JYVÄSKYLÄN YLIOPISTO
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RESPONSE OF THE LYSOSOMAL SYSTEM OF SKELETAL MUSCLE TO EXERCISE

VEIKKO VIHKO

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V. Veikko Vihko, Antero Salminen, and Jari Rantamäki, Oxidative and lysosomal capacity of mouse skeletal muscle after endurance training of different intensities. *Acta physiol. Scand.* 104, 74-81, 1978.
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VI. Veikko Vihko, Antero Salminen, and Jari Rantamäki, Exhaustive exercise, endurance training and acid hydrolase activity in skeletal muscle. *J. appl. Physiol.* (in print), 1979.
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INTRODUCTION

Experimental variation of the contractile activity of skeletal muscles offers a means of inducing and studying cellular adaptation. The capacity of muscle fibres to increase their energy production by a factor of over one hundred times compared to the state of relative rest, as well as the absence of cell division after maturation make them well suited for such studies.

Several treatments have been used to modify the functional activity of muscle fibres. These include a period of exhaustive exercise (loading) and regular series of exercise periods constituting a particular programme (training) intended, for instance, to increase the endurance capacity of muscles.

Even a single moderate exercise period of an endurance training programme affects the homeostasis of exercised fibres and evidently causes a specific induction of protein synthesis, since the mitochondrial capacity of muscle fibres increases. Acute exhaustive physical exercise may disturb the homeostasis of muscle fibres to such an extent that they may undergo necrosis. A muscle fibre which has completely lost its homeostatic ability is lethally injured. Cell injury may also be sublethal or reversible, in which case the homeostatic mechanisms of the cell are first altered, and a new cellular steady state is achieved after certain recovery and repair processes. The new steady state may or may not be the same as the original. It is not known whether exhaustive exercise also causes sublethal fibre injuries in addition to known fibre necrosis.

The lysosomal system is known to participate in both lethal irreversible and sublethal reversible cell injuries in several tissues. Strong activation of the skeletal muscle lysosomal system is a common feature of a variety of

pathological conditions characterized by increased degradation. It is not known whether the lysosomal system participates in the adaptation of muscle fibres to increased physical activity.

The aim of the present investigations was to study the role of the lysosomal system of skeletal muscle in connection with increased physical activity. Two physical exercise treatments with mice were selected: an acute, relatively short-lasting period of exhaustive running and a period of endurance training. The phenomena brought about by exhaustive physical loading in fatigued muscle on the one hand, and the enzymatic and organelle adaptations occurring in endurance-trained muscle on the other, justified the postulation of a hypothesis that the lysosomal system of skeletal muscle participates in exercise-induced adaptation of muscle fibres.

Lysosomal system of skeletal muscle

Acid hydrolases and their distribution in skeletal muscle. Lysosomes are sedimentable intracellular particles composed of a single membrane and a matrix containing hydrolytic enzymes (de Duve and Wattiaux 1966). The pH optima of these hydrolases are generally in the acid range (pH 4-6). Their activity is normally latent and full activity is released when the lysosomal membrane is broken. In addition to the sedimentable activity, the supernatant of skeletal muscle homogenates also contains some acid hydrolase activity (Canonico and Bird 1970, Pollack and Bird 1968, Stauber and Schottelius 1975). This activity most probably results from the disruption of lysosomal membranes during homogenization. In cell fractionation studies a proportion of the total activity of certain acid hydrolases is distributed like the activity of marker enzymes of the sarcoplasmic reticulum (Stauber and Bird 1974).

The lysosomes of most cells possess a common but diverse array of enzymes. About 60 lysosomal enzymes are known (Dean and Barrett 1976). They include several proteinases, glycosidases, nucleases, phosphatases, lipases, and sulphatases. Lysosomal hydrolases evidently build up a hydrolytic entity, which is in general capable of degrading proteins to small peptides and amino acids, carbohydrate moieties from *e.g.* glycoproteins or glycolipids to monosaccharides, nucleic acids to nucleotides, complex lipids to *e.g.* fatty acids and phosphates, and of removing inorganic phosphate from other materials, such as phosphoproteins (Barrett 1972, Dean and Barrett 1976, Tappel 1969).

The specific activity of acid hydrolases in skeletal muscle is normally low when compared to *e.g.* liver (Bird 1975, Weinstock and Iodice 1969). Particles showing latent acid hydrolase activity have been sedimented from skeletal muscle homogenates (Canonico and Bird 1970, Pollack and Bird 1968). Biochemical acid hydrolase activity in muscle homogenates originates from at least two populations of lysosomes, one from muscle fibres and the other from connective tissue including macrophages (Canonico and Bird 1969, 1970). In addition to this bimodal distribution, possible changes in cell populations (by *e.g.* mononuclear cell infiltration or fibroblast proliferation) evidently affect the total activities in skeletal muscle homogenates.

The proportion of the total sedimentable activity accounted for by a particular specific enzymatic activity varies between subfractions originating from muscle fibres and from non-muscle cells (Canonico and Bird 1970). Recent histochemical studies have confirmed the occurrence of acid hydrolase activity both inside muscle fibres and in interstitial tissue (Gutmann *et al.* 1976, Lojda and Gutmann 1976, Shannon *et al.* 1974). Highly oxidative red fibres, both slow and fast twitch, contain more activity than the less oxidative white fibres (Gutmann *et al.* 1976, Lojda and

Gutmann 1976, Peter *et al.* 1972).

Skeletal muscle lysosomes and activation of the lysosomal system. Lysosomes are seldom observed in normal skeletal muscle (Bird 1975, Schiaffino and Hanzlikova 1972), and a clearly differentiated lysosomal system comparable to that occurring in most other cells has not been described in muscle. The sparse lysosomes, containing acid phosphatase and arylsulphatase activities (Gordon *et al.* 1967, Schiaffino and Hanzlikova 1972) are usually located in the neighbourhood of nuclei, as are also the Golgi apparatuses (Price 1973). Acid phosphatase and ß-glucuronidase activity may also be located in the sarcoplasmic reticulum, as has been shown by fractionation and electron microscopic methods (Bird 1975, Canonico and Bird 1970, Seiden 1973, Stauber and Bird 1974, Weglicki *et al.* 1975). In skeletal muscle the lysosome-like particles and acid hydrolase activities generally constitute an integral part of the sarcotubular system. The term sarcotubular-lysosomal system has been suggested by Pearse (1965).

Increased cellular acid hydrolase activity is a sign of the activation of the lysosomal system. In addition to several dystrophic muscle diseases (Bird 1975, Christie and Stoward 1977, Kar and Pearson 1972a, Weinstock and Iodice 1969), the activities are high in connection with experimental atrophies, *e.g.* during denervation (Maskrey *et al.* 1977, Pollack and Bird 1968, Schiaffino and Hanzlikova 1972, Stauber and Schottelius 1975), tenotomy (Pollack and Bird 1968), immobilization (Max *et al.* 1971), starvation (Bird 1975, Canonico and Bird 1970, Stauber *et al.* 1977), and some other nutritional deficiences (Korovkin and Budnyakov 1973, Ruth and Van Vleet 1974, Weinstock and Iodice 1969) and in connection with aging (Gutmann *et al.* 1976, Pilström *et al.* 1978, Yohotsky-Gore and Pathmanathan 1968). Activities also increase during acute and chronic hypoxiae (Arcangeli *et al.* 1973a, b, Digiesi *et al.* 1975, Shannon and Courtice 1975, Shannon *et al.* 1974) and during prolonged physical activity

(Pilström *et al.* 1978, Vihko *et al.* 1974).

Increased acid hydrolase activities and, generally, the activation of the lysosomal system are accompanied by the appearance of primary lysosomes, mostly in the perinuclear area in the close neighbourhood of the Golgi apparatuses but also between myofibrils (Christie and Stoward 1977, Hanzlikova and Gutmann 1975, Hanzlikova and Schiaffino 1973, Manolov and Ovtscharoff 1974, Pellegrino and Franzini 1963, Price 1973, Schiaffino and Hanzlikova 1972). The size and number of the Golgi bodies also increase. During or after the appearance of primary lysosomes, autophagic vacuoles are observed in the perinuclear area of the muscle cells (Christie and Stoward 1977, Hanzlikova and Gutmann 1975, Manolov and Ovtscharoff 1974, Price 1973, Schiaffino and Hanzlikova 1972, Seiden 1973). These vacuoles contain mitochrondria, membrane structures, ribosomes, and glycogen. Lysosomes are in particular abundant in the mitochondria-rich red fibres (Christie and Stoward 1977).

The activation of the lysosomal system often occurs in conjunction with increased muscle breakdown. Protein degradation increases in dystrophies, denervation, immobilization and starvation (Goldspink 1976, 1977, Goldspink and Goldspink 1977, Millward *et al.* 1976). Fibres also contain neutral and alkaline proteases, the activities of which also increase during enhanced breakdown of skeletal muscle, as do the activities of acid proteases (Kar and Pearson 1972b, Noguchi *et al.* 1974, Mayer and Shafrir 1977). Neutral and alkaline proteases probably hydrolyze myofibrils (Mayer and Shafrir 1977, Reddy *et al.* 1975).

Acute exhaustive exercise

Energy consumption and fibre recruitment. Insufficient energy production and the accumulation of acid metabolites in muscle tissue are some reasons for fatigue during muscular

work. The fibre type profile of the muscles involved together with the type, duration, and intensity of the exercise undergone influence the onset of exhaustion during loading.

In rodents it is practical to divide the fibres into three types: slow twitch - oxidative, fast twitch - oxidative - glycolytic, and fast twitch - glycolytic types (Armstrong *et al.* 1974, Barnard *et al.* 1971). In muscle tissue the different fibre types build up motor units, each of which is composed of only one fibre type (Granit 1970).

At low work intensity there seems to be a primary reliance upon oxidative motor units, both slow and fast twitch, for contractile activity (Armstrong *et al.* 1974, Baldwin *et al.* 1973, 1975). Free fatty acids together with a minor proportion of glucose are oxidized to produce energy for contractions. Such light or moderate exercises can be maintained for prolonged periods of time.

Major use of anaerobic fast twitch - glycolytic fibres occurs at high work intensity or after the depletion of glycogen from oxidative fibres during long-lasting low-intensity work (Armstrong *et al.* 1974, Gollnick *et al.* 1973). With increasing work resistance the proportion of fat oxidation decreases and that of carbohydrates increases (Keul *et al.* 1972). Maximal dynamic or static effort depletes the energy supplies of the muscle within a very short time. ATP synthesis occurs *via* anaerobic glycolysis and the work involves fast twitch - glycolytic fibres (Armstrong *et al.* 1974, Terjung 1976). During maximal exercises lactate accumulation causes the cellular pH to decrease and finally, probably as a regulatory mechanism, inhibits phosphofructokinase (Keul *et al.* 1972).

A loading programme composed of intermittent periods of aerobic and anaerobic energy production and causing the re-

cruitment of both oxidative and glycolytic motor units might provide a very effective means of producing skeletal muscle exhaustion, because when prolonged it would evidently result in the depletion of glycogen stores in all three fibre types.

Fibre homeostasis and recovery. The energy homeostasis of a muscle is greatly upset during strenuous physical exercise. However, at the moment of exhaustion the energy state is not similar in all fibre types, not even in those belonging to the same fibre type. When muscle contractile activity terminates, phosphagen stores are rapidly replenished and ATP is consumed for restoration of cellular homeostasis.

Heavy physical exercise may cause immediate swelling of mitochondria (Gollnick and King 1969, Vihko and Arstila 1974, *e.f.* Bowers *et al.* 1974, Gale 1974, Terjung *et al.* 1972), as also does ischaemia (Mäkitie and Teräväinen 1977b, Trump *et al.* 1976). Mitochondria also accumulate calcium during exhaustive exercise (Sembrowich and Gollnick 1976). Calcium may activate mitochondrial phospholipase (Majewska *et al.* 1977), which could disintegrate membrane phospholipids and thus increase the permeability of mitochondria especially during hypoxia (Majewska *et al.* 1977, Trump *et al.* 1976).

The functional deficiences and structural changes of mitochondria probably lead to a diminished capacity to produce ATP *via* oxidative phosphorylation and thus cause the accumulation of lactate. In the succeeding phase ion equilibria and cell homeostasis in general are disturbed. Changes are to some extent reversible but at a certain point the capacity of the cell to recover is lost and the cell dies (Arstila *et al.* 1974, Helminen 1975, Trump *et al.* 1976). The mechanism(s) of lethal or sublethal muscle fibre injuries are not known. Disturbances in calcium homeostasis, brought about by the lack of energy, have been suggested to

damage fibres irreversibely (Wrogeman and Pena 1976, Yarom 1976).

Exhaustive exercise - either brief intensive or prolonged moderate - may damage some muscle fibres so that their homeostasis is completely lost and the fibres undergo necrosis (Altland and Highman 1961, Hecht *et al.* 1975, Highman and Altland 1963, Schuman 1972, Van Linge 1962). Muscle has the potential to repair these injuries. Degeneration may still continue in some necrotic fibres while other fibres are simultaneously regenerating (Mäkitie and Teräväinen 1977a, Reznik 1973, Snow 1977). Myoblasts fuse with each other to constitute a regenerated fibre (Reznik 1973).

During recovery after exhaustive exercise the increasing oxygen tension reintroduces oxidative phosphorylation and ion equilibria. Decreased phosphorylase and phosphofructokinase activities (Boström *et al.* 1974), and depleted glycogen stores return to their initial levels within 24 hours (Pihl 1974). The activities of enzymes of the pentose phosphate shunt, and muscle RNA content, increase after exhaustive exercise (Boström *et al.* 1974), suggesting increased nucleic acid synthesis.

The physiological states of skeletal muscle during periods following exhaustive exercise and experimental ischaemia greatly resemble each other. After a period of temporary ischaemia, protein synthesis increases as do also the activities of the pentose phosphate shunt enzymes (Arcangeli *et al.* 1973b). Ischaemia and exhaustive loading bring about similar disturbances in the energy metabolism of muscles (Haljamäe and Enger 1975, Keul *et al.* 1972). Histopathological and enzymatic manifestations also resemble each other (Altland and Highman 1961, Arcangeli *et al.* 1973a, b, Boström *et al.* 1974, Mäkitie and Teräväinen 1977a, Shannon *et al.* 1974). After transient ischaemia the skeletal muscle lysosomal system is activated along with increase in acid

hydrolase activities (Arcangeli *et al.* 1973a, b, Digiesi *et al.* 1975, Shannon and Courtice 1975, Shannon *et al.* 1974).

Endurance training

Effects on energy metabolism. Physical training brings about biochemical adaptations in skeletal muscle. In the case of endurance training (*i.e.* exercise of low or moderate intensity repeated over long periods) these adaptations include increased muscle myoglobin content (Lawrie 1953) and an improved capacity of muscle to oxidize carbohydrates, fatty acids and ketones (Baldwin *et al.* 1973, Beatty *et al.* 1972, Mole and Holloszy 1970, Salminen *et al.* 1977, Winder *et al.* 1973). Underlying these improvements is an increased level of mitochondrial capacity. The volume fraction, number and size of mitochondria is increased (Hoppeler *et al.* 1973, Kiessling *et al.* 1973, 1974, Vihko *et al.* 1975), as are the contents of mitochondrial protein (Holloszy 1967), lipids (Morgan *et al.* 1971) or cytochrome c (Barnard and Peter 1971, Oscai and Holloszy 1971), in endurance-trained skeletal muscle. Along with the increase in the components of the respiratory chain, the activities of mitochrondrial ATPase and enzymes involved in the oxidation of NADH and succinate increase considerably (Gollnick *et al.* 1972, Holloszy 1967, Oscai and Holloszy 1971). The levels of the activities of mitochondrial citric acid cycle enzymes increase similarly (Baldwin *et al.* 1972, Dohm *et al.* 1972, Holloszy *et al.* 1970, Salminen *et al.* 1977), as do those of enzymes involved in the oxidation of fatty acids and ketones (Mole *et al.* 1971, Winder *et al.* 1974). The mitochondrial and cytoplasmic activities of enzymes of the malate-aspartate shuttle also increase (Holloszy and Oscai 1969, Mole *et al.* 1973). Endurance training does not change fibre size or the total protein content of muscle (Holloszy and Booth 1976).

In contrast to the remarkable adaptive increases in mitochondrial capacity the responses, if any, of glycolytic

enzymes are small, except for an increase in hexokinase activity (Baldwin *et al.* 1973, Lamb *et al.* 1969, Peter *et al.* 1968). The degree to which adaptive responses occur depends on the type, duration and intensity of physical activity applied and on the fibre type composition of the muscles involved during a particular exercise. Mitochondrial enzymes increase in all three (slow - oxidative, fast - oxidative - glygolytic, and fast - glycolytic) fibre types, but the fibre type profile does not change (Baldwin *et al.* 1972, Holloszy 1967, Kiessling *et al.* 1975) although fibres evidently possess the potential for conversion from one type to another (Romanul 1971, Romanul and Van Der Meulen 1967). Furthermore, as for example in the case of glycolytic enzymes in mixed skeletal muscle, training-induced decreases in fast - oxidative - glycolytic fibres are obscured by increased levels in fast - glycolytic fibres (Baldwin *et al.* 1973, Holloszy and Booth 1976).

Summarizing, endurance training brings about adaptations in the energy metabolism of exercised skeletal muscles. These adaptations are directed mainly towards the aerobic component of energy production, and are dependent on both fibre type profile and the type of training involved.

Effects on the lysosomal system. The function and role of the skeletal muscle lysosomal system in relation to physical exercise has evoked only minor research interest. It is, however, known from previous studies that the activities of ß-glucuronidase (Vihko *et al.* 1974) and ß-N-acetylglucosaminidase (Pilström *et al.* 1978) increase in mouse skeletal muscle after programmes of running exercise executed daily of several times *per* week for observation periods ranging from one to three and half months.

AIM OF THIS STUDY

The aim of the present study was to evaluate the role and importance of the lysosomal system of skeletal muscle in relation to physical exercise. The functional state of the lysosomal system was estimated quantitatively by assaying the total activities of a number of acid hydrolases and qualitatively by histochemical staining of suitable marker enzymes. Brief exhaustive loading and/or moderate regular endurance training were the exercise types of physical activity applied. The experiments were designed to suggest answers to the following questions:

(1) What are the levels of the activities of representative acid hydrolases in mouse skeletal muscle, distinguishing between the activities in predominantly white and predominantly red muscle types (I,II,IV,V, VI)?

(2) What is the distribution of representative acid hydrolases between muscle fibres and interstitial structures of muscle tissue (III,VI)?

(3) How does a single period of vigorous, exhausting exercise affect the activity of selected acid hydrolases in untrained skeletal muscle (I,III,IV)?

(4) Is the activity of selected acid hydrolases affected by regular moderate endurance training (I,II, V,VI)?

(5) What are the influences of a single period of exhaustive exercise on the activities of representative acid hydrolases in previously endurance-trained skeletal muscle (I,VI)?

MATERIALS AND METHODS

Animal care

The mouse was selected to serve as experimental animal because its size permitted simultaneous training in reasonably large numbers. Young male NMRI mice from our own laboratory (I,III-VI) or mice of strain B6D2F1/BOM, from G.L. Bomholtgård (Ry, Denmark) (II), were used. The animals were housed in Danish Scanbur Type IV cages with 8-10 mice to a cage. Food pellets (Hankkija, Finland (I,II) or R3, Astra Ewos, Sweden (III-VI)) and tap water were available *ad lib*. Cage temperature (21-22°C) and relative humidity (40 %) were kept constant. The cage-day was artificially divided into a 12 h on-off rhythm with one of the changeover points at 6-8 a.m.

Exhaustive exercise and endurance training

Mice were exhausted and trained by running on a motor-driven treadmill which consisted of an endless belt rolling on two cylinders. Running took place on four separate tracks (length 100 cm, width 30 cm) with 2-8 animals on each. When necessary running was induced by slight electric shocks from electrodes located in the escape gates of each of the four tracks. This motivation was needed only when animals were being taught to run on the treadmill. Speed and track elevation were adjustable.

An intermittent type of running programme was used to exhaust the animals. Exhausting exercise was started at a slow initial speed (18 m/min) intended to familiarize the animals with running on the treadmill. After 30 min the speed was increased stepwise three times (25, 28, and 31 m/min), for a period of 5 min at each time, and then again decreased for 5-10 min to the initial level (18 m/min). The same speed-increase programme was repeated five times. Mice

showed large individual variation in their capacity to complete the programme. The weakest runners were usually exhausted after 90-100 min running and the strongest after 140-150 min. In the case of previously trained animals the running speeds used were remarkably higher (28, 31, 36, and 42 m/min) than with previously untrained mice. The timing of speed changes, however, was similar. The definition of exhaustion was made on the basis of the behaviour of the animal on the running track and later in the cage. An exhausted mouse, when placed on its back, failed to return instantly to its feet and moved very little for some minutes.

The details of exhaustive loadings are given in III, IV, and VI and those of each training programme in I, II, V, and VI, and in Table 3.

Muscle samples and tissue preparation

In preliminary experiments and in study I samples representing most thigh muscles were used in the enzyme assays (I, Vihko *et al.* 1974). In later studies anatomically separate muscles, *m. rectus femoris* (IV-V), or parts of predominantly red or predominatly white *m. quadriceps femoris* (II, IV, and VI) were analyzed. The predominantly white part of *m. quadriceps femoris* was composed of the distal head of *m. vastus lateralis* and the predominantly red sample from proximal heads of *m. vastus lateralis* and *m. vastus medialis* together with red fibres from *m. vastus intermedius*. The red and white muscle samples were not exclusively composed of red or of white fibres, but always included some fibres of the other type. For the preparation procedure, which is described in detail in II, IV, and VI, the samples were as purely red or white as possible.

Muscle samples for biochemical studies were rapidly prepared and weighed, cut into smaller pieces with scissors and homogenized in ice-cold buffer (150 mM KCl, 50 mM $KHCO_3$,

6 mM EDTA, pH 7.4, in IV, V, and VI; 1 M Tris-HCl, pH 7.5 in II, or distilled water in I). Preparative steps were performed at 0-4°C. The homogenates were diluted usually to 3 % (w/v) and stored at -20°C until analyzed (within 2 weeks). In order to confirm the assay of total activities of acid hydrolases, the buffered homogenates were made 0.1 % in respect to Triton X-100 before assay. More details of preparative steps are given in I, II, and IV-VI.

In histological and histochemical studies (III, V, and VI) the contralateral leg than that used in quantitative determinations was used. *M. rectus femoris* was separated and cut into two parts. The proximal portion was embedded on a specimen holder for longitudinal cryostate sectioning and the distal part likewise for transverse sectioning. Samples were frozen in isopentane prechilled with liquid nitrogen. Specimens from *m. vastus lateralis* and from *m. vastus intermedius* were used in study III in addition to *m. rectus femoris*. Drying of samples was prevented and the blocks were kept at -20°C until sectioned and stained (within 1-6 days).

Biochemical methods

The number of main variables, *i.e.* acid hydrolase activities, was enlarged during the study (Table 1) in order to obtain a good representation of the acid hydrolytic capacity of the lysosomal system of skeletal muscle. The activities of certain energy metabolism enzymes were selected to serve as background variables (Table 2). These reference activities were used (1) to characterize the metabolic properties of the muscle samples under study, (2) to relate, approximately, the changes observed in main variables to adaptations in energy metabolism, and (3) to compare quantitatively the effects of different training programmes, and earlier results obtained with other animal species, with the present results.

Table 1

List of acid hydrolase activities used as main variables in the separate studies together with selected data as to assay conditions and natural substrates of enzymes

Main variable (*)	E.C. number	pH	Experimental substrate	Major natural substrates	Paper
ß-Glucuronidase	3.2.1.31	4.2	p-nitrophenylglucuronide (Sigma)	polysaccharides, mucopoly-saccharides, steroid glucur-onides	I-VI
ß-Acetylglucosaminidase	3.2.1.30	4.8	p-nitrophenyl-N-acetyl-glucosamide (Sigma)	ß-N-acetylhesoxaminides in glycoproteins and glycolipids	I-VI
Arylsulphatase	3.1.6.1	5.0	nitrocatecholsulphate (Sigma)	arylsulphates, cerebrosides, condroitin sulphate	IV-VI
Acid ribonuclease	2.7.7.16	5.0	dialyzed RNA (Fluka)	RNA	IV-VI
Acid deoxyribonuclease	3.1.4.5	5.0	salmon DNA (Sigma)	DNA	VI
Cathepsin D	3.4.23.5	3.6	urea-denaturated hemo-globin (Riedel)	proteins	II, IV-VI
Cathepsin C	3.4.14.1	6.0	glycyl-L-phenylalanine ß-naphtylamide (Sigma)	peptides	V,VI
p-Nitrophenylphosphatase (acid phosphatase)	3.1.3.2	5.0	p-nitrophenylphosphate (Sigma)	most ortophosphoric mono-esters	I,II, IV-VI
ß-Glycerophosphatase (acid phosphatase)	3.1.3.2	5.0	ß-glycerophosphate (Sigma)	most ortophosphoric mono-esters	IV

*) assays performed esscntially as described by Barrett (1972)

Table 2

List of enzyme activities used as reference variables in the separate studies together with references to the methods employed

Reference variables	E.C. number	Method	Paper
Malate dehydrogenase	1.1.1.37	Ochoa 1955	I,IV,V
Succinate dehydrogenase	1.3.99.1	Pennington 1961	I
Cytochrome c oxidase	1.9.3.1	Whereat *et al.* 1969	II,V
Citrate synthase	4.1.3.7	Srere 1969	V,VI
Lactate dehydrogenase	1.1.1.27	Kornberg 1955	II,V,VI
Creatine kinase	2.7.3.2	TC 15926/Biochimica Boehringer	II
Protein	--------	Lowry *et al.* 1951	I,II,IV-VI

The selected *main variables* were lysosomal acid hydrolase activities. The term "lysosomal capacity" is used interchangeably with "acid hydrolytic capacity" to mean the activity of all or some of the main variables. The selected *reference variables* included activities representing the aerobic and anaerobic capacities of skeletal muscle. The following activities were used as estimates of the oxidative capacity: malate dehydrogenase, succinate dehydrogenase, cytochrome c oxidase, and citrate synthase. The activities of lactate dehydrogenase and creatine kinase were used to estimate the anaerobic capacity of the muscle samples. Protein content was determined from all muscle samples.

Histochemical and histological methods

Serial cryostate sections were cut for the staining of NADH-tetrazolium reductase (NADH-diaphorase) (Novikoff *et al.* 1961) (III,V,VI), β-glucuronidase (Chayen *et al.* 1972) (III, V,VI), β-N-acetylglucosaminidase (Shannon 1975) (III), β-glycerophosphatase (Pearse 1972) (III), and arylsulphatase (Pearse 1973) (V) activities.

Acid hydrolase stainings were aimed at determining the localization and distribution of the enzymes. NADH-diaphorase staining was used to classify muscle cells as either red or white, *i.e.* as fibres having high or low oxidative capacity, respectively. Histological staining (hematoxylin-eosin) was also carried out on serial cryostate sections in order to identify degenerating and regenerating fibres. A fibre was classified as degenerating or necrotic if the staining was pale, no intracellular regular structure was observed and in many cases if the fibre contained mononuclear cells inside basal lamina. Fibres, usually small sized, with central nuclei and slight basophilia were classified as regenerating. The number of degenerating and regenerating fibres was counted per cross-sectional area of *m. rectus femoris*, which contained on average 2 200 fibres (III).

Hematoxylin-eosin staining was also used, together with β-glycerophosphatase staining, to estimate the strength of inflammatory reactions. Inflammation was inferred from the presence of a highly increased number of mononuclear cells occurring diffusively in the interstitial area and/or locally inside degenerating fibres.

Statistical methods

Standard procedures were used to calculate means and standard deviations. The significances of the differences between means were tested by Student's t-test. Correlations between enzyme activities (IV) were calculated by the least square method.

RESULTS

Acid hydrolase activity in normal skeletal muscle

Figure 1 summarizes and compares the activities of selected acid hydrolases and certain reference enzymes in the three representative muscle samples: in predominantly white and predominantly red parts of *m. quadriceps femoris* and in "mixed" *m. rectus femoris*.

The main results (I-VI) were in short:

(1) Acid hydrolase activities in crude skeletal muscle homogenates of mice varied from approximately 7 pmoles substrate consumed/min/mg fresh muscle (β-glucuronidase in white *m. quadriceps femoris*) to approximately 2 nmoles/min/mg fresh muscle (p-nitrophenylphosphatase in red *m. quadriceps femoris*).

(2) Activities were regularly somewhat (20-60 %) higher in the predominantly red than in the predominantly white skeletal muscle.

(3) The distribution and localization of histochemically examined (III,V,VI) acid hydrolase activities (β-glucuronidase, β-N-acetylaminidase, and arylsulphatase) were rather similar. Activity in control samples was usually slight. Activity granules were observed inside fibres and they were always more numerous in the highly oxidative red compared to the less oxidative white fibres. The number of activity granules in the perinuclear area of red fibres exceeded that in the interfibrillar zone. In white fibres β-glucuronidase and β-N-acetylglucosaminidase activities were distributed rather evenly. Arylsulphatase activity was typically observed in red fibres only. Its intracellular distribution was more random than that of

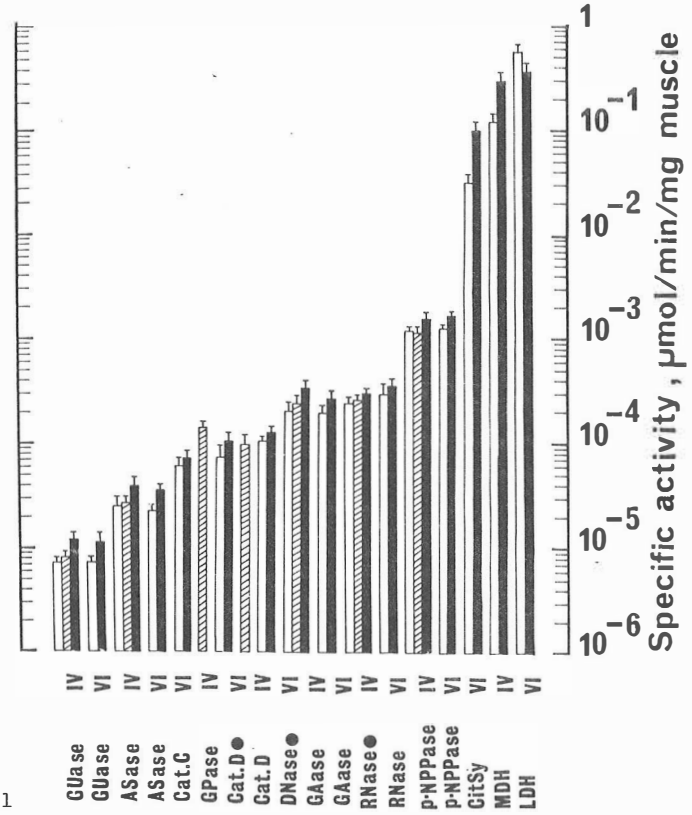

Figure 1

Comparison of activities of acid hydrolases studied and selected energy metabolism enzymes in predominantly white (white columns) and predominantly red (black columns) parts of *m. quadriceps femoris* and in "mixed" *m. rectus femoris* (hatched columns) of mice. Enzyme activities, given on the logarithmic scale, are expressed as specific activity (µmol substrate/min/mg fresh muscle) at 37°C for β-glucuronidase (GUase), arylsulphatase (ASase), β-N-acetylglucosaminidase (GAase), p-nitrophenylphosphatase (p-NPPase), cathepsin C (Cat. C), and β-glycerophosphatase (GPase), and at 25°C for citrate synthase (CitSy), malate dehydrogenase (MDH), and lactate dehydrogenase (LDH). (●): Cathepsin D (Cat. D) activity is given as µmoles tyrosine equivalents solubilized at 37°C/min/mg fresh muscle, and acid ribonuclease (RNase) and acid deoxyribonuclease (DNase) activities as µmoles respective nucleotides solubilized at 37°C/min/mg fresh muscle. Figure also compares data (controls) between studies IV and VI.

β-N-acetylglucosaminidase or β-glucuronidase. In the longitudinal sections the activities of all three acid hydrolases seemed in red fibres to be arranged between the myofibrils in a chainlike manner.

β-Glycerophosphatase activity (III) was not observed inside histologically normal fibres with the assay method used.

Effects of exhaustive exercise on untrained skeletal muscle

The pilot study showed that β-glucuronidase activity increased 2,3-fold three days after exhaustive exercise in previously untrained muscle (I). Untrained animals were therefore used for the quantitative characterization of the increase of acid hydrolytic capacity (IV) and for histochemical studies aimed at determining the localization of increased acid hydrolase activities and at tracing histopathological alterations (III) in the exercised skeletal muscles.

The main quantitative results are summarized in Figure 2:

(1) The activities of β-glucuronidase, β-N-acetylglucosaminidase, arylsulphatase, acid ribonuclease and cathepsin D in *m. rectus femoris* increased during the period following exhaustive exercise. The highest increase in the activities usually occurred five days after the exercise. Thereafter the activities slowly returned to the level found in controls. The activities of β-glucuronidase and acid ribonuclease were still higher than in the controls 15 days after the exercise. The highest relative increases in *m. rectus femoris* were 230 % for β-glucuronidase, 60 % for β-N-acetylglucosaminidase, 70 % for arylsulphatase, 40 % for acid ribonuclease, and 20 % for cathepsin D.

The activity of acid phosphatases, p-nitrophenylphosphatase and β-glycerophosphatase did not increase

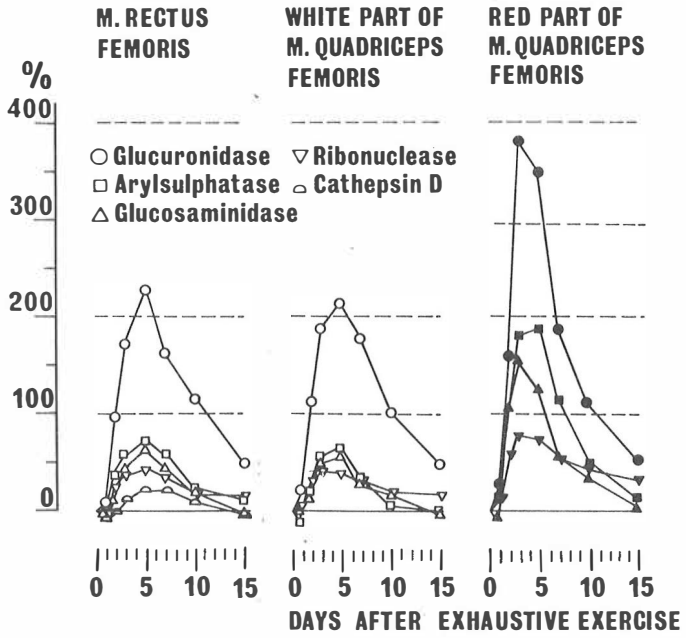

Figure 2

Summary of the relative changes of specific activities of five acid hydrolases in *m. rectus femoris* and in predominantly white and predominantly red parts of *m. quadriceps femoris* during a 15-day period following acute exhaustive running exercise. Solid line gives the level of control activity. Data are taken from study IV.

after exhaustive exercise (IV).

(2) Changes in the activity of acid phosphatases were very similar to those found in the reference variables, *i.e.* in protein content and in malate dehydrogenase activity (IV). Two days after the exercise the protein concentrations in muscle samples were less than in the controls. Similar decreases in the activity of acid phosphatases, expressed per muscle fresh weight, were observed (IV).

(3) Activity changes in *m. rectus femoris* were very similar to those in predominantly white skeletal muscle (Fig. 2). The increases were, however, far more prominent in the red than in the white part of *m. quadriceps femoris* or in *m. rectus femoris*. In addition, the highest activities of ß-glucuronidase, ß-N-acetylglucosaminidase, and acid ribonuclease were recorded three days after the loading in red and five days after the loading in white muscle. Thereafter the activities decreased, the change being again most prominent in red muscle. ß-Glucuronidase and acid ribonuclease activities remained higher than the respective control values for 15 days after the exercise in both red and white skeletal muscle.

The gross histological effects of exhaustive loading in skeletal muscle were the following:

(1) Fibre degeneration and necrosis (Figs. 3, 4). One day after the exercise samples from exhausted animals were usually histologically similar to control samples. Two days after exhaustion some degenerating fibres and intercellular oedema were observed (Fig. 4). Three days after the exercise a few fibres were classified as necrotic (together with degenerating fibres usually below 1 % of all fibres). Inflammatory reaction (highly increased numbers of mononuclear cells in the interstitial

Number of degenerating plus necrotic, and regenerating fibres per cross-sectional area of *m. rectus femoris*

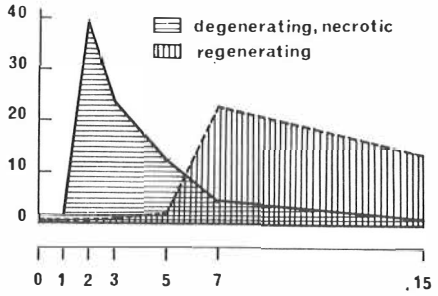

Figure 3

The time sequence of histopathological changes in *m. rectus femoris* of mice during a two-week period following acute exhaustive running exercise. The cross-sectional area of *m. rectus femoris* averaged 2 200 fibres. Data are taken from study III.

area) was maximal on the fifth day after exhaustion. Mononuclear cells were then observed mainly inside necrotic fibres. The relative number of these fibres had now increased when compared to the situation three days after exhaustion. Seven days after the exercise the number of degenerating and necrotic cells clearly decreased (Fig. 3). Some samples still contained mononuclear, evidently phagocytizing, cells. Two weeks after exhaustion mononuclear cells or degenerative reactions were not observed more frequently than in the control samples.

(2) Fibre regeneration. Five days after the exercise the number of degenerating and necrotic fibres had diminished while that of regenerating fibres had simultaneously increased to such an extent that they were found in almost all samples (Figs. 3, 4). Seven days after the exercise the number of regenerating fibres

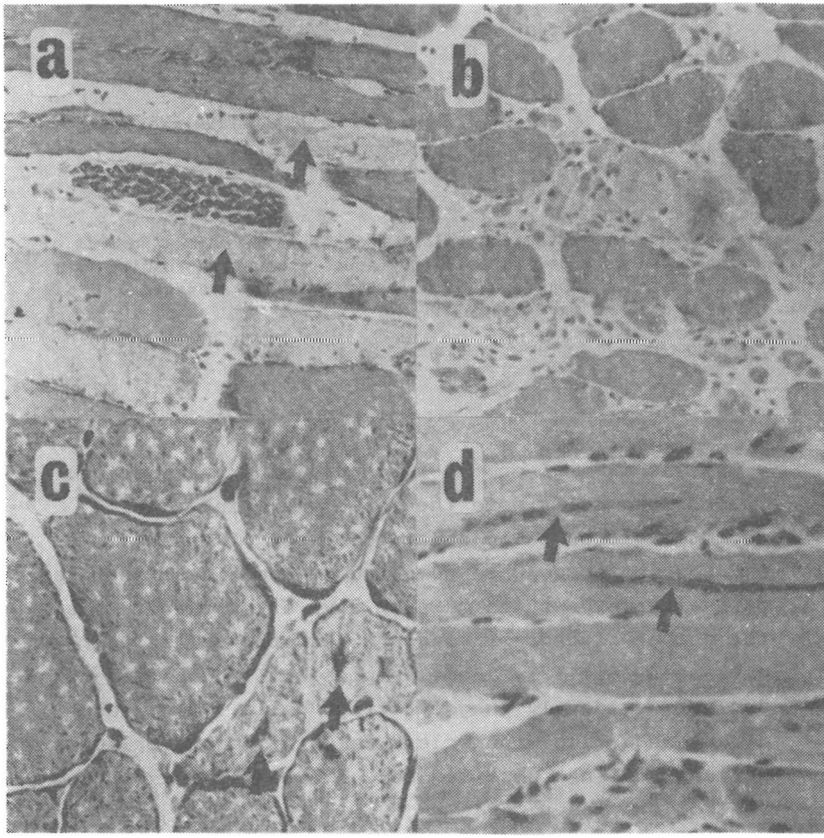
a
b
c
d

Figure 4

Examples of histological changes in mouse *m. rectus femoris* during a period following acute exhaustive running exercise.
(a) A longitudinal section two days after exercise showing pale degenerating fibres (arrows). Hematoxylin-eosin staining, magnification x 260.
(b) A cross-section five days after exercise showing degenerating and necrotic fibres together with increased number of mononuclear cells in the muscle. Hematoxylin-eosin staining, magnification x 320.
(c) Fibre regeneration. A cross-section seven days after exercise showing small-sized regenerating fibres with centrally located nuclei (arrows). Hematoxylin-eosin staining, magnification x 410.
(d) A longitudinal section five days after exercise showing regenerating fibres with central nuclei (arrows). Hematoxylin-eosin staining, magnification x 510. Examples a - c are taken from study III and d from study VI.

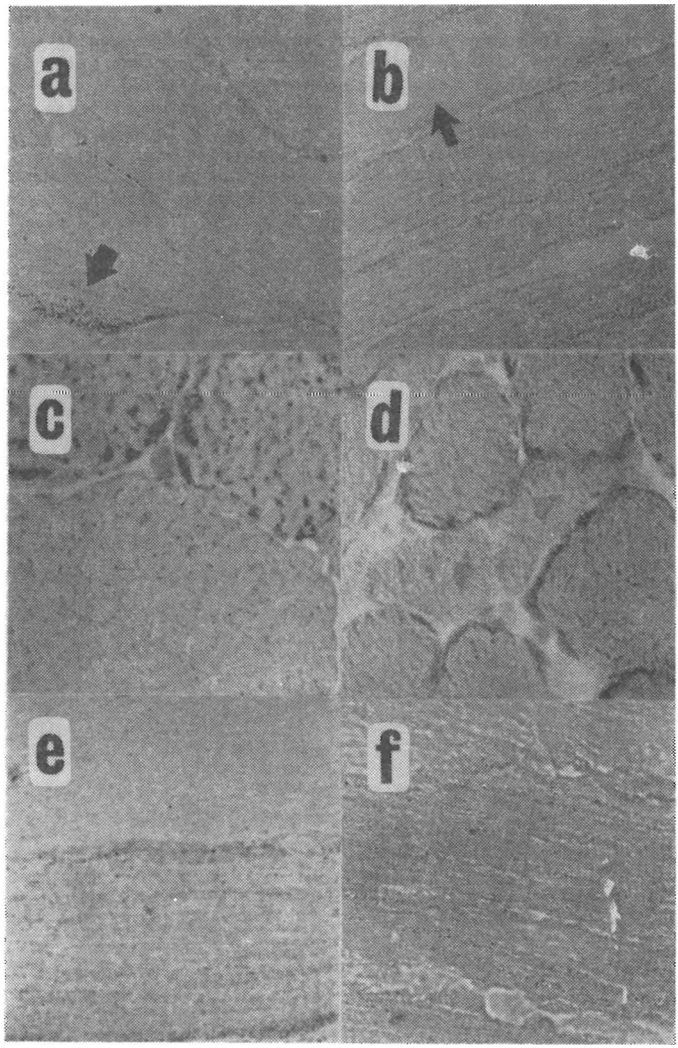
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Figure 5

Some examples of histochemical stainings of representative acid hydrolase activities in mouse *m. rectus femoris* during a period following acute exhaustive running exercise.
(a) β-N-Acetylglucosaminidase staining one day after exercise showing abundant activity in muscle - connective tissue junction (arrow). Magnification x 380.
(b) β-N-Acetylglucosaminidase staining two days after exercise; a longitudinally sectioned degenerating fibre (arrow) together with fibres showing activity, which is arranged in a chainlike manner. Magnification x 320.
(c) β-N-Acetylglucosaminidase activity three days after exhaustive exercise. Activity is strong especially in red fibres (upper part of the figure) compared to a white fibre (lower part of the figure). Magnification x 920.
(d) β-N-Acetylglucosaminidase staining together with hemalumin-staining showing fibre regeneration seven days after exhaustive exercise. Serial section from the same sample as in Fig. 4 c. Magnification x 360.
(e) An example of abundant β-glucuronidase activity five days after exercise. Increased activity is observed especially in red fibres (upper part of the figure). Magnification x 680.
(f) An example of arylsulphatase staining, longitudinal section from a control sample. Activity is typically seen in red fibres (the lower two fibres in the figure). Magnification x 710. Data in a - e are taken from study III and in f from study V.

was further increased, and regenerating fibres were still observed in all samples taken from exhausted animals two weeks after exhaustion. They were, however, less prevalent than one week after the exercise.

Histochemical studies showed that:

(1) In good agreement with the quantitative results the activities of selected acid hydrolases representing lysosomal capacity (ß-glucuronidase, ß-N-acetylglucosaminidase, and arylsulphatase) increased 3-7 days after exhaustion (III,IV). Activity changes were more prominent in highly oxidative red than in less oxidative white fibres. Activity granules were most numerous in the perinuclear area of red fibres. In the interfibrillar area and in white fibres activity granules were rather evenly distributed. In longitudinal sections granules seemed to be arranged in a chainlike manner between myofibrils.

(2) ß-Glycerophosphatase activity was not demonstrated inside fibres of histologically normal appearance by the method used. In histologically damaged fibres this activity, originating evidently from phagocytizing mononuclear cells (III), was very evident.

(3) ß-Glucuronidase or ß-N-acetylglucosaminidase activity in connective tissue was usually observed only 3-5 days after exhaustion. The activity in muscle fibres usually exceeded that simultaneously occurring in connective tissue (Fig. 5).

Effects of training

Preliminary experiments showed that ß-glucuronidase activity increased in mouse mixed skeletal muscle after regular endurance training (I). In study II the effects of training

on the activities of four acid hydrolases in predominantly white and predominantly red skeletal muscle, and in study V the effects of different intensities of endurance training on the activities of seven acid hydrolases in mixed skeletal muscle, were investigated. In study VI the effects of training on the activities of eight acid hydrolases in predominantly white and predominantly red skeletal muscle were investigated.

The most important observations concerning the effects of endurance training on acid hydrolase and reference enzyme activities were the following (Table 3):

(1) A typical increase in the activity of ß-glucuronidase. The activities of ß-N-acetylglucosaminidase and cathepsin D also regularly increased but the increases were not always statistically significant. Acid ribonuclease and p-nitrophenylphosphatase activities were not affected by training. Arylsulphatase, acid deoxyribonuclease and cathepsin C activities usually increased but this effect was not regular (V,VI) and was therefore difficult to interprete (Table 3).

(2) The regular increase in the activities of ß-glucuronidase and cathepsin D occurred rather similarly in both *m. rectus femoris* and the red and white parts of *m. quadriceps femoris*. In specific activity units the increase in red muscle was, however, more pronounced than in white muscle.

(3) The magnitudes of the increase in ß-glucuronidase, or of the changes in the activities of the other hydrolases, were not in mixed muscle affected by the following training intensity factors, if the factors were modified one at a time: (a) a daily exercise period of two hours instead of one hour (I), (b) a running speed of 25 m instead of 20 m/min (V), (c) uphill (inclina-

Table 3

Summary of the effects of endurance training on activities of acid hydrolases and selected enzymes of energy metabolism, together with data on experiment animals and their training

Data on animals and their training								Relative effect on main variables								Relative effect on reference variables					
Paper No	Number of animals[1]	Age of mice[2] (weeks)	Length of training period (days)	Daily exercise (hours/day)	Exercise frequence (days/week)	Running speed (m/min)	Muscle studied	β-Glucuronidase	β-N-Acetylglucosaminidase	β-Nitrophenylphosphatase	Cathepsin D	Aryisulphatase	Acid ribonuclease	Acid deoxyribonuclease	Cathepsin C[9]	Succinate dehydrogenase	Malate dehydrogenase	Cytochrome c oxidase	Citrate synthase	Lactate dehydrogenase	Protein
I	6	8	35	1	5	21	Mixed thigh	+24[x][8]	+12	-2	-	-	-	-	-	-	+26[x]	-	-	-	-
I	5	8	35	2	5	21	Mixed thigh	+26[x]	+3	-4	-	-	-	-	-	-	+25[x]	-	-	-	-
I	10	14	35	1	5	21	Mixed thigh	+65[x]	+15	+3	-	-	-	-	-	+17	-	-	-	-	-
II	18	18.5	25-70	1.5	5	18	Red *MQF*[4]	+48[x]	+4	+3	+10	-	-	-	-	-	-	+15	-	-4	-
II	18	18.5	25-70	1.5	5	18	White *MQF*	+30[x]	+7	+2	+10	-	-	-	-	-	-	+13	-	-8	-
V	15	10	30	1	5	20	*MRF*[5]	+18[x]	+2	0	+19	-15[x]	-5	+2	-5	-	+23[x]	+33[x]	+23[x]	-7	+2
V	14	10	30	1	5	25	*MRF*	+33[x]	+5	+4	+14	-4	+6	+5	+2	-	+29[x]	+29[x]	+23[x]	-12	+1
V	16	10	30	1	5	20[6]	*MRF*	+17[x]	+6	+2	+17	+1	+6	-5	+2	-	+30[x]	+30[x]	+28[x]	-8	+1
V	16	10	30	1	5	25[6]	*MRF*	+35[x]	+1	0	+13	-6	+1	+1	-1	-	+37[x]	+37[x]	+30[x]	-9	0
VI	20	10	20[3]	1.5	5	25[7]	Red *MQF*	+31[x]	+3	-3[x]	+19[x]	+3	-8	+12[x]	0	-	-	-	+24[x]	-17[x]	-1
VI	20	10	20[3]	1.5	5	25[7]	White *MQF*	+42[x]	+14[x]	-2	+25[x]	+14[x]	+1	+8	+17[x]	-	-	-	+55[x]	-6	-2

[1] Plus a corresponding number of control animals

[2] At commencement of training period

[3] 20 periods of exercise per 28 days

[4] *M. quadriceps femoris*

[5] *M. rectus femoris*

[6] 8° uphill

[7] 6° uphill

[8] Statistically significant difference ($p < .05$ at least) compared to control

[9] Results from V previously unpublished

tion 8°) instead of horizontal running (V). A simultaneous rather long (1.5 h) running time and relatively high intensity of training (running speed 25 m/min, uphill inclination 6°) produced a broad response of acid hydrolytic capacity in terms of the number of different activities affected (VI).

(4) Reference variables showed typical and expected effects of endurance training, namely an increase in the activities of the markers of oxidative capacity, a slight and usually insignificant decrease in anaerobic capacity and no changes in protein content (Table 3).

Effects of exhaustive exercise on trained muscle

The results of study VI showed that exhausting exercise, which in untrained animals caused an extensive and highly significant increase in the activities of all acid hydrolases studied except acid phosphatase activity, did not affect these activities in trained skeletal muscle, *i.e.* in muscle which had previously been adapted to moderate exercise by regular training (Table 4).

Table 4

Relative effect of exhaustive exercise on activities of acid hydrolases in untrained and trained, predominantly white and predominantly red parts of m. quadriceps femoris of mice five days after exhaustion. Comparison between effects on untrained muscle in studies IV *and* VI *is also included*

Activity	Animal group	White part of *m. quadriceps fem.* % Increase		Red part of *m. quadriceps fem.* % Increase	
		Study IV	Study VI	Study IV	Study VI
β-Glucuronidase	Untrained	213	141	248	434
	Trained	-	-7	-	2
β-N-acetylglucosaminidase	Untrained	56	15	126	179
	Trained	-	-1	-	3
Arylsulphatase	Untrained	65	44	188	308
	Trained	-	1	-	4
Acid ribonuclease	Untrained	32	32	69	126
	Trained	-	0	-	0
Acid deoxyribonuclease	Untrained	-	31	-	184
	Trained	-	-4	-	-6
p-Nitrophenylphosphatase	Untrained	8	-1	8	8
	Trained	-	1	-	2
Cathepsin D	Untrained	21*)	43	-	93
	Trained	-	-1	-	12
Cathepsin C	Untrained	-	43	-	207
	Trained	-	1	-	11
Protein	Untrained	-5	-6	-4	-7
	Trained	-	2	-	4

(*Value from *m. rectus femoris*

DISCUSSION

The most important new observations during the present investigations were the following: (1) the total activities of all the acid hydrolases studied, with the exception of acid phosphatase, strongly increased in skeletal muscle 3-7 days after acute exhaustive exercise (I,III,IV,VI), (2) regular endurance training induced a moderate increase in the total activities of certain acid hydrolases (I,II,V,VI), (3) acute exhaustive exercise did not increase acid hydrolytic capacity of muscle, which had previously been adapted to exercise by regular training (I,VI), and (4) the exhaustion-induced activity increase occurred principally in muscle fibres *per se*, particularly in the highly oxidative red fibres, and to a lesser extent in other - interstitial - structures (III). Of these observations the effect of training on certain acid hydrolase activities in the thigh or calf muscles of mice has been earlier reported (Pilström *et al.* 1978, Vihko *et al.* 1974).

Exhaustive exercise

Lysosomal hydrolases have a dominant role in a variety of lethal and sublethal, acute and chronic cell injuries (Arstila *et al.* 1974, Helminen 1975). Previous studies (Altland and Highman 1961, Hecht *et al.* 1975, Schuman 1972, Van Linge 1962) and the present results (III,VI) show that exhaustive exercise causes fibre necrosis, which indicates that lethal fibre injuries take place during or immediately after exercise. The strong response in acid hydrolytic capacity of skeletal muscle 3-7 days after exhausting exercise can be examined in relation to preceeding sublethal fibre injuries just as degenerative and necrotic fibre alterations in relation to previous lethal fibre injuries (III). By assuming that a condition which produces irreversible or lethal fibre injuries also produces reversible or sublethal injuries, the observed lysosomal reaction can be interpreted as a phase of

removal of damaged structures from sublethally injured fibres during a process of subcellular regeneration. In lethally injured fibres the removal of degenerative material occurs *via* phagocytozing mononuclear cells and cellular regeneration then takes place (III).

In histological studies relatively few fibres were classified as necrotic (III,VI) whereas others were sublethally injured if judged on the basis of acid hydrolase response (III,VI). Some fibres were evidently not at all affected by exhaustive exercise (III,VI), indicating that the homeostasis of individual fibres, even that of fibres belonging to the same fibre type, is not equally disturbed by the exercise. The potential of individual fibres for rapid metabolic recovery may also differ. A remarkable difference in the strength of the lysosomal response was observed between red and white fibres (III,IV,VI).

The inherent capacity of fibres to resist changes in homeostasis may vary. Necrotic fibres occurred in the red areas of muscles studied (III) and acid hydrolase activity changes were remarkably more prominent in the red than in the white muscle samples (IV). The reason for these injuries may, at least partly, be the lack of available energy (see below). The energy state at the moment of exhaustion is not the same in all fibres. Different recruitment during exercise, specialized energy production and different capacities and possibilities for rapid metabolic recovery after exhaustion distinguish between red and white fibres. Highly oxidative red fibres are probably continually recruited during such exercise as was used (IV). The recruitment causes extensive diminution of *e.g.* their glycogen stores and makes them more suspectible than white fibres to mechanical injuries. Red fibres are also more sensitive than white to the effects of ischaemia (Mäkitie and Teräväinen 1977), denervation (Fidzianska *et al.* 1974), and vitamin E deficiency (Ruth and Van Vleet 1974).

Endurance training

The training programmes applied (I,II,V,VI) caused typical and expected effects of endurance training on the activities of the reference variables, namely an increase in the oxidative and a decrease in the anaerobic capacity of exercised skeletal muscles.

The fibre composition of the muscle ultimately determines the basic activity levels of enzymes. Habitual contractile activity evidently maintains a certain profile of enzyme activities in the fibres. This dynamic profile is altered if the level of physical activity is changed, as in the case of immobilization or training. Even a moderate loading, such as a single exercise bout in a long-term endurance training programme, evidently affects the homeostasis of exercised fibres and causes a specific induction of protein synthesis. Regular repetitions of the exercise stimulus lead to teleologically meaningful adaptations, which manifest themselves *e.g.* as increased skeletal muscle activities of certain energy metabolism enzymes, as increased cardiopulmonary capacity and, finally, as the improved physical performance capacity of a trained individual.

Endurance training-induced high aerobic capacity in skeletal muscle was accompanied by specifically increased acid hydrolytic capacity (I,II,V,VI). The most pronounced effects occurred in ß-glucuronidase activity, although most of the activities studied were to some extent affected (VI). Acid phosphatase activity was a remarkable exception. Neither p-nitrophenyl- nor ß-glycerophosphatase activity increased during training or after exhaustive exercise in young mice (I-VI, Pilström *et al.* 1978). p-Nitrophenylphosphatase is only partly lysosomal in origin (Barrett 1972), which may explain to some extent the different response of this activity (IV). Acid phosphatase is, however, a widely used marker for lysosomal capacity (Barrett 1972,

Christie and Stoward 1977, Weinstock and Iodice 1969).

Higher than normal acid hydrolytic capacity in endurance-trained muscle might reflect increased cellular degradation, which would compensate for enhanced biosynthesis (Jacques 1969) in maintaining the proper muscle homeostasis. The training-induced moderate increase in acid hydrolytic capacity could also be interpreted as a cumulative effect of minor cell injuries caused by daily exercise bouts. This interpretation is actually equivalent to a new state of cellular homeostasis.

The responses of acid hydrolytic capacity appear to be flexible in relation to changes in the continuity of physical activity, as is shown by the termination of training. One week after the cessation of training the activities of β-glucuronidase and β-N-acetylglucosaminidase are below those observed in trained mice (Pilström *et al.* 1978, VI). Decreasing glycosidase activities and decreasing activities of the enzymes of energy metabolism (Guy and Snow 1977) could reflect diminished biosynthesis resulting from the decreased need for energy production in muscles functioning less than earlier. On the other hand, both reference enzymes and acid hydrolases were rather insensitive to moderate variation in the intensity of training applied (V).

Skeletal muscle contains a full complement of acid hydrolases (Dean and Barrett 1976), which probably function also in normal cellular turnover. The hydrolases probably participate in the breakdown of sarcoplasmic material (Lockshin and Beaulaton 1974), whereas myofibrils are evidently hydrolyzed by neutral and alkaline proteases (Mayer and Shafrir 1977, Reddy *et al.* 1975). Different training responses in the components of acid hydrolytic capacity (II, V,VI) might reflect specific adjustment of the degradating system according to the material to be hydrolyzed.

Type of injury

Similarities in the responses of the acid hydrolytic capacity of skeletal muscle to transient ligation ischaemia (Shannon and Courtice 1975, Shannon *et al.* 1974) and to heavy physical exercise are striking both histologically and biochemically (III,IV,VI). A common feature of both ischaemia and exhaustion of skeletal muscle is a disturbed energy balance (Haljamäe and Enger 1975). Because of these similarities it may tentatively be concluded that the agent or factor causing the strong increase in acid hydrolytic capacity is myogenic and at least partly related to energy depletion and concomitant phenomena (III,IV,VI).

Trump *et al.* (1976) have suggested that there are only two, closely interacting mechanisms, which may cause acute cell injury leading to cell death. These are inhibition of ATP synthesis and changes in membrane properties followed by irreversible disturbances in ion balances. Ion equilibria are disturbed due to lack of ATP both in sarcolemma and sarcoplasmic reticulum (Trump *et al.* 1976, Wrogeman and Pena 1976, Yarom 1976). This is probably true also in the case of ischaemia or exhaustive exercise. One possible factor causing injuries might be calcium (IV). Disturbances in calcium equilibrium may cause fibre necrosis (Wrogeman and Pena 1976, Yarom 1976) and possibly also sublethal injuries in exhausted muscle. These effects might arise from calcium accumulation in mitochondria (Sembrowich and Gollnick 1977, Wrogeman and Pena 1976), with subsequent functional (Trump *et al.* 1976) and later structural (Gollnick and King 1969) mitochondrial alterations (III,IV). The damaged structures induce *de novo* acid hydrolase synthesis, directly or by some unknown mechanism. Injured structures are removed by autophagy, resulting in restored cellular homeostasis at the earlier or at a changed level. Lethally injured fibres are abolished from the tissue by heterophagy in macrophages (Helminen 1975) and tissue homeostasis is

re-attained by fibre regeneration, which probably starts from myoblasts originating from degenerating fibres. It must, however, be emphasized that the considerations expressed above are partly hypothetical.

Protective effect of previous exercise

In endurance-trained skeletal muscle the strong increase in acid hydrolytic capacity which occurred in untrained muscle as a response to exhaustive exercise was not observed. It was, however, apparent that both trained and untrained animals were equally exhausted after the loading (VI).

It can tentatively be suggested that a hypothetical myogenic stimulus or agent(s), which varies in strength, acts during exhaustive exercise. At worst this may in untrained muscle cause fibre necrosis, and in less severe cases hereto unidentified sublethal fibre injuries, which manifest themselves as increased lysosomal capacity a few days later. There are at least three possible explanations for the obvious resistance of endurance-trained fibres to this hypothetical stimulus.

As discussed earlier, the resistance of individual fibres (or organelles, supramolecular structures *etc.*) may vary. Repeated training exercise might eliminate all "fragile" fibres or structures and thus cause the apparent resistance of trained muscle to exhaustion. Another possibility is that the damaging stimulus does not develop in a trained individual. The reason for exhaustion might be extramuscular and therefore prevent the onset of the stimulus in trained muscle. The difference in response of trained and untrained muscle might also occur after exhaustion, *i.e.* during the phase of recovery. The well-known endurance training-induced adaptations, both cardiovascular and muscular, add to the capacity of trained muscle for rapid metabolic recovery after exhaustion (VI), *e.g.* by

permitting a higher level of oxidative phosphorylation than in sedentary muscle and thus facilitating the re-attaining of homeostasis. The damaging stimulus could thus be a delay in ATP production immediately after exercise. A third possibility could be that endurance training induces a specific protective mechanism, which is directly linked to the type of injury sustained by the skeletal muscle fibres. The role of lipid peroxidation in ischaemic injury of sarcoplasmic reticulum (Arkhipenko *et al.* 1977) could be an example of such injury, which can be prevented by specific scavengers (Slater 1972). All the three possible causes of the resistance of endurance-trained fibres to the damaging effects of exhaustive exercise are so far unexplored.

A sharp distinction between the responses of the lysosomal system to "sublethal injury" (loading effect) and to what could be called "normal physiological adaptation" (training effect), is probably partly misleading. Data concerning the importance of acid hydrolases, and the lysosomal system in general, in the turnover of cellular contents are scanty. The difference between "injurious effect" and "normal adaptation" might be quantitative, depending on the strength of the stimulus and of the response. During moderate training the lysosomal system participates in the process of adaptation if the process is within the homeostatic ability of the muscle fibres. In the new, trained, steady state the homeostatic ability of the fibres is also changed, and the fibres are no longer affected even by exhaustive exercise.

SUMMARY

The responses of the skeletal muscle lysosomal system, estimated by the activity of selected lysosomal acid hydrolases, to a bout of acute exhaustive exercise and to prolonged endurance training was studied in young mice by enzymological methods and a histological study was also conducted. The following main results were obtained:

(1) Acute exhaustive exercise increased the acid hydrolytic capacity of the muscles exercised during the several days immediately following the loading. The response was most prominent 3-7 days later and occurred particularly in highly oxidative red muscle fibres. The activities of ß-glucuronidase, ß-N-acetylglucosaminidase and arylsulphatase increased relatively most abundantly and those of acid ribonuclease, acid deoxyribonuclease, cathepsin D and cathepsin C somewhat less, while the activities of p-nitrophenylphosphatase and ß-glycerophosphatase were unaffected or slightly decreased two days after the exercise. In histochemical studies the representative enzymes (ß-glucuronidase, ß-N-acetylglucosaminidase, and arylsulphatase) showed that the activity increase occurred mainly inside red fibres and to a lesser extent inside white fibres or in connective tissue. ß-Glucuronidase and acid ribonuclease activities were still higher than the respective control values two weeks after exhaustion.

(2) In addition to fibres showing acid hydrolase responses, histopathological phenomena were also observed. The gross pathological effects of exhaustive exercise in skeletal muscle were oedema, fibre degeneration and necrosis, and inflammation. These phenomena were followed by fibre regeneration.

(3) Endurance training always increased the activities

of ß-glucuronidase and cathepsin D, whereas the effects on the activities of ß-N-acetylglucosaminidase, arylsulphatase, acid deoxyribonuclease and cathepsin C were not so distinct. Acid phosphatase and acid ribonuclease activities were not affected by training. Reference variables (succinate dehydrogenase, malate dehydrogenase, cytochrome c oxidase, citrate synthase, and lactate dehydrogenase) exhibited well-attested effects of endurance training, *i.e.* increase in the oxidative capacity of mucle and unchanged or slightly decreased lactate dehydrogenase activity.

(4) The strong response in acid hydrolytic capacity of skeletal muscle to exhaustive exercise occurred only in previously untrained sedentary animals. In the muscles of trained mice no increase in the activities of acid hydrolases or increase in the number of degenerating fibres were observed after exhaustive loading.

Exhaustive exercise in untrained muscle caused a remarkable activation of the skeletal muscle lysosomal system as evidenced by highly increased acid hydrolytic capacity. The reported results can be explained by a hypothetical stimulus which acts in muscles during exhaustive physical loading. The stimulus is probably intimately associated with available chemical energy in contractile fibres. In untrained muscle the effect of the stimulus is particularly evident in highly oxidative red fibres and their homeostasis may be seriously upset. At the worst this leads to fibre necrosis and in less severe cases the disturbance appears as increased acid hydrolytic capacity a few days after exhaustion. Increased acid hydrolase activities constitute a manifestation of previous sublethal fibre injuries. Increased acid hydrolytic capacity can be considered as an indication of autophagy, which removes damaged structures. Cellular homeostasis is thus restored without the regeneration which is needed in the case of fibre necrosis.

In endurance-trained muscle the activities of certain acid hydrolases increase. Higher than normal acid hydrolytic capacity in endurance-trained muscle might reflect increased cellular breakdown, compensating for enhanced biosynthesis.

In endurance trained muscle the strong response of acid hydrolytic capacity to exhaustive exercise does not occur. Histopathological phenomena are also absent showing an increased resistance of trained fibres to the hypothetical myogenic stimulus. Endurance training-induced adaptations, both cardiovascular and muscular, add to the capacity of trained muscle to resist the damaging effects of exhaustive exercise possibly by enhancing the recovery of efficient energy production and by re-attaining ion equilibria after exhaustion. Another, also so far unexplored possibility is that training eliminates all "fragile" fibres and organelles, thus causing the apparently increased resistance. Furthermore, it is also possible that endurance training induces a specific protective mechanism, which is directly linked to the type of injury sustained by the skeletal muscle fibres.

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