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# DEVELOPMENT AND ADAPTABILITY OF MICROVASCULATURE IN SKELETAL MUSCLE

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## SUMMARY

In skeletal muscle, the size of the capillary bed is adapted to the type of muscle metabolism and can be altered by adaptation to different environments or increased activity. Muscle fibres with high aerobic metabolism have more capillaries, and an increase in aerobic metabolism is usually followed by capillary growth. It is assumed that local hypoxia – created by increased demand for oxygen during growth, cold exposure or increased activity – can stimulate proliferation of capillaries. Capillary density is reduced in parallel with enhanced glycolytic metabolism.

The size of the capillary bed can also increase without any apparent change in the oxidative metabolism (e.g. in the early stages of chronic electrical muscle stimulation or as a result of long-term administration of vasodilating drugs), and it is argued that the growth of capillaries in these cases may be due to various mechanical factors connected with increased blood flow.

## INTRODUCTION

It has been assumed since the pioneering work of Krogh (1919*a*,*b*) that the capillary bed in skeletal muscle serves mainly to supply oxygen. This idea is supported by the observation that red muscle, characterized by a highly aerobic metabolism, has a much more dense capillary network than white muscle, with high glycolytic metabolism. However, capillaries are also needed to remove different metabolites from muscles, one of the most important being lactate, and to reduce the heat produced mainly during muscle contraction. It is still not clear what induces the development and adaptation of the capillary bed which differs greatly between different muscles and different species.

Adaptation of the capillary bed according to oxygen demand or consumption has been disputed by Maxwell, White & Faulkner (1980), who found a very poor correlation between capillary density and chemically estimated succinate dehydrogenase in a variety of muscles and animals. On the other hand, there is a good correlation between citrate synthase and capillary density both in different muscles and in the heart (Hudlická, 1982), and an excellent correlation between capillary density and volume density of mitochondria in various muscles of 20 different mammalian species (Hoppeler *et al.* 1981*b*). Moreover, it has been shown that changes in muscle oxidative

metabolism, induced either as a consequence of altered activity during cross-innervation or by administration of thyroxine, produce an adaptation in the vascular bed with more numerous capillaries around highly oxidative fibres and lower density around the glycolytic ones (Romanul & Pollock, 1969), although Capo & Sillau (1983) described increased capillary density without any change in the oxidative enzymes after administration of thyroid hormones.

Comparisons of the size of the capillary bed are usually made either on the basis of capillaries $mm^{-2}$ (capillary density, CD), or on the number of capillaries per muscle fibre (capillary/fibre ratio, C/F). Ideally, capillary supply should be described in terms of capillary surface area or capillary length per volume of tissue. Since capillaries in most muscles are arranged parallel to the fibre axis, it is possible to use cross-sections for the evaluation of the number of profiles. Mathieu, Cruz-Orive, Hoppeler & Weibel (1983) showed that the contribution of tortuosity towards the total capillary length represents only 12% in cat soleus – a muscle with the most tortuous capillaries – and it is thus justifiable to compare and describe capillary supply under most circumstances either as CD or C/F.

Capillary density is inevitably dependent on the size of fibres – muscle composed of larger fibres having lower capillary density (Plyley & Groom, 1975; Ripoll, Sillau & Banchero, 1979; Aquin, Sillau, Lechner & Banchero, 1980). It is therefore better to compare the extent of capillarization in terms of C/F ratio which is relatively independent of fibre area (Fig. 1).

## CAPILLARY SUPPLY TO VARIOUS MUSCLES IN DIFFERENT SPECIES

Comparison of capillary supply to different types of fibre can be readily made in fish, most of which have highly oxidative red fibres in superficial muscle, and glycolytic white fibres in the deep part of the trunk. There is a C/F of 1·9–2·5 in red muscles and 0·16–0·3 in white muscles of several seawater teleosts (Mosse, 1978) and a similar situation in the juvenile European eel (Eggington & Johnston, 1982). In freshwater fish there is a higher C/F in white muscles (0·8 in tench, Johnston & Bernard, 1982; 1·4 in carp, Johnston, 1982). This difference can be explained by different activity, as higher teleosts can recruit white fibres over a wide range of swimming speed.

Birds also have a higher proportion of capillaries supplying red rather than white muscles. In one study of chicken flight muscles, the red anterior latissimus dorsi (ALD) were found to have a C/F of 1·77 while the white posterior latissimus dorsi (PLD) had a value of 1·34 (Cotter, 1975). In another study, a C/F of 1·11 was reported for the red muscle, and 1·08 for the white (Gray, McDonagh & Gore, 1983).

In mammals, the highest capillary density is in the diaphragm and in the masseter muscle (Hoppeler *et al.* 1981*a*). The values in these two muscles are rather similar in different species of greatly different body weight (Schmidt-Nielsen & Pennycuick, 1961). The C/F ratio of muscles involved in locomotion increases with the proportion of oxidative fibres (Fig. 2) and a higher ratio is found in the same muscle (e.g. soleus or

gastrocnemius) in wild animals than in domesticated ones (Wachtlová & Pařízková, 1972).

Even in mixed muscles (most animal muscles and all human muscles are mixed) fibres with a high oxidative capacity are surrounded by a greater number of capillaries than those with a high content of glycolytic enzymes (Romanul, 1965; Gray & Renkin, 1978).

The data for capillary density in man are very variable (Table 1). This is partly due to different methods (depicting the capillaries either by histochemical staining or by electron microscopy) or to different sampling, but is obviously mainly due to inter-individual variability, because even data published by the same group of authors show great differences. The emerging pattern shows a higher C/F ratio in the soleus, a lower C/F in women in most muscles that have been studied, and decreasing C/F ratio in old age, when fibre diameters are smaller. The last observation is in agreement with studies of animals during development (Ripoll *et al.* 1979; Aquin *et al.* 1980).

## DEVELOPMENT OF THE VASCULAR BED

There are surprisingly few data on the development of the microvascular bed. The capillary network of rats on the 17th day of gestation is formed by polygonal loops

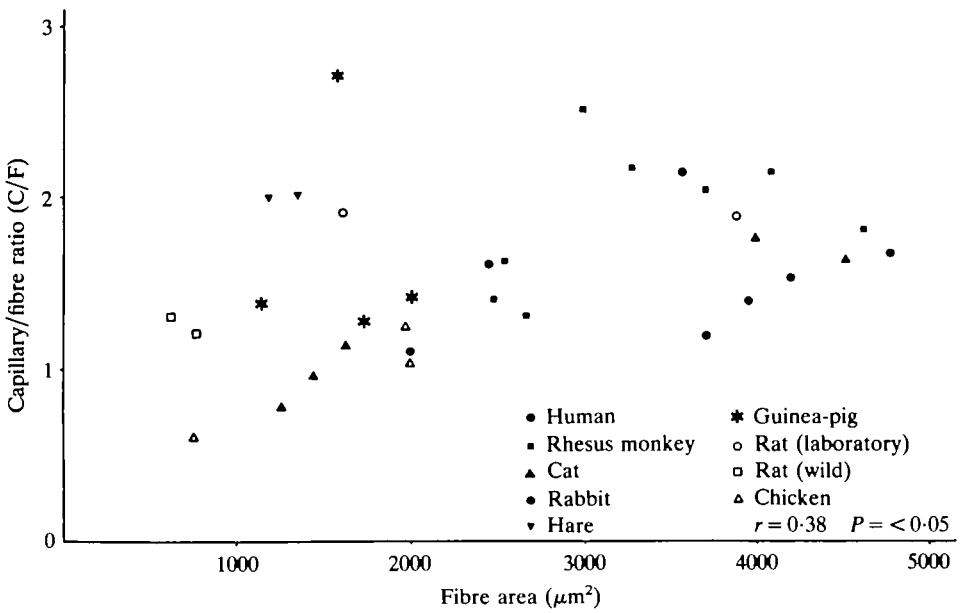


Fig. 1. Relationship between fibre area and capillary/fibre ratio (C/F) in a variety of muscles and animals. Data in man from Ingjer (1979); Ingjer & Brodal (1978) and Andersen & Kroese (1978); in monkeys from Maxwell, Carlson, McNamara & Faulkner (1979); in cats from Myrhage (1978) and Plyley & Groom (1975) (also rabbit); in rabbit and hare, Wachtlová & Pařízková (1972); in guinea-pig, Plyley & Groom (1975) and Schmidt-Nielsen & Pennycuick (1961); laboratory and wild rat, Wachtlová & Pařízková (1972); chicken, Gray, McDonagh & Gore (1983) and Holly *et al.* (1980).

which gradually stretch under the tension of the growing muscle fibres (Wolff, Goerz, Bär & Goldnerf, 1975). There are quite a number of capillary sprouts representing up to 50% of the capillary network; these gradually disappear by 21 days *postpartum* (Welt, Scheller, Schippel & Schippel, 1975).

Capillary growth gradually slows although fibre growth continues; CD decreases while C/F increases and is linearly related to fibre size in different muscles in chicken (Cotter, 1975), guinea-pigs (Aquin *et al.* 1980), rats (Ripoll *et al.* 1979) and hamsters

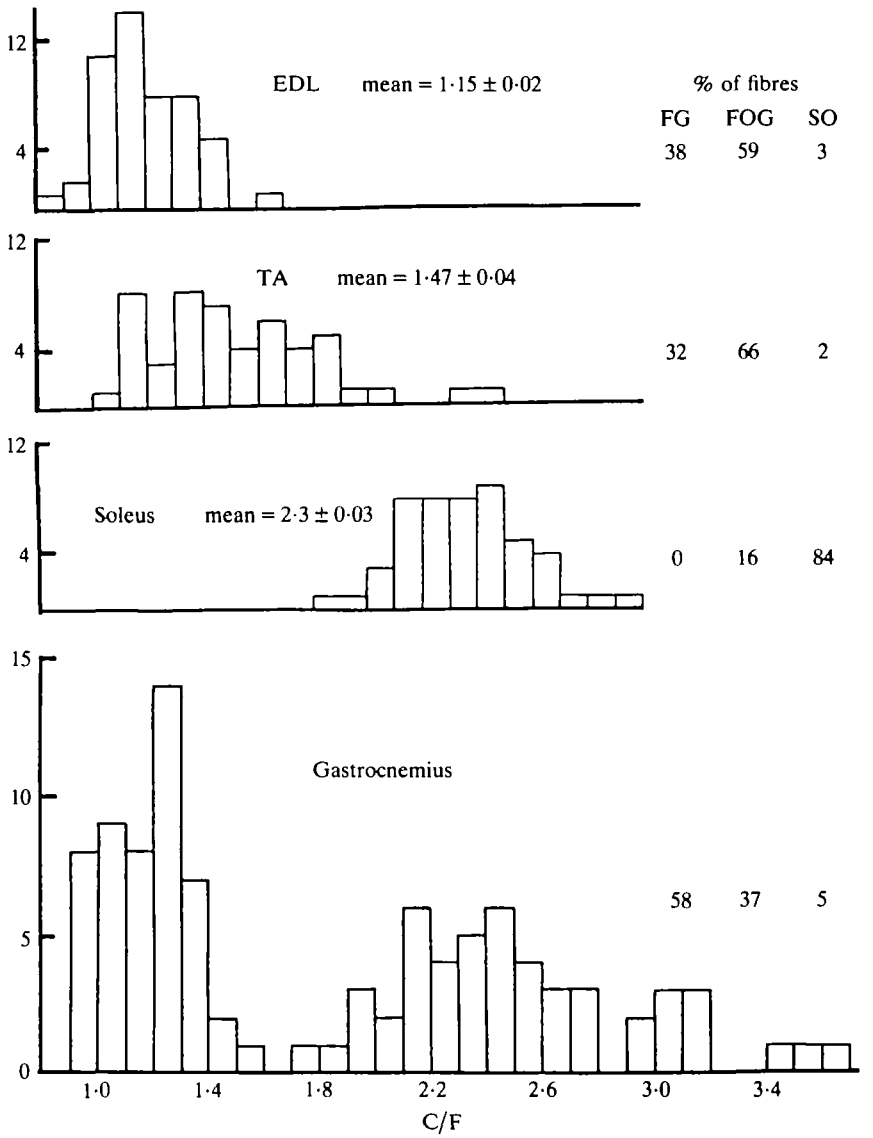


Fig. 2. Left side, C/F ratio; right side, percentage of fast glycolytic (FG), fast oxidative (FOG), and slow oxidative (SO) fibres, in various rat muscles (from Cotter, 1975).

(Sarelius, Damon & Duling, 1981). The C/F ratio increases equally during development in dogs, but in this species capillary density remains constant irrespective of the more than 70-fold increase in fibre area. Thus many more smaller fibres in a 6-day-old dog are supplied by the same number of capillaries as relatively few fibres per $mm^2$ in adults. This can probably be explained by the observation that all fibres in puppy leg muscles are glycolytic, and they are replaced by an entirely oxidative population in adult dogs (Aquin & Banchero, 1981).

Capillaries in younger animals are more tortuous (Cotter, 1975; Sarelius *et al.* 1981) and gradually straighten, elongate and become more parallel to muscle fibres. The decrease in capillary density in chickens and hamsters is accompanied by decreased blood flow (Hudlická, 1969; Sarelius *et al.* 1981). It is also accompanied by a decrease in oxidative capacity during development in chickens (Hudlická, Pette & Staudte, 1973), rabbits and kittens (Cotter, 1975). However, the increase in C/F in kittens is faster in the soleus than in the gastrocnemius even if the increase in the activity of oxidative enzymes is similar in both muscles (Fig. 3). It is possible that the high glycolytic activity in the gastrocnemius (Fig. 3) suppresses capillary development in this muscle.

## ADAPTATION OF THE CAPILLARY BED TO INCREASED ACTIVITY

The discovery that contracting muscles had a capillary density up to ten times higher than resting muscles (Krogh, 1919*b*) initiated a great number of investigations to find out whether the capillary bed enlarges with training (for review see Hudlická, 1977). However, most of the studies showing an increase in the number of perfused capillaries were not really indicative of capillary growth, as capillaries were visualized either by dye injection or by counting capillaries filled with erythrocytes. It was only recently, when either histochemical staining or electron microscopy was used, that capillary growth has been demonstrated to result from training. Andersen & Henriksson (1977) found a significant increase in both cytochrome oxidase and succinate dehydrogenase (SDH) together with an increase in capillary density in biopsies from human vastus lateralis after 8 weeks of endurance training. The C/F increased earlier, after only 5 weeks, a result of muscle fibre hypertrophy. There is a very close correlation between C/F ratio and maximal $V_{O_2}$ (Ingjer, 1978; Zumstein, Mathieu, Howald & Hoppeler, 1983) or volume density of mitochondria (Zumstein *et al.* 1983). There is a higher capillary density, as well as higher C/F ratio in vastus lateralis and deltoid muscle in female swimmers, with a greater increase in relation to oxidative fibres (Nygaard, 1982). The C/F ratio in the vastus lateralis has been found to increase with heavy resistance training in two studies (Schantz, 1982, 1983) but not in another (Tesch, Daniels & Sharp, 1984). The C/F ratio is inversely correlated with both muscle and blood lactate concentration during cycling (Tesch, Sharp & Daniels, 1982). The increased capillary density in the vastus lateralis of endurance-trained men and women is accompanied by very little increase in fibre diameters thus indicating real capillary growth (Brodal, Ingjer & Hermansen, 1977; Ingjer & Brodal, 1978). The increase is also higher around slow oxidative fibres than around fast glycolytic

Table 1. *Capillary/fibre ratio in human muscles*

| Reference | Vastus lateralis | Gastrocnemius | Soleus | Triceps brachii | Deltoid |
|---|---|---|---|---|---|
| Andersen & Henriksson (1977) | 1·36 ± 0·07 | | | | |
| Brodal, Ingjer & Hermansen (1977) | 1·77 ± 0·10 | | | | |
| Ingjer & Brodal (1978) | 1·11 ± 0·07 W | | | | |
| Ingjer (1979) | 1·39 ± 0·06 | | | | |
| Andersen & Kroese (1978) | | 1·53 ± 0·009 | 2·23 ± 0·19 | | |
| Henriksson, Nygaard, Andersson & Eklof (1980) | | 1·48 ± 0·1 | | | |
| Tesch, Sharp & Daniels (1982) | 1·7 ± 0·08 | | | | |
| Tesch, Daniels & Sharp (1984) | 2·16 ± 0·34 | | | | |
| Klausen, Andersen & Pelle (1981) | 2·07 ± 0·11 | | | | |
| Sjøgaard (1982) | 2·50 | | 2·90 | 1·94 | |
| Zumstein, Mathieu, Howald & Hoppeler (1983) | 1·63 W | | 3·06 W | 1·37 W | |
| | 2·07 ± 0·08 | | | | |
| | 1·37 ± 0·13 W | | | | |
| Nygaard (1982) | 1·20 ± 0·09 W | | | | 1·4 ± 0·09 W |
| Saltin & Gollnick (1984) | 0·85–1·9 | | | | |
| Pařízková, Eiselt, Šprynarová & Wachtlová (1971) | 0·81 ± 0·22 | | | | |
| Aniansson, Grimby, Hedberg & Krotkiewski (1981) | 0·59 ± 0·08 O | | | | |
| | 1·3 ± 0·07 O | | | | |
| | 1·1 ± 0·10 OW | | | | |

W, women; O, old. Means ± S.E.

fibres (Ingjer, 1979). Similar findings have been made in guinea-pigs (Mai, Edgerton & Barnard, 1970) and rats (Zika, Lojda & Kučera, 1973). In humans, there was a considerably greater increase in the number of subsarcolemmal mitochondria in slow oxidative than in fast glycolytic fibres, and it appears that the increase in capillary density was related more closely to the mitochondrial content than to fibre types (Ingjer, 1979). All these findings can be explained by the fact that submaximal work performed during endurance training activates predominantly slow oxidative fibres whereas fast glycolytic fibres are only active during maximal or supramaximal contraction of a very short duration (e.g. Gollnick, Piehl & Saltin, 1974). In trained rat soleus (84 % slow oxidative) in which the area of subsarcolemmal mitochondria is increased, there is hardly any change in C/F (Pařízková, Wachtlová & Soukupová,

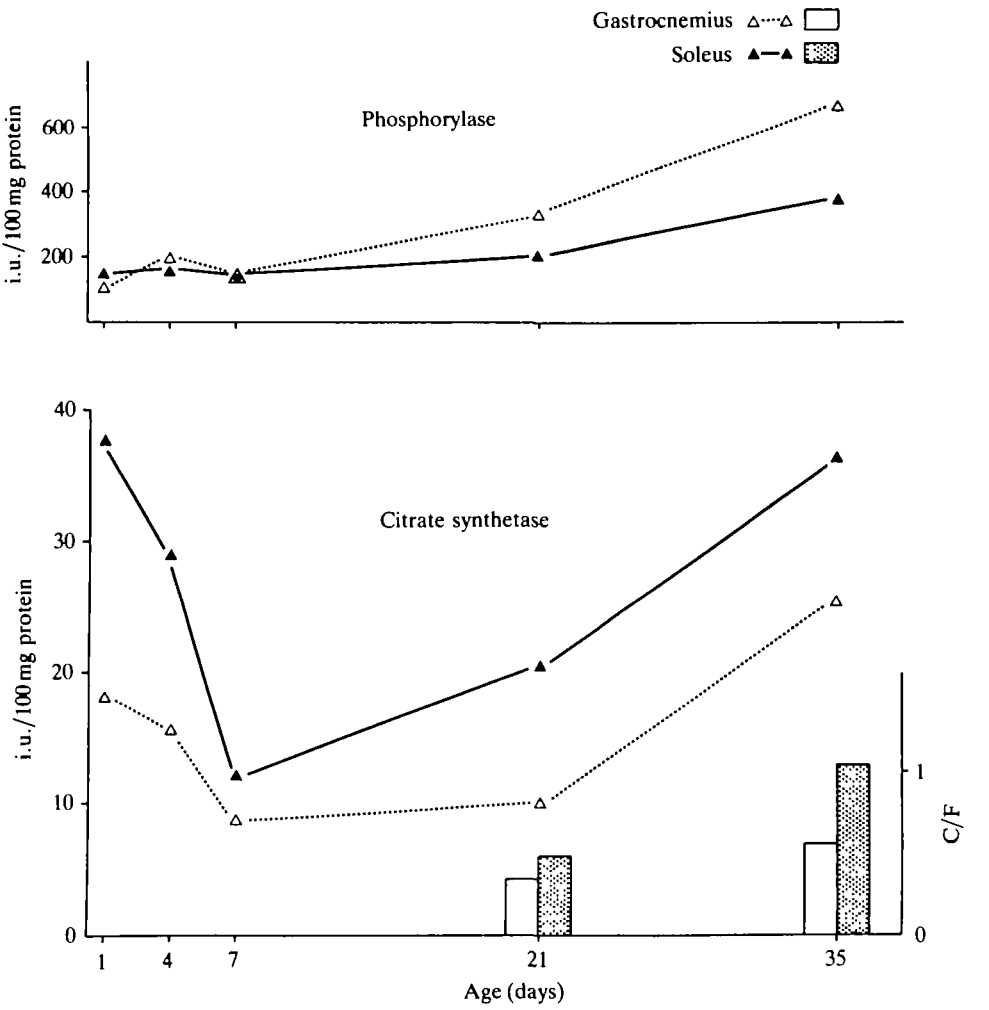


Fig. 3. Changes in the activity of a glycolytic and an oxidative enzyme in kitten gastrocnemius and soleus muscles during development. Data from Hudlicka, Pette & Staudte (1973).

1972; Müller, 1976). This suggests that the amount of activity superimposed on normally very active soleus is not great enough to produce an increase in capillary density, although it is sufficient to produce an increase in aerobic capacity. Unfortunately, the experiments were not carried out for a sufficiently long period to show whether any capillary growth would have been found later. Increased capillary density has also been described in young, but not in adult, rats trained by swimming (Adolfsson, Ljungqvist, Tornling & Unge (1981). There were no changes in the incorporation of [$^3$H]thymidine into capillary endothelium in skeletal muscles of adult rats under similar conditions even though there was capillary proliferation in the heart (Ljungqvist & Unge, 1977).

Exercise thus seems to produce an increase in capillary density that can be explained by capillary growth in young animals, and, if performed for a sufficiently long time (minimum 8 weeks), in adults. This growth appears in the vicinity of permanently active slow oxidative fibres and may perhaps be preceded by an increase in the oxidative capacity.

Another situation when the capillary bed is adjusted to increased activity can be achieved by long-term electrical stimulation, when the increase in activity is similar in all muscle fibres. When fast rabbit muscles were indirectly stimulated by implanted electrodes for 8 h day$^{-1}$, at a frequency naturally occurring in nerves to slow muscles (10 Hz), the activity of oxidative enzymes was increased and that of glycolytic decreased (Pette, Smith, Staudte & Vrbová, 1973), and C/F was almost doubled within 28 days (Cotter, Hudlická & Vrbová, 1973*b*; Brown, Cotter, Hudlická & Vrbová, 1976). The muscles did not hypertrophy as during exercise. The average fibre diameter in extensor digitorum longus (EDL) stimulated for 28 days decreased from $72 \pm 1{\cdot}25\,\mu$m to $65 \pm 1{\cdot}0\,\mu$m and was accompanied by the transformation of large fast glycolytic into small oxidative fibres. Consequently, C/F increased to the value normally found in slow soleus muscle. A small increase in both CD and C/F appeared after only 4 days of such stimulation ( Fig. 4). This preceded the increase in the activity of oxidative enzymes estimated chemically in muscle homogenates (Cotter *et al.* 1973*a*). The doubling of capillary density after 28 days of stimulation could only be explained by capillary growth and this was confirmed by identification of sprouts in rat extensor hallucis proprius and rabbit tenuissimus muscles stimulated for 7–12 days (Myrhage & Hudlická, 1978): both sac-like and tapering sprouts, similar in appearance to previously observed sprouts (e.g. Cliff, 1963) were found in muscles stimulated for 7 days, and their incidence increased to four sprouts per 10 capillaries (a percentage similar to that found for C/F) after 12–14 days of stimulation. The sprouts were more numerous on the venular side of the capillaries, which were also wider and more tortuous. In these studies the growth of sprouts invariably started from a point at which the pre-existing capillary was bent. Sprouts made connection to other capillaries whose lumens were too narrow for erythrocytes to pass through initially but which eventually widened and resembled normal capillaries. No signs of capillary growth were found in control or sham-operated muscles.

Long-term stimulation of rabbit fast muscles at a pattern of frequency more similar to that occurring naturally in their supplying nerves (intermittent bursts of tetani) did

not change capillary density or C/F even after 28 days when contractions of 40 Hz were applied for 5 s every 100 s for 8 h day$^{-1}$ (Brown *et al.* 1976). When the total number of impulses was increased to give the same number of stimuli per hour as the stimulation at 10 Hz (contractions at 40 Hz for 5 s, three times per minute), no change in CD or C/F was found after 4 days, but prolonged stimulation for 14 and 28 days had a similar effect as stimulation at 10 Hz (Fig. 4). At the same time, the muscles change from a mixed population of glycolytic and oxidative fibres to an apparently uniform population of highly oxidative fibres (Hudlická & Tyler, 1984). Any type of electrical stimulation – provided it lasts long enough and is sufficiently intensive – thus produces capillary growth in fast muscles (Fig. 4), and it is therefore the total amount of activity which is important for capillary growth.

However, the adaptation in the capillary bed varies with different frequencies of stimulation in the early stages of stimulation, and it also varies in fast muscles with the different proportion of oxidative and glycolytic fibres: stimulation at 10 Hz induces very early on – after only 4 days – an increase in the proportion of oxidative fibres (Hudlická, Dodd, Renkin & Gray, 1982) and increases SDH activity in IIb fibres in tibialis anterior (TA) which has a higher percentage of oxidative fibres than EDL. Muscles stimulated at 40 Hz show a gradual increase in SDH up to 14 days (Pette & Tyler, 1983). In EDL, qualitative histochemistry shows no changes in the proportion

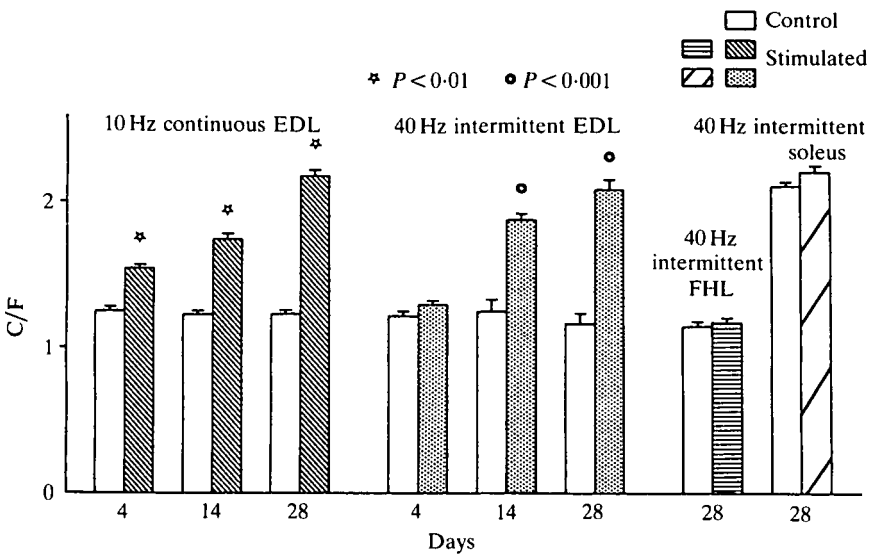


Fig. 4. Capillary/fibre ratio (C/F) in rabbit extensor digitorum longus (EDL), flexor hallucis longus (FHL) and soleus. Unhatched columns represent control muscles. '10 Hz continuous stimulation' represents values in muscles stimulated continuously at 10 Hz, 36 000 impulses h$^{-1}$. '40 Hz intermittent' represents values in muscles stimulated with 36 000 impulses h$^{-1}$ using three trains (each at 40 Hz, 5 s duration) per minute. FHL and soleus muscles stimulated with 1440 impulses h$^{-1}$, trains at 40 Hz of 5 s duration every 100 s. All muscles were stimulated for 8 h day$^{-1}$ for the number of days marked on the abscissa.

of glycolytic or oxidative fibres in muscles stimulated for 4 days at 10 Hz (Hudlická *et al.* 1982), and a lower proportion of glycolytic fibres in muscles stimulated at 40 Hz (Hudlická & Tyler, 1984). In this muscle C/F increases first in the vicinity of predominantly glycolytic fibres with both types of stimulation. Why the two muscles show slightly different changes in response to long-term stimulation is open to speculation: rabbit TA has a slightly lower proportion of glycolytic fibres than EDL, and it is possible that the increased metabolic demands during the increased activity could be met in the first place by increased blood supply to the oxidative fibres with their already high capillary supply and the possibility of higher oxygen extraction. On the other hand, this increased activity could produce local hypoxia in EDL which has a higher proportion of glycolytic fibres not adapted to long-lasting contractions. Activity of oxidative enzymes has previously been shown to increase in muscles with decreased blood supply (Holm, Björntorp & Scherstén, 1972). When the stimulation was discontinued, capillary density remained high for as long as 4 weeks and gradually returned to control values 6 weeks after the cessation of stimulation (Cotter, 1975). Similar detraining effects were described by Houston, Betzen & Larsen (1979), Klausen, Andersen & Pelle (1981) and Saltin & Rowell (1981) in endurance-trained subjects.

## FACTORS INVOLVED IN THE ADAPTATION OF THE VASCULAR BED

Possible factors responsible for capillary growth – whether during normal development, changes in environment or increased activity – could be either metabolic or mechanical. Changes in the capillary bed during development, exercise or chronic electrical stimulation are almost always accompanied by changes in the activity of oxidative enzymes and by changes in the volume density of mitochondria, with an increase or decrease in the oxidative capacity usually preceding the increase or decrease in the size of the vascular bed. Ashton (1961) suggested that endothelial cells are in some way directly sensitive to oxygen, multiplying at low oxygen levels, resting at normal, and dying at high oxygen concentrations. In muscles stimulated chronically at 10 Hz, capillary growth appeared to be induced by local hypoxia (Hudlická & Schroeder, 1978; Hudlická *et al.* 1982), but in muscles stimulated at 40 Hz, $P_{O_2}$ was found to increase after 4 or 7 days (Kanabus, Hudlická & Tyler, 1980). However, there is a greater accumulation of lactate in rat muscles stimulated for 7 days at 40 Hz than at 10 Hz (Cotter & Hudlická, 1979). It was also shown that in a predominantly glycolytic muscle – cat gracilis – the size of the capillary bed bears no relationship to the volume density of mitochondria, but is related to the lactate output (Hoppeler & Hudlická, 1984).

Increased blood flow and factors connected with it were suggested as being important for vessel growth during normal development by Thoma (1911) and Clark (1918). Whereas the former thought that capillaries grow when blood through the terminal vascular bed is increased because of its mechanical expansion, the latter supposed that capillary growth is induced by mechanical friction connected with the increased amount of filtered fluid.

Exercise, as well as long-term stimulation, produces a long-lasting increase in blood flow (see Hudlická, 1977), connected with an increased proportion of perfused capillaries (e.g. Petrén, Sjöstrand & Sylvén, 1937; Cotter & Hudlická, 1979). Increased capillary density was found during endurance exercise around oxidative fibres, while it appeared first around glycolytic fibres in chronically stimulated muscles. Blood flow during exercise is increased much more in muscles, or parts of muscles, composed of oxidative fibres which are recruited first (Laughlin & Armstrong, 1982). Even in chronic stimulation, which activates all fibres at the same time, there is a greater and more consistent increase in blood flow to glycolytic rather than oxidative fibres (Dawson, Hudlická & Tyler, 1983).

Different patterns of blood flow can perhaps also explain the different onset of capillary growth with different patterns of stimulation: 10 Hz continuous contractions lead to a long-lasting increase, intermittent 40 Hz stimulation not only causes a short period without blood flow at the peak of each tetanus, but the total increase in blood flow per minute is not well maintained over a long period of isotonic contractions during the first hours of stimulation (Aitman, Hudlická & Tyler, 1979). These differences disappear with continuing stimulation, and both resting flow and functional hyperaemia are very similar in muscles stimulated at either frequency for 14 days (Hudlická, Tyler & Aitman, 1980). At this time, the increase in both capillary density and capillary/fibre ratio is virtually the same with either frequency.

The factors connected with increased blood flow that would stimulate capillary proliferation are not quite clear. Since capillary sprouts in chronically stimulated muscles are always found at bends of pre-existing capillaries (Myrhage & Hudlická, 1978) it is quite possible that mechanical factors of a nature different even from those suggested by Thoma (1911) or Clark (1918) can be involved: slight damage to endothelial cells exposed to more frequently moving erythrocytes could induce their proliferation; proliferation of damaged endothelial cells has been found in the aorta (Payling-Wright, 1972) and DNA synthesis is increased in tissue culture in areas with damaged cell surfaces (Gimbrone, Cotran & Folkman, 1974). Brånemark (1965) suggested that pulsatory movements of erythrocytes during the first phases of vascularization are of primary importance in capillary growth. If any of the factors connected with increased blood flow are involved in capillary growth, it should be possible to elicit growth by long-lasting vasodilation, and this has been reported in rats treated with dipyridamole (Tornling, Adolfsson, Unge & Ljungqvist, 1980), in rats and rabbits treated with axanthine derivate (Wright, Hudlická, Tyler & Ziada, 1981), in rabbits on long-term administration of adenosine (Hudlická, Tyler, Wright & Ziada, 1983) and in rats treated with prazosin (Tyler & Ziada, 1983).

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