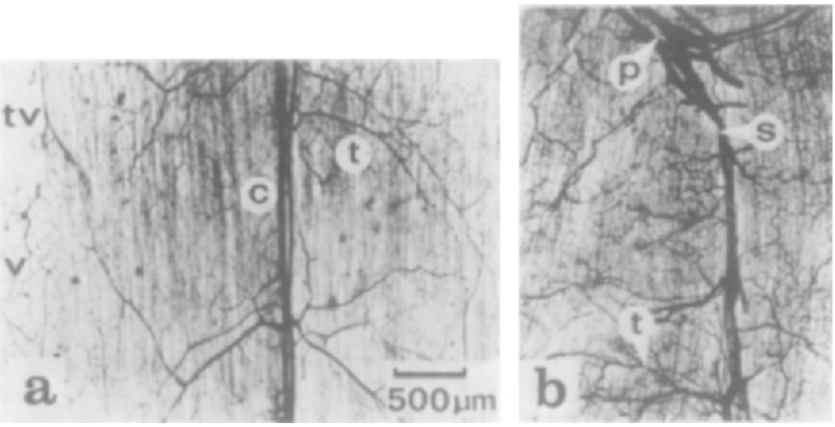

Fig. 3 a) Spalteholz preparation of the tenuissimus muscle showing the "central" artery and vein (c) and transverse (terminal) arterioles and venules (t). A terminal venule (tv) runs close to the muscle fascia. The vascular network (v) supplies fascia-embedded adipose tissue.
b) Longitudinal section of the LG muscle, prepared ad modum Spalteholz. Obliquely running *primary* vessels (p) subdivide into *secondary* vessels (s), which give rise to terminal arterioles and venules (t).

obliquely to the muscle fibres and are in the periphery subdivided into capillaries which are oriented parallel to the direction of the muscle fibres.

The central vessels of the tenuissimus correspond directly to the *secondary* generation of branches from the large intramuscular vessels in the biceps femoris (Fig. 2), the LG (Fig. 3b) and the MG as well as the soleus muscle.

In these muscles the supplying arteries subdivide into a *primary* generation of branches (Fig. 2). These vessels had a similar diameter range, 150-350 µm. The *secondary* arteries of thicker muscles were slightly wider (60-100 µm) than the corresponding vessels of the tenuissimus. The endpoints of the *primary* arteries were interconnected by *arterio-arterial anastomoses*, slightly wider (80-150 µm) than the *secondary* vessels (Fig.2). *Terminal* arterioles branche off from the *arterio-arterial anastomoses* as well as the *secondary* arteries in a three-dimensional pattern. The diameter range of the *terminal* arterioles was 25-40 µm, i.e. they were a little wider than in the tenuissimus.

The primary as well as the secondary veins follow the course of the corresponding arteries. Duplicated *primary veins* are common only in the soleus muscle,while duplicated *secondary veins* occur both in the soleus and in the MG muscles.

Terminal venules running close to the muscle fascia were found frequently in the tenuissimus muscle (Fig. 3a). Such vessels were also found in the other muscles, especially in the MG.

The average distance between secondary vessels was 1600-1800 µm in the biceps and the LG muscles, about 1200 µm in the MG, and 800-900 µm in the soleus. The cross-sectional area of the tissue cylinder (Fig. 4) supplied by one secondary artery in the MG muscle is about 1 mm^2. This value is in the range of the total cross-sectional area of the tenuissimus.

Arterio-venous anastomoses were not observed at any level within the muscles examined. This finding is in accordance with observations by Hammersen (1968). In the tenuissimus muscle, however, several of the terminal vessels penetrate the epimysium and enter the surrounding connective tissue. This type of vascular communication was also found in the other muscles, e.g. in the biceps and the soleus, where some primary vessels penetrated the muscle fascia and supplied the subcutaneous tissue and the skin (Fig. 2).

Both the tenuissimus and the biceps contain several anastomoses between the endpoints of the primary vessels. This is a common feature of flat and extended muscles (Stingl, 1973). These anastomoses were less frequent in the LG muscle. In the MG and the soleus, anastomoses were only seen connecting the largest of the primary vessel branches.

The present investigation shows that *the vascular arrangement found in the tenuissimus muscle exists as a "basic unit" in thicker muscles* (Fig. 4). The terminal arterioles and venules, which branch off from the secondary vessels in a three-dimensional pattern (two-dimensional in the tenuissimus), supply a cylinder of muscle fibres.

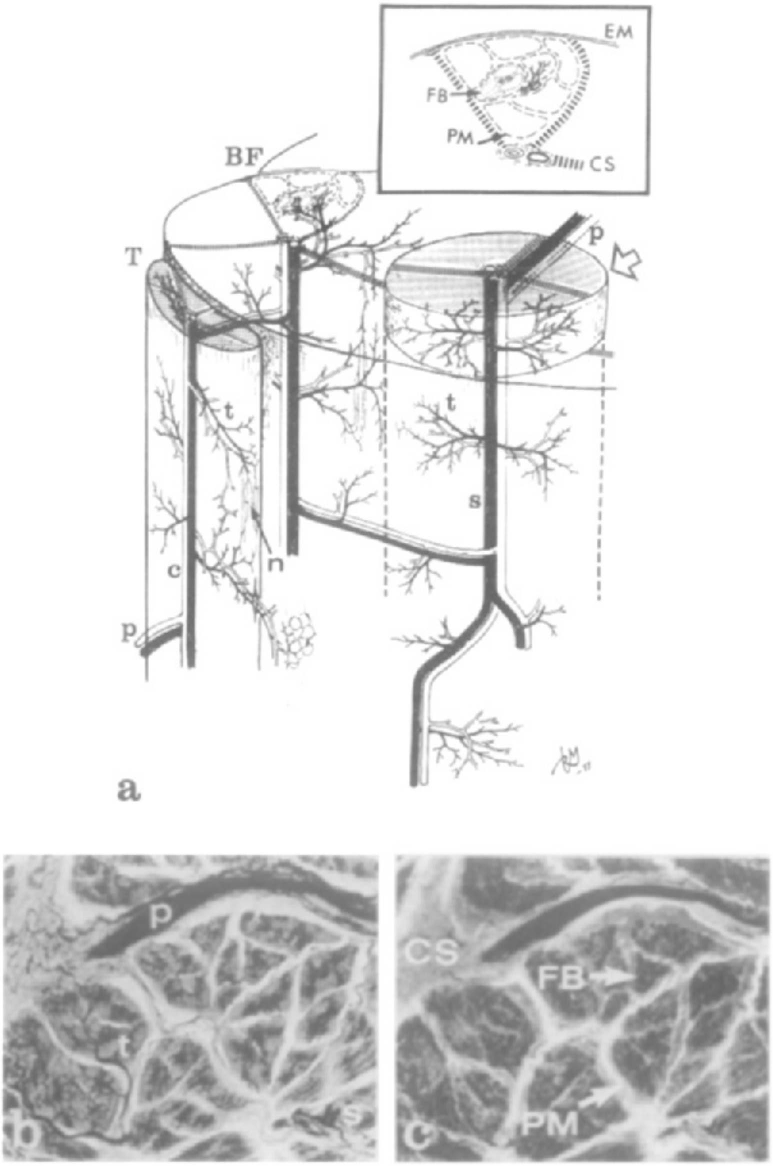

Fig. 4 a)The vascular arrangement of the tenuissimus muscle (T) is found as a "basic unit" in thicker muscles (here represented by the biceps femoris, BF). The terminal arterioles and venules(t) of this vascular basic unit are supplying the capillary network within a cylinder of muscle tissue (indicated by open arrow). p= primary-, c= "central"- and s= secondary vessels; n= capillary network. The fibre mass is first grouped by connective tissue septa (CS) and then subgrouped by two levels of perimysium (PM), enclosing the fibre bundles (FB). EM= epimysium.
b) Cross-section of the biceps muscle, clarified *ad modum* Spalteholz - conventional condenser. c) Phase contrast condenser.

Such cylinders in the MG muscle have an average cross-sectional area which corresponds fairly well to the total area of a cross-section through the tenuissimus.

Types of muscle fibres and capillary supply (II)

Freezing in liquid propane-propene preserves the skeletal muscle with a minimum of tissue artefacts. Serial cryostat cross-sections stained alternately for $NADH_2$- diaphorase and alkaline ATPase permit a qualitative classification of types of muscle fibre (Fig. 5).

The nomenclature used by Burke *et al.* (1971) merely describes the functional characteristics of the muscle fibres (Table 1). The functional properties in relation to the histochemical staining pattern of individual motor units have been described in detail for the cat gastrocnemius muscle (Burke & Tsairis, 1973; Burke *et al.*, 1973). It would therefore seem reasonable

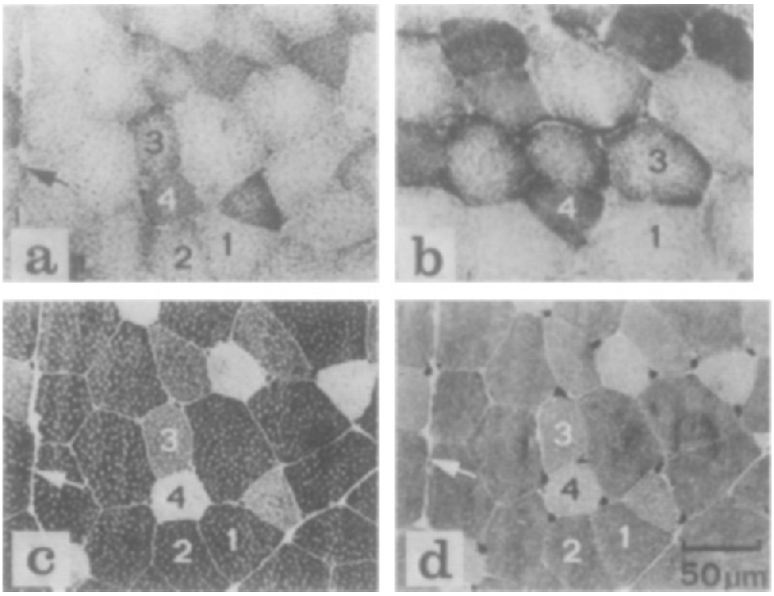

Fig. 5. Cryostat sections of the LG (a, c, and d) and of the MG muscle (b). Histochemical staining for $NADH_2$-diaphorase activity (a, and b) and alkaline ATPase activity, ad modum Padykula & Herman (c), and Guth & Samaha (d), respectively. 1 = FG; 2 = FG/FOG; 3 = FOG; 4 = SO. Arrows indicate the border-line of a muscle fibre bundle.

to use the more explicit nomenclature of Peter *et al.* (1972), which also indicates the metabolic capacity of each type of fibres. The fast contracting fibres are either mainly glycolytic (FG) or both glycolytic and oxidative (FOG), and the slow contracting fibres are mainly oxidative (SO).

In accordance with the findings of Ariano *et al.* (1973), the biceps femoris and the LG muscles were found to be dominated by FG fibres (40-50% of the total fibre population). The major part of all muscles in this study had a higher percentage of FOG fibres than was reported by Ariano. This discrepancy might be due to different levels of exercise of the investigated animals, since endurance training is known to increase the number of FOG fibres (Barnard *et al.*, 1970; Jansson & Kaiser, 1977). In the present study the highest number of FOG fibres was found in the tenuissimus muscle (45-50% of the fibres). The MG muscles had about 35% of SO fibres, twice as much as the LG, biceps femoris and tenuissimus. The soleus consisted to almost 100% of SO fibres.

Some fibres with high alkaline ATPase activity (which is typical for FG fibres) had a $NADH_2$-activity corresponding to the FOG fibres. This intermediate type of fibre, called FG/FOG fibres, generally formed 10-15% of the fibre populations in the biceps femoris and the LG muscles. A similar fibre type has been found in cat LG and tibialis anterior muscles by Hammarberg (1974).

The MG and the soleus generally had larger fibres than the other muscles (Fig. 5). The average fibre diameter reflects the contractile force of the muscle, but is also influenced by the muscle fibre orientation. In the MG and the soleus, both ends of all fibres are attached to the epimysium (Burke & Tsairis, 1973; Murphy & Beardsley, 1974). Sections of these muscles, perpendicular to the fibres, thus contain few fibres which are cut close to the fibre endpoint. The other muscles have fibres oriented parallel to the longitudinal axis of the muscle, the length of which exceeds the fibre length. Consequently, these fibres are interdigitated and several of them will be cut close to the fibre end points in a muscle cross-section. This would explain the low values of average fibre diameter found especially in the tenuissimus.

The soleus muscles contained quite large SO fibres. In the other three muscles studied, this type of fibre had a smaller average diameter than the fast contracting fibres. The FG fibres were the largest fibres in all evaluated muscles, with the exception of the soleus which did not contain these fibres (Fig. 6).

Endothelial cells have a high alkaline phosphatase activity (Gomori, 1939; Romanul, 1965). According to e.g. Freiman & Kaplan (1960), this enzyme is capable of hydrolyzing ATP, which is used as substrate in histochemical staining of ATPase activity. In the present investigation the alkaline ATPase activity was examined with a histochemical method containing no blocking agent for alkaline phosphatase (Guth & Samaha, 1969; 1970). This enabled quantification of the different types of muscle fibres as well as the total number of capillaries in the same tissue section.

Romanul (1965) suggested that the number of capillaries around each muscle fibre is directly correlated to the oxidative capacity of the muscle fibres. It has later been stated that the fibre diameter is the only factor determining the capillarization of muscle fibres. The present calculations revealed a fairly linear relationship between fibre size and number of fibre-surrounding capillaries for all types of muscle fibres. However, the large FG fibres were surrounded by fewer capillaries than the smaller FOG and SO fibres (Fig.6). The values of capillaries around fibres were lower in all the biceps femoris and the LG muscles studied as compared to the tenuissimus muscles, which in turn had lower values than the MG and the soleus muscles. This difference in capillarization was confirmed by calculations of the capillary/muscle-fibre ratio.

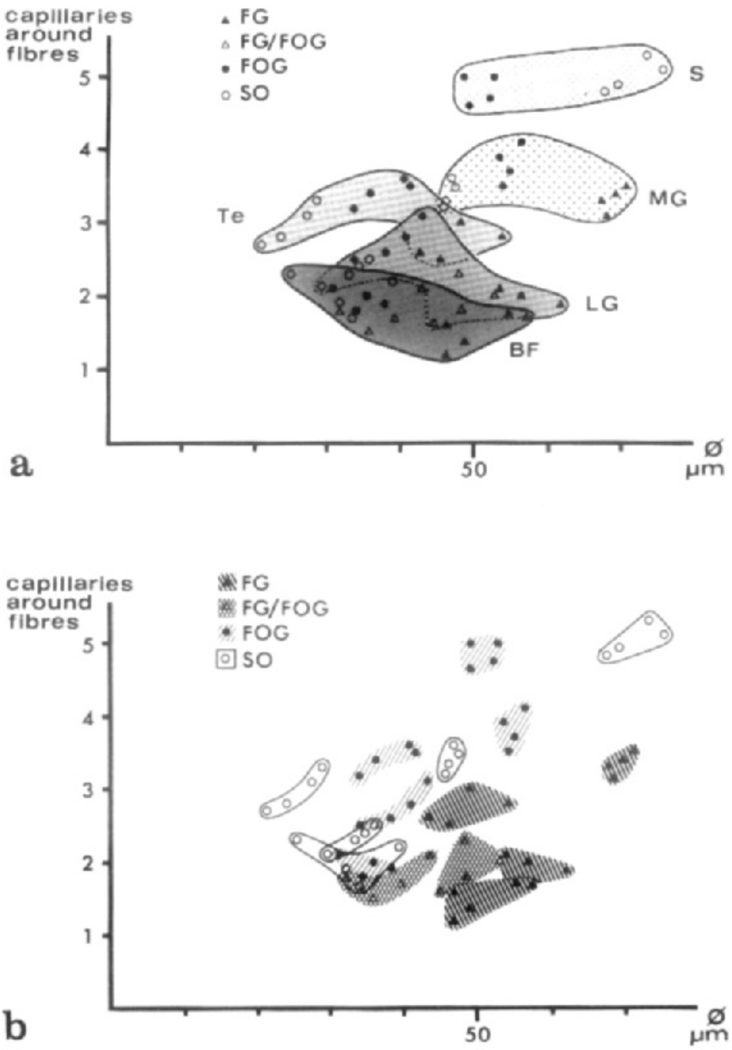

Fig. 6. The average number of capillaries around individual muscle fibres, in relation to fibre type and average diameter of the fibres (in four animals). a) values grouped for different muscles, b) values grouped for different fibre types. Te = the tenuissimus muscle; BF = the biceps femoris; LG = the lateral head and MG = the medial head of the gastrocnemius; S = the soleus.

Thus *the capillary supply of muscle fibres seems to be directly proportional to the oxidative enzyme activity as well as the diameter of individual muscle fibres.* The thin tenuissimus muscle does not differ from thicker muscles in this respect. The comparably large number of capillaries in relation to the average fibre diameter in the tenuissimus can reasonably be explained by the high proportion of FOG fibres in this muscle.

THE EXTENSOR HALLUCIS PROPRIUS MUSCLE (EHP) OF THE RAT (III).

There seems to be no major discrepancies between the tenuissimus and thicker hind limb muscles of the cat as regards the architecture of the vascular bed and the capillary supply of the muscle fibres. However, considering the point of insertion in relation to the knee joint, it is questionable whether the tenuissimus muscle acts primarily as a muscle of locomotion.

Only the marginal parts of thick, fusiformed or polygonal muscles that are of importance for locmotion can be used for *in vivo* observations of skeletal muscle circulation (see Gray, 1971). In the case of the fusiformed EHP muscle of adult rats, even the belly is thin enough to be transilluminated for intravital microscopy.

From its origin on the central part of the fibula and the interosseous membrane, the EHP muscle runs parallel to the extensor digitorum longus on the deep side of the tibialis anterior muscle. The tendons of the EHP and latter muscle pass together under the transverse crural ligament, whereafter the EHP tendon follows the medial side of the paw and inserts on the second phalanx of the first digit. By dividing the ligament and the tendon of the tibialis anterior muscle, the EHP can be held out ventrally and exposed for intravital microscopy (Fig. 7). The major part of the vascular bed can be analysed with an adequate resolution.

The EHP muscle is supplied by three small branches from the anterior tibial artery. Most of the veins leave the proximal part of the muscle to the anterior tibial vein. However, three to four veins (30-50 µm in diameter) drain the distal part of the muscle to the deep fascia and into the tendon. Usually, the EHP contains one central artery, about 30 µm in diameter and running parallel to the longitudinal axis of the muscle. Proximally, this vessel is accompanied by a vein (about 40 µm in diameter), which distally is a fusion of venous branches running close to the margins of the muscle. Terminal arterioles and venules branch off in a pattern similar to that in the tenuissimus (Eriksson & Myrhage, 1972). The capillaries have a few intercapillary branches. The capillary blood-flow is intermittent, with periods of varying flow and periods (10-60 sec.) of circulatory arrest (cf. Burton & Johnsson, 1972). The average internal diameter of the capillaries is 4.0 µm at the arteriolar end and 5.5 µm close to the collecting venule. Measured from the branching off from the terminal arteriole down to the collecting venule, the average capillary length is 535 µm.

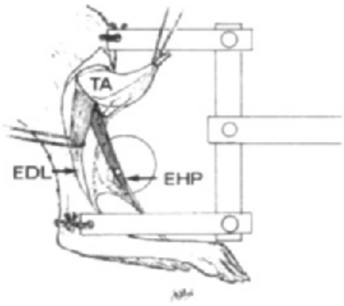

Fig. 7. The EHP muscle exposed for intravital microscopy. The lower leg of the rat is stabilized with a holder and the EHP muscle, with intact deep fascia, is placed on a glass cone and transilluminated. Both of the adjacent muscles (extensor digitorum longus, EDL, and the tibialis anterior, TA) are gently held aside.

The muscle fibre mass of the EHP is dominated by fast glycolytic (FG) fibres (about 60%; average diameter: 35 µm). Slow oxidative fibres (SO) constitute 15-20% (diam. about 20 µm) and the rest of the fibres are of the FOG type (diam. 25-30 µm).

The proximal cross-section of the muscle contains on average 1350 capillaries/mm^2, and close to the tendon the value is about 1050. The discrepancy might be explained by the decreased dimension of the muscle fibres as they insert into the tendon.

The total capillary surface area (TCSA), as calculated from *in vivo* measurements of individual capillaries and histochemical data on capillary density and related to the total dimensions of the muscle, amounts to an average value of 1.6 m^2/100 g of muscle tissue. A considerably lower value of TCSA (0.7) was found by Pappenheimer *et al.* (1951) in India-ink-perfused cat muscles. Species differences, e.g. differing body size, might explain this discrepancy (Schmidt-Nielsen & Pennycuik, 1961). It should be noted, however, that evaluations of capillary density from injection specimens may easily underestimate the total number of capillaries, since it is difficult to fill all capillaries present in the tissue with this technique.

The anatomy and the location of the EHP muscle is quite favourable for structural and functional *in vivo* analyses of the skeletal muscle circulation. Being a well-defined locomotor muscle with a proper tendon, the *EHP would be more suitable than the tenuissimus for studies of tissue nutritional adaptation to various levels of muscle activity.*

CHRONIC STIMULATION OF THE RABBIT TENUISSIMUS AND THE RAT EHP MUSCLES (IV).

Postnatal differentiation of the muscle fibre metabolism and the vascular bed in skeletal muscles is closely connected with the functional activity of the muscles (Hudlická, 1973). A direct correlation between the fibre metabolism, the capillary density, and the physiological characteristics of the nerve innervating the muscle is indicated from experiments on "cross-innervation" (Romanul & Pollock, 1969).

Chronic stimulation, at a low impulse frequency, of the intact nerve to a fast muscle has a slowing effect on the speed of contraction. Simultaneously the glycolytic enzyme activity decreases while the glucose phosphorylation and the fatty acid activation increase. This is later followed by an increased oxidative capacity (Pette *et al.* , 1973). After 28 days of stimulation the histochemical fibre staining pattern in the fast muscle can not be distinguished from that of a slow muscle (Brown *et al.*, 1976).

In the chronically stimulated rat EHP muscle, the percentage of glycolytic fibres (FG) decreased steadily with the time of stimulation. Parallel to this, there was an increase in the number of oxidative fibres (SO and FOG), which showed a slightly intesified staining for SDH activity as compared to the corresponding fibres in the control muscles. The average diameter of FG fibres was smaller after stimulation, while the dimensions of SO and FOG fibres were not changed (Fig. 8). This is in accordance with observations from stimulated large rabbit muscles (Cotter *et al.*, 1973).

In vivo observations of the microvascular bed in stimulated muscles revealed an increased internal diameter of the capillaries. This was most prominent in the tenuissimus muscles stimulated for 7 days, where the average capillary diameter was 6.1 µm at the arteriolar and 9.5 µm at the venular end of the capillary as compared to 4.7 and 6.1 µm, respectively, in the control animals. It should be emphasized that these figures represent relative values. But the differencies are real since the measurement error is the same.

In the stimulated EHP muscles the increase in diameter of the capillaries was smaller, but still detectable by the simple fact that the erythrocytes did not deform to the same extent during passage through the capillaries in the stimulated as compared to the control muscles (Fig. 9).

Growth of new capillaries in the stimulated muscles was observed as *buds* or *sprouts* going off from the bendings of the pre-existing capillaries. The sprouts often contained trapped erythrocytes and thrombocytes, which showed small oscillatory movements (Fig. 10).

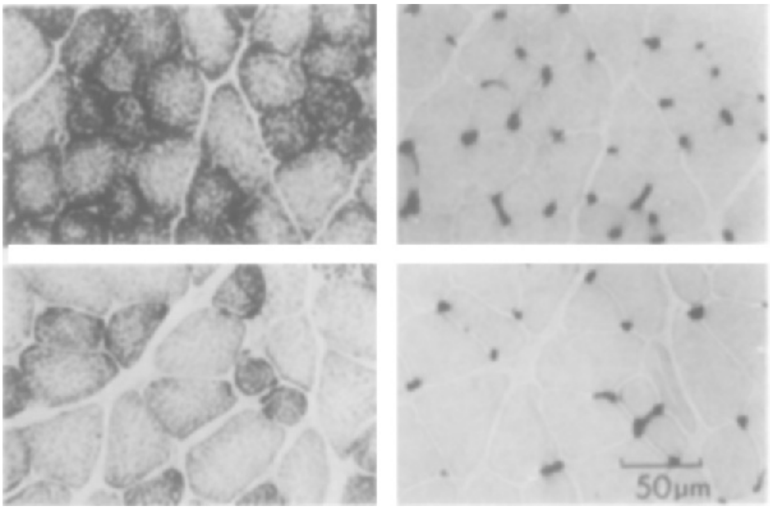

Fig. 8. EHP muscle chronically stimulated for 7 days (above) and contralateral control muscle (below). Cryostat sections, histochemically stained for SDH (left) and alkaline phosphatase activity (right).

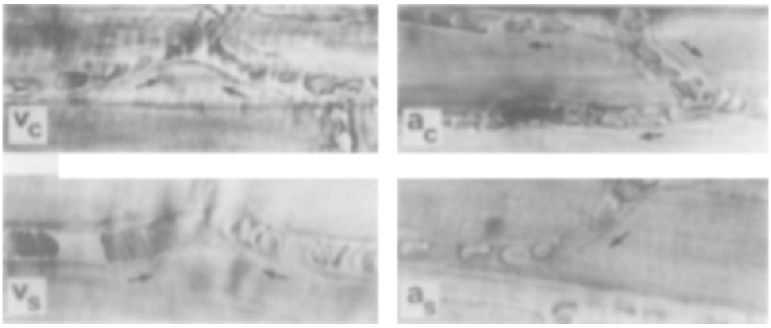

Fig. 9. Arteriolar (a_c) and venular end (v_c) of a capillary in the contralateral *control* EHP muscle, and corresponding parts (a_s, and v_s) after 7 days of chronic *stimulation*. Arrows indicate blood flow direction.

Recent fusion of two sprouts was indicated by hourglass-shaped capillary segments. Erythrocytes were frequently observed being very slowly squeezed through these narrow passages. A similar pattern of capillary growth has been described for the tadpole tail (Meyer, 1853), the rabbit ear chamber (Clark & Clark, 1939; Cliff, 1963; Brånemark, 1965; Lindhe & Brånemark, 1970) and for healing muscle wounds (Schoefl, 1963; McKinney & Panner, 1972). The proportion of sprouts to preexisting capillaries was about 40% in both the tenuissimus and the EHP muscles after 14 days of stimulation. This figure corresponds roughly to the values of increased capillary density found in stimulated rabbit muscles (Cotter *et al.*, 1973; Brown *et al.*, 1976).

The newly formed capillaries often had a winding or tortuous appearance, similar to the capillaries normally found in slow muscles (Romanul, 1965).

The total capillary surface area (TCSA) had an average value of 1.41 m^2/100 g of muscle tissue in the contralateral control muscles. This value was in-

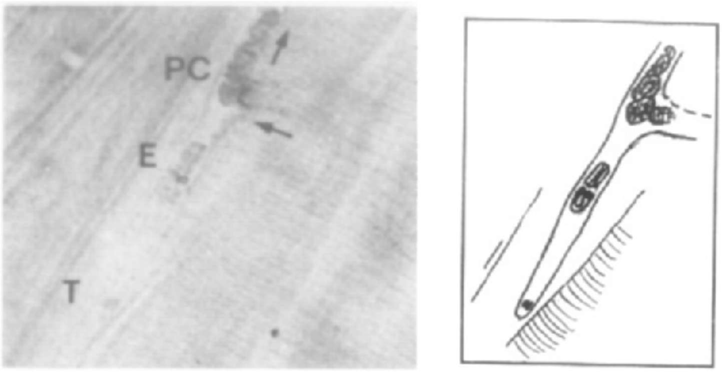

Fig. 10. Capillary *sprout* in a rat EHP muscle, stimulated for 12 days. The sprout originates from a sharp bending of a pre-existing capillary (PC), and runs along the muscle fibres. T is a trapped thrombocyte close to the tip of the sprout, and E = two trapped erythrocytes. Arrows indicate blood flow direction. From 16 mm film.

creased by 29% (1.81) in muscles stimulated for 7 days and by 46% (2.08) in muscles stimulated for 14 days.

Factors connected with tissue injury, e.g. granulocyte-released substances (Brånemark, 1965) and histamine (Smith, 1961; Schoefl, 1963), have been suggested as initiating capillary growth during tissue regeneration. In the chronically stimulated muscles this type of stimulus is probably not present, since histological examinations showed no signs of inflammation and mast cells were only occasionally observed in close vicinity to the sprouts. Furthermore, there was no change in capillary diameter and no capillary sprouts in the sham-operated tenuissimus muscles.

Collagen, which has been observed close to the tip of capillary sprouts, might support the growth of new capillary segments (Cliff, 1963; McKinney & Panner, 1972). In the stimulated muscles, we frequently found a bright, wedge-like zone around the tip of the sprouts. The location of this zone corresponds well to the site of the collagen fibres in the previous studies.

It has been postulated by Ashton (1961), that endothelial cells are directly sensitive to oxygen and start multiplying at low oxygen levels. A high capillary density has also been found in animals living continuously at high altitudes (Valdivia, 1958). Increased muscle activity, by prolonged contractions at 10 Hz, seems to be associated with a considerable increase in oxygen consumption (Cotter & Hudlická, unpublished data). Thus, it cannot be excluded that chronic stimulation causes a relative hypoxia in the muscle during the first few days of stimulation, which might provoke the gradual increase in TCSA.

Endurance muscle training increases the diameter of the muscle fibres parallel to the number of capillaries per fibre (Hermansen & Wachtlova, 1971). In contrast to this, the muscle fibres have a smaller average diameter after chronic stimulation at 10 Hz but still an increased capillary/fibre ratio.

Neither the diameter nor the metabolism of the muscle fibres, not even the capillary/fibre ratio are changed in fast contracting muscles subjected to chronic stimulation at an impulse frequency *exceeding* 10 Hz. Similarly, no changes in the fibre population or the capillary/fibre ratio of slow contracting muscles, were found after chronic stimulation at 40 Hz - the impulse frequency normally occurring in nerves to fast muscles - (Brown *et al.,* 1976).

It should be noted, however, that in the case of chronic stimulation an altered pattern of activity is *superimposed* on the normal motor nerve activity. In "cross-innervation" the *total* activity of the motor nerve is altered, which actually changes the metabolism and the number of capillaries even in slow contracting muscles (Romanul & Pollock, 1969).

In view of this, the pattern of activity in the motor nerve seems to be more important than the level of muscle exercise as a regulating factor for the metabolism and the *capillary supply* of muscle fibres.

COMMENTS ON VASCULAR INTERCONNECTIONS BETWEEN SKELETAL MUSCLES AND OTHER MESENCHYMAL TISSUES

The main trunks of about 30% of the "transverse" arterioles and venules regularly penetrate the fascia of the tenuissimus muscle and ramify into the deep extended part of the fascia (Fig. 7). These vessels form a capillary network supplying the adipose tissue, which is embedded in the fascia (cf. Myrhage *et al.,* 1973).

Similarly, 4-5 branches of primary arteries and veins were found to leave the belly of all examined biceps femoris muscles. Careful dissection revealed that these vessels subdivided in the subcutaneous tissue adjacent to the muscle. Such interconnecting vessels were also observed in the LG and the MG muscles,where they, however, are smaller and less frequent.

The main trunk of a primary artery penetrated the distal part of the soleus in about 75% of all the examined muscles. This vessel (followed by two parallel primary vein branches) ramifies in the crural fascia and also gives branches to the periosteum of the tibia.

Corresponding vascular interconnections have also been found in the lower leg of man (Saunders *et al.*, 1957). From investigations of injured rabbit EHP muscles, Zucman (1960) concluded that intact vascular anastomoses between muscle and periosteum are essential for successful revascularization of the muscle.

Thus, vascular interconnections between muscles and other mesenchymal tissues might be more important for the blood flow distribution than is generally appreciated.

Intravital microscopy has been found quite suitable for investigations of these vascular pathways. Pilot studies of EHP muscles, subjected to various periods of denervation, indicate that vessels of this type are involved in the circulatory adaptation during muscle atrophy.

SUMMARY AND CONCLUSION

Hind limb muscles, from cats subjected to intravascular perfusion of India ink, were prepared according to the method of Spalteholz. The vascular tree in the unsectioned tenuissimus muscles was analysed and compared with the arrangement of vessels in sectioned, thicker hind limb muscles (the biceps femoris, the gastrocnemius - both heads, separately, and the soleus).

Quantification of different fibre types (FG, FOG, and SO) was performed in serial cryostat sections, histochemically stained for the activity of $NADH_2$-diaphorase (or SDH) and alkaline ATPase. The capillary endothelium was identified by staining for the activity of alkaline phosphatase, and the capillary density was evaluated as the number of fibre surrounding capillaries and as the number of capillaries per muscle fibre.

A method was developed for intravital microscopy and estimation of the total capillary surface area (TCSA) of the rat extensor hallucis proprius muscle (EHP).

Blood flow distribution in the microvascular bed, the structure of the capillary network, and changes in TCSA were evaluated in chronically stimulated, adult rabbit tenuissimus and rat EHP muscles.

From the results the following conclusions are made:

1. A vessel arrangement, similar to that found in tenuissimus, is present as a "basic unit" even in the thicker hind limb muscles.
2. The average cross-sectional area of this unit in the MG muscle is in the range of the total cross-sectional area of the tenuissimus (about 1 mm^2). This value is about 80% greater in the biceps femoris and the LG muscles, but 50% less in the soleus.
3. Generally, all fibres in the MG are 20-40% larger than the corresponding fibres in the tenuissimus, the biceps and the LG. The soleus has quite large SO fibres (about 70 μm in diameter) as compared to this type of fibre (25-45 μm) in the other muscles.
4. Cat muscles with large muscle fibres have a higher number of capillaries per fibre (1-2) than muscles with a smaller average fibre diameter (relative value, less than 1 capillary). In spite of a larger diameter, the FG fibres are surrounded by fewer capillaries (1-3) as compared to the FOG, and the SO fibres (2-4). It seems most likely that the oxidative capacity of the fibre is more important than the fibre diameter as a regulatory factor for the fibre capillarization.

5. The tenuissimus muscle does not differ from thicker muscles in respect of the vascular arrangement and the capillary supply of muscle fibres, although the tenuissimus seems to be of minor importance for locomotion.

6. The rat EHP is a typical locomotor muscle and is also quite suitable for combined intravital and histochemical studies. FG fibres dominate in this muscle. The vascular bed has an arrangement similar to that of the tenuissimus. However, the capillaries have fewer interconnections, which is in advantage for evaluations of the TCSA.

7. Chronic muscle stimulation of fast contracting muscles at 10 Hz results in widening of the capillaries, especially close to the collecting venules. The capillary density is also increased. These rearrangements of the capillary bed enhance the TCSA in the rat EHP by 46% after 14 days of stimulation.

8. Growth of new capillaries in the stimulated muscles was observed as *sprouts* emanating from pre-existing capillaries. The proportion of sprouts to pre-existing capillaries is about 40% after 14 days of stimulation.

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