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The Effect of Long-Term Stimulation of Fast Muscles on Their Blood Flow, Metabolism and Ability to Withstand Fatigue

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Summary. Chronic stimulation of fast rabbit muscles (tibialis anterior, extensor digitorum longus and the peroneal muscle group) at a frequency naturally occurring in nerves to slow muscles increased their ability to withstand fatigue. Isometric tension decreased during a 10-min period of contractions at 4 Hz by 75% in control muscles, but only 55% in muscles chronically stimulated for 4 days, and 23% in muscles stimulated for 28 days.

Chronic stimulation had little effect on resting blood flow, oxygen or glucose consumption. The output or consumption of lactate and free fatty acids (FFA) at rest were also unaffected. The glycogen content was regularly increased, and was apparent after only 2 days of stimulation. The activity of fatty acid activating enzyme was increased after 28 days.

During a 10-min period of isometric contractions at 4 Hz, there was a markedly greater increase in blood flow and oxygen consumption in muscles stimulated for 14–28 days than in control muscles; lactic acid output was lower in muscles stimulated for 28 days, and the uptake of FFA was significantly higher. It is therefore suggested that muscles chronically stimulated for 14–28 days use fats as the main source of energy during isometric contractions. The predominantly oxidative metabolism is probably facilitated by the higher density of capillaries. The latter also enables more efficient delivery of oxygen, and therefore smaller fatiguability, already after 4 days of chronic stimulation.

Key words: Fast muscles – Chronic stimulation – Fatigue – Muscle blood flow – Muscle metabolism.

INTRODUCTION

It has long been known that muscles involved in the maintenance of posture contract more slowly (e.g. Ranvier, 1875; Denny-Brown, 1929), fatigue less (e.g. del Pozo, 1942), have a higher capillary density (e.g. Ranvier, 1874; Romanul, 1965; for review see Hudlická, 1973) and higher blood flow (Hilton, 1966; Folkow and Halicka, 1968; Hudlická, 1969; Hilton et al., 1970) than fast-contracting muscles involved in rapid movements. Pette and Bücher (1963) and Romanul (1965) have also shown that slow muscles have a higher activity of oxidative enzymes and lower activity of glycolytic enzymes than fast muscles.

Chronic stimulation of fast muscles at the low frequency naturally occurring in the nerves to slow muscles (i.e. continuous activation at 5–10 Hz) results in a prolongation of contraction time (Salmons and Vrbová, 1969), increased oxidative and decreased glycolytic enzyme activities (Pette et al., 1973) decreased fatiguability (Brown et al., 1974, 1975; Pette et al., 1976) and an increased capillary density which precedes any changes in the activity of oxidative enzymes (Cotter et al., 1973). The increase in the capillary density is not due simply to an increase in muscle activity, since it does not occur in muscles stimulated by short bursts of tetani (Brown et al., 1976). The purpose of this investigation was to find out whether the increased capillary density in fast muscles chronically stimulated at a low frequency is accompanied by any changes in local blood flow and fatiguability, and whether the changes in the activities of oxidative and glycolytic enzymes can be related to the way in which the muscles utilize different substrates. Preliminary reports on some of these experiments have been published (Brown et al., 1974, 1975).

METHODS

Experiments were performed on 30 New Zealand red rabbits weighing 2.5–3.5 kg.

Chronic Stimulation

Animals were anaesthetized by injection of pentobarbitone sodium (50 mg/kg) or thiopentone (50 mg/kg) through an ear vein. Stimu-

lating electrodes of coiled, multistranded stainless steel wire (Devices Ltd.) were implanted into one leg so as to stimulate the lateral popliteal nerve, as described by Pette et al. (1973). Tibialis anterior (TA) and extensor digitorum longus (EDL) muscles were thus stimulated continuously for 8 h each day with rectangular pulses at a frequency of 10 Hz and of 0.3 ms duration; the voltage was adjusted to give contractions which could be felt and observed in the appropriate muscles without any noticeable discomfort to the animals.

Final experiments were performed after different periods of stimulation: 2, 4, 14 and 28 days.

Experimental Procedures

In the final experiments, the rabbits were anaesthetized by i.v. injection of pentobarbitone sodium (50 mg/kg). Blood pressure was recorded via the carotid artery using a pressure transducer (Bell and Howell Ltd.) 5–10 ml dextran in 5% sucrose, and heparin (1000 I.U./kg) were injected i.v. just before cannulation of the carotid artery. Arterial samples were taken via the cannula in the carotid artery for the measurement of oxygen-content, glucose, lactate and free fatty acids.

1. Isometric Contractions and the Evaluation of Fatigue. Isometric contractions of TA and EDL were elicited and recorded as described by Salmons and Vrbová (1969). The muscles were stimulated via the motor nerve with supramaximal (8–10 V) rectangular pulses of 0.3 ms duration at a frequency of 4 Hz, over a period of 10 min, and the tension was recorded on a pen recorder (Devices). Fatigue was assessed by measuring the tension produced at the end of the 10-min period of contractions, as a percentage of the peak tension.

2. Measurement of Blood Flow. The venous outflow from TA, EDL and the peroneal muscles were isolated in both legs. In a number of experiments, the isolation of the vascular supply to the muscles was checked at the end of the experiment by an injection of India ink-gelatin solution into the femoral artery after previously washing out the vascular bed with warm saline. This showed that such an isolation procedure allowed the measurement of blood flow from only TA, EDL and the peronei. Venous outflow was continuously recorded by means of a photo-electric drop counter (Hilton and Lywood, 1954). Blood was diverted via a cannula from the popliteal vein into a drop-chamber and returned via the femoral vein. The cannula receiving blood from the muscles was used for collecting venous samples for the measurement of oxygen-content, glucose, lactate and free fatty acids. The drop-chambers were calibrated at the end of each experiment by measuring the volume of 50 drops of blood. TA, EDL and the peronei muscles were weighed at the end of each experiment and blood flow calculated per 100 g muscle.

3. Uptake and Release of Substrates and Metabolites. Uptake or release of oxygen, glucose, lactate and free fatty acids (FFA) was estimated from the respective A–V differences and blood flow. Blood samples were taken at rest and at 2 and 10 min after the start of isometric contractions.

a) Estimation of oxygen: Blood was taken into a capillary tube, with vinyl tubing over each end, under anaerobic conditions. The tubes were clamped at either end to prevent contact with air and stored on ice for as short a time as possible. The samples were then injected under anaerobic conditions with a Hamilton syringe into a Lex O_2 CON (Lexington Instruments, Waltham, Mass.) direct-read-out oxygen content analyser (Valeri et al., 1972).

b) Glucose estimation: Glucose was estimated using Boehringer's standard enzymatic method. 0.1 ml blood was deproteinized in 1.0 ml uranyl acetate (Urac®, Boehringer). After centrifugation, duplicates (50 µl each) were taken for glucose estimation using Boehringer's standard reagent solution (containing 100 mM phosphate buffer pH 7.0, 20 g/ml peroxidase, 180 µg/ml glucose oxidase and 1.0 mg/ml chromogen PERID). The optical densities of a blank, standards of known concentration of glucose, and the samples were read at 420 nm. The error between the readings of duplicates was less than ± 2%.

c) Lactate estimation: Blood lactate was estimated using Boehringer's standard enzymatic method. 0.1 ml samples of blood were deproteinized in 1.0 ml perchloric acid. After centrifugation, lactate was estimated in duplicates (0.1 ml each) according to Hohorst and Bergmeyer (1962) using Boehringer's standard reagents (0.5 M glycine buffer, pH 9.0, containing 0.4 M hydrazine, 27 mM NAD and lactate dehydrogenase). The extinctions of NADH in samples, blanks and standards were read at 340 nm using a Pye Spectrophotometer 1800, and a calibration curve with known concentrations of lactate was plotted for each experiment. The error between readings of duplicates was ± 3%.

d) Free fatty acid estimation: Plasma free fatty acids were estimated in duplicates (0.1 ml plasma each) by the method of Duncombe (1964), using Boehringer's standard reagents (0.27 M copper sulphate, 0.45 M triethanolamine buffer pH 7.8, 9 mM diethyldithiocarbamate in butanol).

e) Fatty acid activating enzyme estimation (palmityl-CoA-synthetase): 500 mg muscle was minced and homogenized in 10 ml ice-cold 0.1 M phosphate buffer, pH 7.2, containing 2 mM EDTA, using a Polytron PT 10 homogenizer for 4 × 30 s periods. The homogenate was centrifuged for 60 min at 140000 g. The sediment was resuspended in the phosphate buffer and the activity of palmityl-CoA-synthetase determined by the method of Kornberg and Pricer (1953). The incubation system contained ATP (0.02 M), coenzyme A (0.00045 M), $MgCl_2$ (0.004 M), KF (0.025 M), cysteine (0.015 M), tris buffer pH 7.4 (0.2 M) hydroxyammonium chloride pH 7.4 (1 M), potassium palmitate (0.002 M) and 0.2 ml muscle suspension in a final volume of 1.0 ml.

f) Glycogen: Glycogen was determined by an anthrone method modified from that of Seifter et al. (1950). The glycogen content of individual muscle fibres was also estimated qualitatively after staining by the periodic-acid-Schiff (PAS) reaction (McManus, 1946). The sections (12 µm thick) were counterstained with Ehrlich haematoxylin.

Statistical Evaluation

All data from chronically stimulated muscles were compared with the contralateral muscles as controls; for it had been shown in a series of preliminary experiments that the values of blood flow, uptake and release of substrates and glycogen content in muscles from unstimulated animals did not differ from those in the unstimulated control muscles of chronically stimulated animals. Unpaired Student's *t*-tests were used for statistical evaluation. All values in the tables and text are expressed as the mean ± S.E.

RESULTS

Changes of Fatiguability in Chronically Stimulated Muscles

When tibialis anterior and extensor digitorum longus muscles which had been chronically stimulated were subjected to a period of isometric contractions they fatigued less than the contralateral control muscles: the fatiguability was smaller, the longer the period of chronic stimulation. Figure 1 shows part of an experimental record depicting the tension developed by contractions at 4 Hz for 10 min, in a control

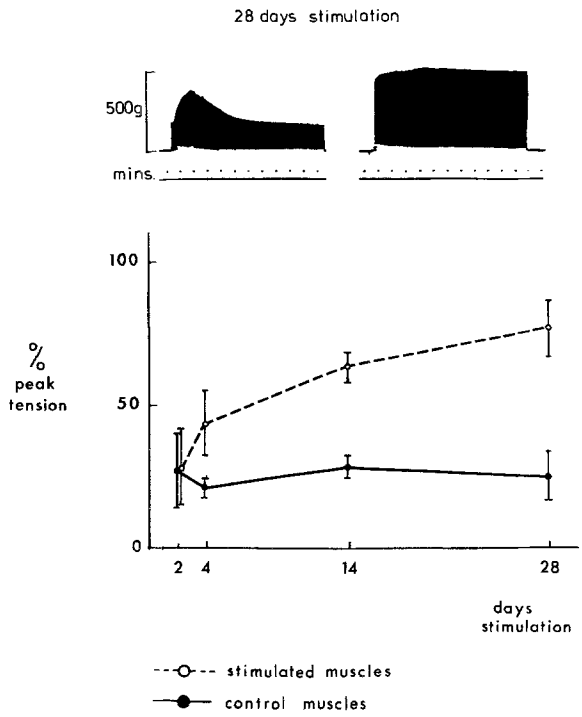


Fig. 1. Top: Original record of isometric tension during 10-min period of contractions at 4 Hz from a tibialis anterior muscle chronically stimulated for 28 days (right) and from a contralateral control muscle (left). Bottom: Ordinate – tension at the end of a 10-min period of isometric contractions expressed as percentage of the peak tension developed during the contractions in chronically stimulated muscles (broken line) and contralateral control muscles (solid line). Days of chronic stimulation on the abscissa. The vertical bars represent S.E. Minimum of 4 experiments for each point

muscle and a muscle which had been stimulated for 28 days. During the 10-min period, the tension of the control muscles decreased by 75% (Fig. 1).

After 4 days of chronic stimulation the reduction in tension at the end of a 10-min period of contraction was 64%, while after 14 days and 28 days stimulation it was only reduced by 37% and 23% respectively. Some reduction in fatigue was occasionally seen in muscles which had only been stimulated for 2 days.

Blood Flow and Oxygen Consumption

The blood flow at rest in muscles that had been stimulated for 2–4 days was similar to that of the controls; it was slightly higher in muscles stimulated for a longer period of time (16.1 ± 1.95 ml/100 g × min in muscles stimulated for 28 days compared with 12.7 ± 1.73 in control muscles). This difference between the blood flow at rest in control muscles and those stimulated for 14 and 28 days was significant when expressed as ΔBF for each animal separately (Fig. 3). During isometric contraction the increase in blood flow was greater in the stimulated than in control muscles, but only after comparatively long periods of stimulation. Figure 2 shows blood flow in contracting muscles that had been stimulated for 28 days which was much higher than normal, and Figure 3 shows that already after 14 days of stimulation the functional hyperaemia was greater in the stimulated than the unstimulated muscles. The time-course of the onset and decline of functional hyperaemia was also different: the maximal increase occurred sooner in chronically stimulated than control muscles while the duration of postcontraction hyperaemia was reduced.

Oxygen consumption at rest was similar in all muscles (Fig. 2 and 3), the values being 0.76 ± 0.09 ml/100 g/min in control muscles and 0.93 ± 0.16 ml/100 g/min in muscles stimulated for 28 days. During isometric contractions, oxygen consumption was similar in control muscles and muscles stimulated for 2–4 days. However, there was a marked increase in oxygen uptake in muscles stimulated for 14 and 28 days (Fig. 3). Figure 2 shows the oxygen uptake during the isometric contraction period in control and 28-day stimulated muscles. It can be seen that oxygen uptake follows the blood flow closely. Indeed, the extraction of oxygen [(A – V)/A] was similar in control muscles and muscles stimulated for 28 days. At rest, the values were 0.288 ± 0.047 in the former and 0.366 ± 1.22 in the latter, while during muscle contractions they were 0.77 ± 0.07 and 0.74 ± 0.1 respectively. Thus the demand for more oxygen by the stimulated muscles is met by the increase in blood flow.

Uptake and Release of Glucose and Lactate, and Changes in Glycogen Content

Table 1 shows the consumption of glucose by control and stimulated muscles at rest and after 10 min of twitch contractions. At rest only the 2-day stimulated muscles had a slightly increased glucose consumption compared with the control muscles. 10 min after the beginning of contractions glucose consumption increased both in control and in stimulated muscles, though less in the latter than in the former. The variations between individual rabbits were so large, however, that the differences were not statistically significant. These variations were probably due to the fact that no attempt was made to standardize the diet or the amount of activity immediately prior to the experiment.

There was no significant difference between the release of lactate from the control and chronically stimulated muscles at rest and during contractions up to 14 days of chronic stimulation (Table 1). Figure 4

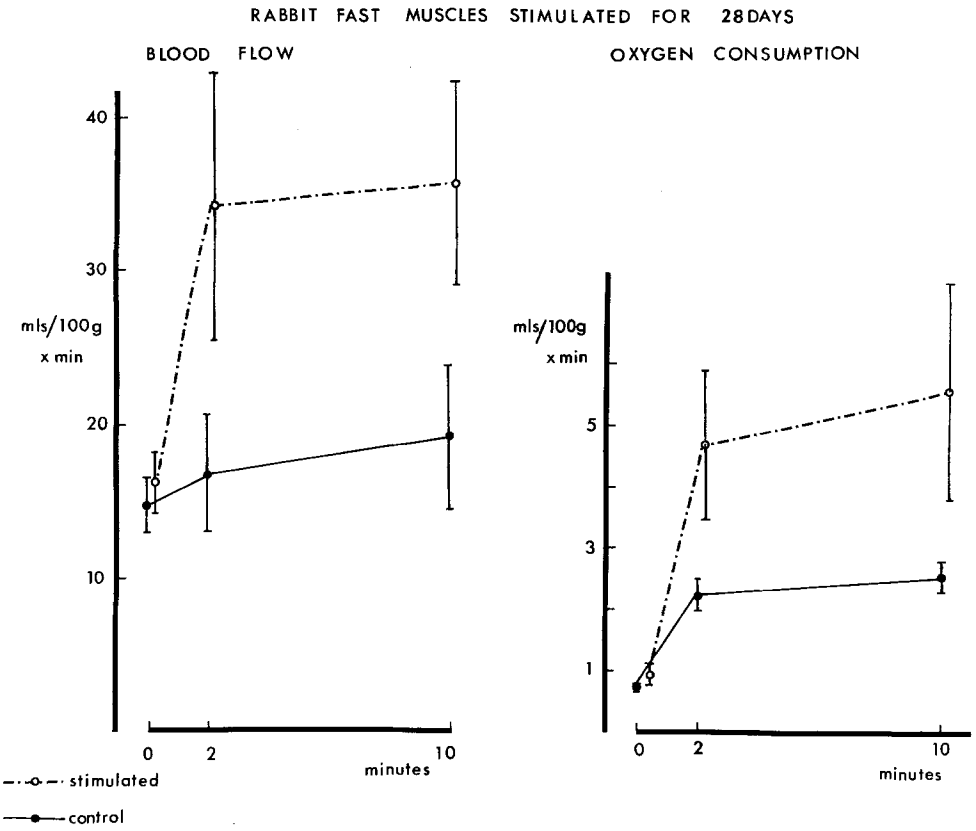


Fig. 2. Blood flow (left side) and oxygen consumption (right side) in ml/100 g × min in muscles stimulated for 28 days (broken lines) and contralateral control muscles (solid lines) at rest (time 0), and during a 10-min period of isometric contractions. Samples for estimation of oxygen consumption taken at 2 and 10 min after the beginning of contractions

shows values of lactate output from control muscles and muscles stimulated for 28 days, both at rest and during contractions when stimulated muscles clearly released less lactate than control muscles when they were contracting (Table 1, Fig. 4).

The glycogen content of chronically stimulated muscles was always increased. Because of the large variations in the values between individual rabbits, those in chronically stimulated muscles were expressed as a percentage of those in the contralateral control muscles. In muscles stimulated for only 2 days the glycogen content was increased by 70 ± 36% as compared with the control muscles, and this increase persisted in muscles stimulated for longer periods (96 ± 27% in muscles stimulated for 14 days, and 140 ± 55% in those stimulated for 28 days).

The higher content of glycogen in chronically stimulated muscles was also shown by histochemical staining for PAS. The distribution of glycogen in fibres of these muscles was homogenous and unrelated to fibre size (Fig. 5b, c); fibres with a low glycogen content were found only exceptionally. In control muscles, the intensity of staining for glycogen was higher in small fibres (Fig. 5a). The glycogen content was measured about 2 h after a period of contractions, so it is possible that the large fibres were still depleted of glycogen as a result of the previous contractions while glycogen was not depleted from the small fibres, or that the resynthesis of glycogen in the smaller fibres was considerably faster.

Uptake and Release of Free Fatty Acids and Changes in Fatty Acid Activating Enzyme

The activity of fatty acid activating enzyme, palmityl-CoA-synthetase was higher in muscles (TA + EDL) stimulated for 28 days than in control muscles, by 59 ± 12%. There was slightly higher increase in EDL (73 ± 21%) than in TA (45 ± 10%). The uptake of FFA was not different in muscles at rest or during contractions up to 14 days. In muscles stimulated for 28 days, uptake of FFA during contractions was significantly greater than in control muscles, and uptake was detectable after only 2 min of contractions (Fig. 4).

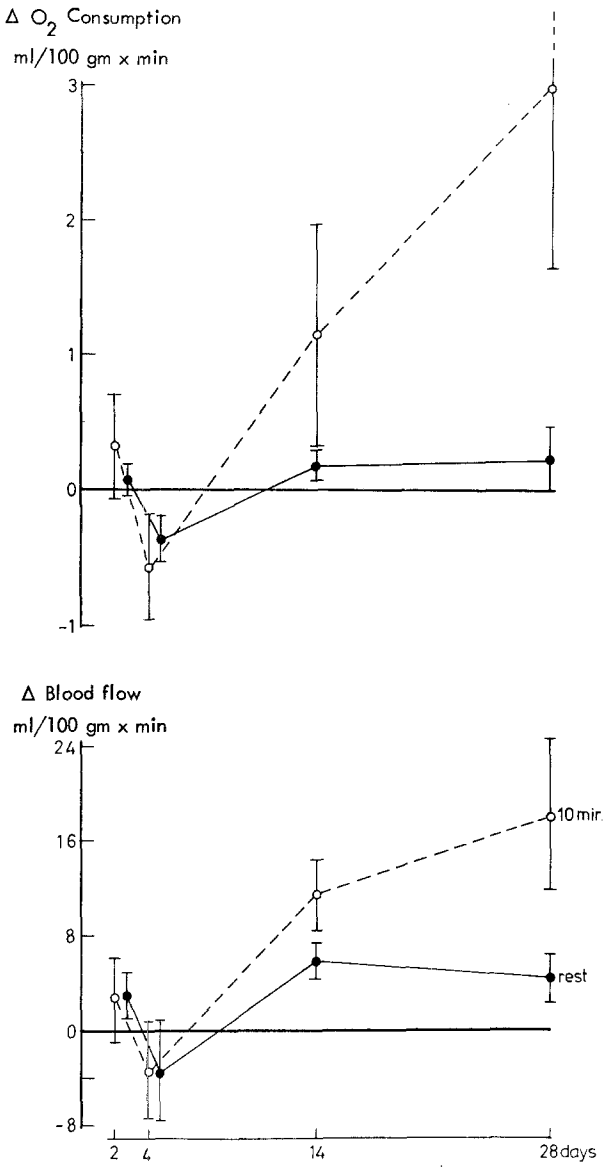


Fig. 3. Differences in blood flow (bottom) and oxygen consumption (top) between chronically stimulated muscles and contralateral control muscles after different periods of chronic stimulation (days on the abscissa). Solid line—values at rest. Broken lines—values at 10 min after the beginning of isometric contractions. Each point represents a mean (± S.E.) of 4 experiments of the differences in blood flow or oxygen consumption between the chronically stimulated and control muscles measured in each animal

DISCUSSION

The present results show that stimulating fast muscles at a frequency normally occurring in nerves to slow muscles produces changes in blood flow and fatigability, as well as metabolism. When the time-course of the development of these changes was compared, the only alteration found during the first few days of stimulation was a higher content of glycogen, higher capillary density (Brown et al., 1976) and slightly slower speed of contraction (Pette et al., 1973; Brown et al., 1976). Nevertheless even at this early stage the stimulated muscles fatigued less than the control muscles.

There are several possible explanations of the reduced fatiguability at this time. It has often been reported that muscle performance is related to the glycogen content of the respective muscles and that increased glycogen levels improve muscle performance (Ahlborg et al., 1967; Bergström et al., 1967). It is possible that in the stimulated muscle the activity of glycogen synthetase is higher than in the unstimulated control. The stimulated muscles were slightly slower than the control ones, and it is known that glycogen synthetase activity increases during muscle contraction more in slow than in fast muscles (Stubbs and Blanchaer, 1965). However, whether in the present experiments glycogen levels in the stimulated muscles were increased at the time when the fatiguability of the muscles was tested is uncertain, since the glycogen content of the experimental and control muscles was measured 2 h after the muscles were activated, at a time when it is known that even in normal muscles glycogen synthesis is very rapid (Yampolskaya, 1950).

A contribution to the greater resistance to fatigue could be made by the increase in capillary density that is known to take place as early as 4 days after the beginning of chronic stimulation (Brown et al., 1976). It was suggested by Schroeder (1972) that more homogenously distributed blood flow in trained muscles ensures better performance even if the total blood flow is not increased, probably due to higher pO_2 in trained muscles (Schroeder et al., 1976). It is thus possible that even in the absence of any change in total blood flow, an increase in capillary density would favour a more homogenous distribution of blood, and shortening of the diffusion pathway for oxygen. Thus a larger number of muscle fibres would have access to oxygen, and this could facilitate rephosphorylation of ATP and creatine phosphate. The latter has indeed been found to take place (Cooper and Hudlická, 1976). That the main contribution to the reduced fatiguability is really made by an increased capillary density was also brought out in experiments where fatiguability of stimulated and control muscles was compared in vivo and vitro. Whereas stimulated muscles always showed less fatigue than control muscles in vivo, there was no difference in vitro (Cooper and Hudlická, 1976).

After longer periods of stimulation the muscle and its vascular bed became even better adjusted to activity. Blood flow and oxygen consumption were already slightly increased at rest—more so in muscles stimulated for longer periods of time. During muscle

Table 1. Glucose consumption and lactate output in control and chronically stimulated muscles at rest and at 10 min after the beginning of isometric contractions at 4 Hz

Days of stimulation	Glucose consumption (mg/100 g × min)				Lactate output (mg/100 g × min)			
	rest		10 min contraction		rest		10 min contraction	
	control	stimulated	control	stimulated	control	stimulated	control	stimulated
2 $n = 3$	1.60 ± 0.52	3.27 ± 0.79	4.96 ± 2.37	2.18 ± 0.62	0.46 ± 0.14	0.49 ± 0.23	9.09 ± 0.24	7.91 ± 3.48
4 $n = 7$	2.03 ± 0.765	1.813 ± 0.995	4.65 ± 0.94	5.75 ± 1.64	1.48 ± 1.10	2.04 ± 0.70	10.48 ± 3.72	10.95 ± 4.46
14 $n = 4$	1.11 ± 1.09		4.44 ± 3.36	0.78 ± 1.71	1.31 ± 0.83	1.90 ± 0.73	11.28 ± 2.95	11.74 ± 2.59
28 $n = 4$	2.34 ± 0.86		7.40 ± 2.73	4.91 ± 2.54	2.89 ± 1.39	1.45 ± 0.65	14.31 ± 2.24	6.70 ± 3.93

contractions, the increase in blood flow and oxygen consumption increased considerably more than in control muscles due to a considerably decreased resistance (Allum et al., 1974) and fatty acids were taken up, and the output of lactate was smaller.

It was shown previously that the activity of some oxidative enzymes is considerably increased in muscles stimulated for a longer period of time, and the activity of glycolytic enzymes considerably decreased (Pette et al., 1973). Since the glycogen levels were higher this could indicate a considerably smaller breakdown of glycogen. The reduced output of lactate would be in agreement with this conclusion, although it is possible that lactate is more readily oxidized than in control muscles. In normal mixed muscles, only about 5% of lactate is oxidized during contraction (Corsi et al., 1971). It was suggested that fibres with high aerobic capacity might take up lactate particularly during prolonged exercise, and oxidize it since they have LDH isozymes similar to those found in heart muscle (Essén et al., 1975). During chronic stimulation the proportion of such fibres with LDH isozymes more similar to those of heart muscle increases and it can therefore be assumed that more lactate is taken up and subsequently oxidized.

Glucose consumption did not change noticeably, in spite of the high activity of hexokinase (Pette et al., 1973) and high levels of glycogen. It is possible that a greater proportion of glucose was used for glycogen synthesis and less converted to lactate than in normal muscles where about 33% of the total glucose consumed is metabolised to lactate (McGilvery, 1975).

All these changes together with the increase in the activity of fatty acid activating enzyme indicate that there is a shift from the predominantly anaerobic breakdown of glycogen in normal fast muscles during contractions towards oxidative metabolism and utilization of fats which has been shown to occur both in slow muscles (Baldwin and Tipton, 1972; Hudlická, 1975) and in muscles subjected to endurance training (Holloszy, 1967; Holloszy et al., 1975; Molé et al., 1971). This shift towards aerobic metabolism could be largely responsible for the increased resistance to fatigue in muscles stimulated for several weeks, as has also been suggested by Pette et al. (1976).

The changes described in long-term stimulated muscles are, however, somewhat different from the changes in muscle metabolism during training. In this situation, the activity of oxidative enzymes increases (see Holloszy et al., 1975), but the capillary density/area remains the same while the muscle fibres hypertrophy (Hermansen and Wachtlová, 1971). Thus the diffusion distance for oxygen is increased. In stimulated muscles not only is the capillary density doubled after 28 days but the fibre diameters are reduced (Brown et al., 1976). So, in the latter case the diffusion distance is very much reduced; the muscle can therefore take full advantage of the large increase in blood flow during muscle contraction, and utilise the increase in the activity of oxidative enzymes more efficiently.

It was shown previously, that after 28 days of chronic stimulation of fast muscles at a frequency naturally occurring in nerves to slow muscles the contraction times were considerably prolonged and the transformation of the metabolic pattern and capillary density towards slow muscles was almost complete (Pette et al., 1973; Brown et al., 1976). The fast muscles came to resemble slow muscles in many respects. The present results show that these stimulated muscles are in many ways very different from slow mammalian muscles, at least at rest. The blood flow at rest was only very slightly higher even after 28 days of stimulation, and it considerably increased during contractions – unlike the flow in slow muscles (Hilton et al., 1970). Oxygen and glucose consumption at rest was much less than in slow muscles, and the increase in oxygen consumption during muscle con-

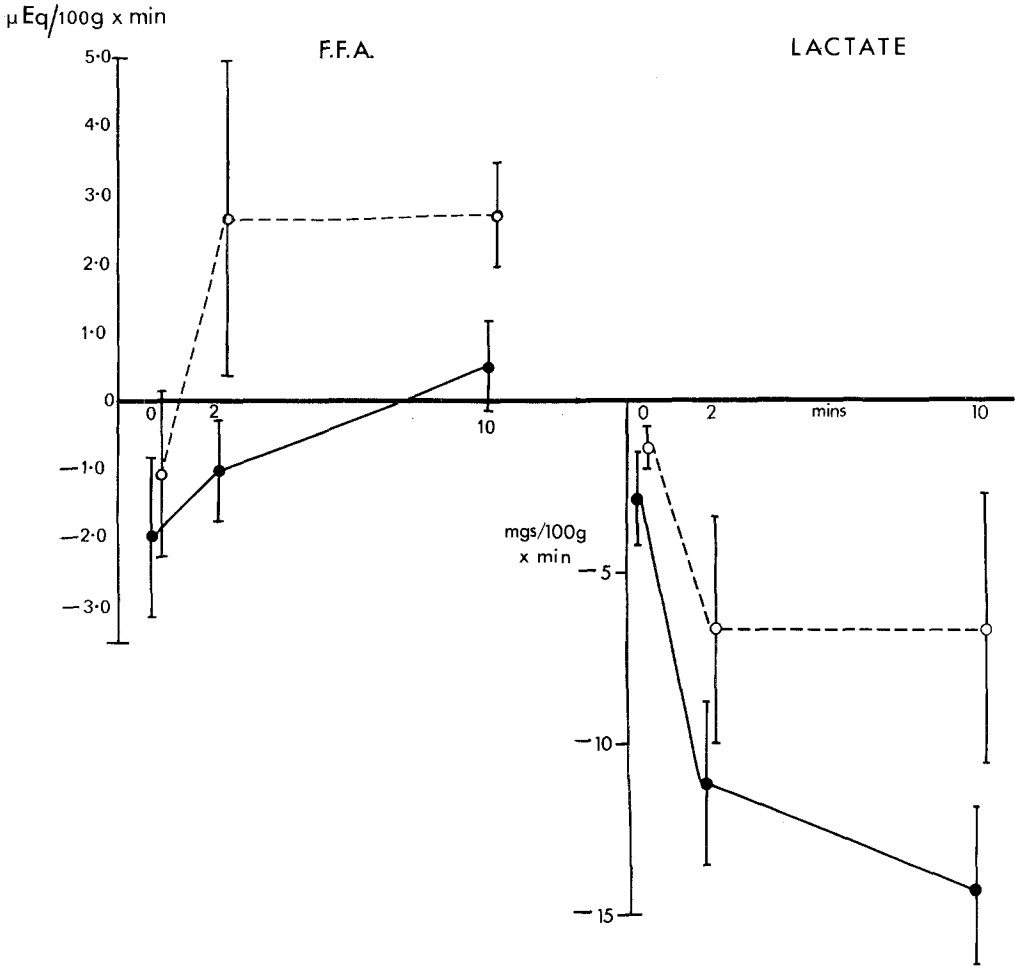
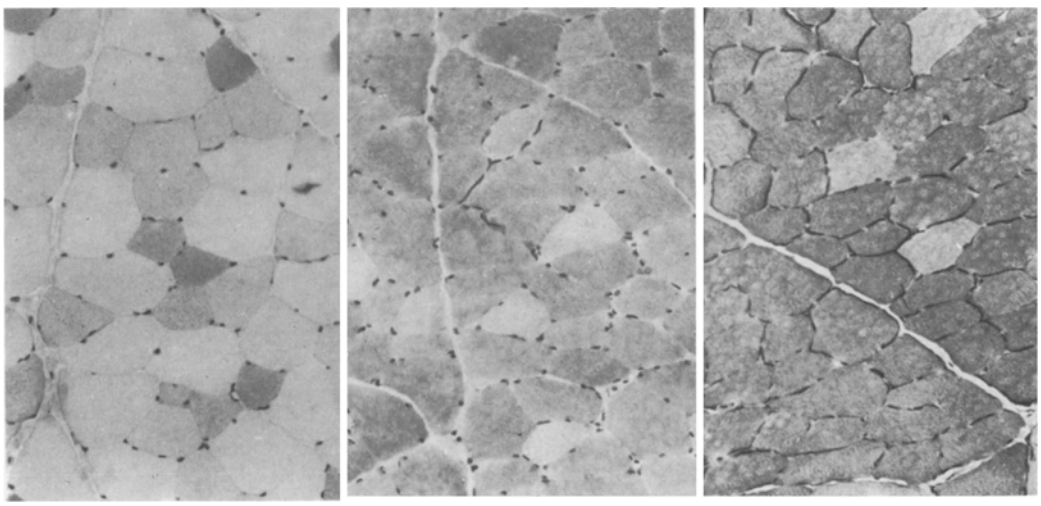


Fig. 4. Uptake of free fatty acids (left side) and release of lactate (right side) from muscles chronically stimulated for 28 days (broken lines) and control muscles (solid lines). Time after the beginning of isometric contractions (rest = time 0) at 4 Hz on the abscissa. Samples were taken at 2 and 10 min after the beginning of contractions. On the ordinate release (negative values) or uptake (positive values) of FFA (μEq/100 g × min) and lactic acid (mg/100 g × min)



a b c

Fig. 5 a–c. Transverse sections of rabbit EDL muscles stained for PAS and counterstained with haematoxylin. Darkly stained fibres indicate a high glycogen content. (a) Section of control EDL muscle showing a substantial depletion of glycogen. (b) Section from EDL muscle stimulated for 2 days and (c) 28 days

traction was much greater (Hudlická, 1975). The levels of glycogen were high, unlike those in slow muscles (Kugelberg and Edstrom, 1968). Output of lactate, and uptake of FFA, on the other hand, were similar to values found in slow muscles (Hudlická, 1975; Beatty et al., 1963).

During contraction, however, the consumption of oxygen in muscles stimulated for 28 days almost reached levels found in contracting slow muscles, and the blood flow was comparable with that in slow muscles at rest. It is therefore possible that the chronically stimulated muscles could utilize the full capacity of the enlarged vascular bed when contracting, but that, under resting conditions, their metabolic turnover was not very much increased and a large part of the newly-formed capillaries was not perfused. It would be interesting to find out whether considerably longer periods of chronic stimulation would eventually result in a more complete change towards the features characteristic of slow muscles.

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