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Exhaustive Physical Exercise and Acid Hydrolase Activity in Mouse Skeletal Muscle

A Histochemical Study

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Summary. Adult, untrained NMRI mice were exhausted on a motor-driven treadmill by an intermittent-type running programme. Serial cryostate sections for the staining of NADH-tetrazolium reductase, β-glucuronidase, β-N-acetylglucosaminidase, and β-glycerophosphatase activities and for making hematoxylin-eosin staining were cut from *m. quadriceps femoris* 1, 2, 3, 5, 7, and 15 days after physical exhaustion. A strong increase in the activities of β-glucuronidase and β-N-acetylglucosaminidase was observed 7 days after exhaustion and the activity changes, which were similar for the both glycosidases, were more prominent in the highly oxidative red compared to less oxidative white fibres. Activity granules were more numerous in the perinuclear than the interfibrillar area of red fibres. Spots were arranged like longitudinal chains between myofibrils. Activity in connective tissue was usually observed only in animals exhausted 3–7 days earlier. Simultaneous activity in fibres exceeded that in connective tissue. β-Glycerophosphatase activity was not, by the method used, seen in histologically "healthy" or normal-looking fibres. In samples taken 2–5 days after exhaustion some degenerating and necrotic fibres were observed. Inflammatory reaction was also observed being at its strongest five days after loading when mononuclear cells were seen inside necrotic fibres. The number of regenerating muscle cells was most abundant 7 days after exhaustion. It is suggested that temporary hypoxia, which accompanies exhaustive physical exercise in skeletal muscle, upsets the energy metabolism and homeostasis of fibres and causes the observed histological and histochemical alterations, which posses features typical of both lethal and sublethal acute cell injury.

Introduction

A single bout of exhaustive exercise – either short-lasting intensive (Hecht et al., 1975; Schumann, 1972; Van Linge, 1962) or long-lasting moderate (Altland and Highman, 1961) – causes transient pathological lesions in exercised skeletal

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muscles. Such lesions include small foci of inflammation and degenerative changes together with fibre necrosis followed by regeneration.

The lysosomal system of skeletal muscle is activated under various pathological and physiological stress conditions (e.g. Weinstock and Iodice, 1969; Max et al., 1971; Schiaffino and Hanzlikova, 1972; Bird, 1975; Maskrey et al., 1977). A temporary ligation ischemia in the hindpaw of rabbits causes a strong activation of the lysosomal system of skeletal muscle. This is evidenced as strongly increased activities of β-glucuronidase, β-N-acetylglucosaminidase and acid phosphatase (Shannon et al., 1974; Shannon and Courtice, 1975).

Ischemia and exhaustive physical loading resemble each other by producing in the energy metabolism of skeletal muscle a condition (Haljamäe and Enger, 1975), which heavily upsets the homeostasis of muscle (Simonson, 1971). Several morphological and enzymological manifestations of injury during the recovery process are also very similar (Highman and Altland, 1963; Arcangeli et al., 1973; Reznik, 1973; Boström et al., 1974; Hecht et al., 1975; Mäkitie and Teräväinen, 1977).

The present investigation was performed to learn if the lysosomal system of skeletal muscle is activated during periods following exhaustive physical loading. Previous studies have shown that repeated moderate physical exercise increase the skeletal muscle activity of β-glucuronidase (Vihko et al., 1974b; Pilström et al., 1978) and that of β-N-acetylglucosaminidase and p-nitrophenylphosphatase in older mice (Pilström et al., 1978). In preliminary biochemical studies it was also shown that even a single bout of exhaustive exercise causes a twofold increase in the activity of β-glucuronidase (Vihko et al., 1978a).

The histochemical method was selected in order to resolve the localization of the normal and post-exhaustion increased activities of representative acid hydrolases. The activities of two glycosidases (β-glucuronidase and β-N-acetylglucosaminidase) and acid phosphatase (β-glycerophosphatase) were stained from muscle samples collected during a two-week period following strenuous physical loading. Histological alterations in muscle were also traced. A simultaneous quantitative study, the results of which are published elsewhere, was also performed (Vihko et al., 1978b).

Material and Methods

Animals. Male untrained NMRI-mice, aged 10–11 weeks, were used in the study. The animals were housed in cages (Type IV, Scanbur, Denmark), 8–10 to each, and fed with solid food pellets (R3, Astra Ewos, Sweden) and water *ad lib.* Temperature (22–23° C) and humidity (40%) were kept constant. The day was artificially divided into 12 h of light and 12 h of darkness changing at 6 a.m. and p.m.

Exhaustive Exercise. 1, 2, 3, 5, 7, or 15 days before killing the animals were subjected to running on a motor-driven treadmill until exhaustion. The exercise was started usually at 8.00 a.m. during the active phase of the mice at an initial speed of 18 m/min, 6–8 mice exercising at each time. After 30 min the speed was increased to 25 m/min for 5 min and thereafter to 28 and 31 m/min, respectively, at 5 min intervals. After 5 min run at a speed of 31 m/min the speed was decreased to 18 m/min for 5–10 min and the complete speed increment programme repeated five times. Running was motivated, when necessary, by slight electrical shocks from electrodes located in the escape gates of each of the four separate running tracks of the treadmill. During the last repetition

of the programme the speed was maintained at 31 m/min until the exhaustion of even the best runners. The weekest runners were exhausted within 90–100 min and the best within 140–150 min. Some animals could not tolerate the highest speed(s) and they were allowed to rest during those periods and set again to run at slower speeds. Before being killed exhausted animals were housed normally. The number of animals in each time group is given in Table 1.

Muscle Samples. Animals were killed by dislocation of the neck and the skin was removed. *M. quadriceps femoris* (MQF) from the right hind leg was excised and the muscle complex divided into *m. vastus lateralis* (MVL) and *m. rectus femoris* (MRF). The latter was cut into two parts. The proximal part was embedded with OCT-compound (®Tissue Tech, Ames) on a specimen holder for longitudinal sectioning and the distal likewise for transverse sectioning. In later phases longitudinal sections from MVL and from *m. vastus intermedius* (MVI) were taken, in addition to the proximal cross-sections from MRF, in order to study also the effects in predominantly white or predominantly red muscle samples, respectively.

Muscle samples were frozen in isopentane prechilled by liquid nitrogen. Drying of samples was prevented and blocks were kept at −20° C until sectioned and stained within 1–6 days from killing.

Histochemical and Histological Methods. Serial cryostate sections for the staining of NADH-tetrazolium reductase (NADH-diaphorase) (Novikoff et al., 1961), β-glucuronidase (Chayen et al., 1973), β-N-acetylglucosaminidase (Shannon, 1975), and β-glycerophosphatase (Lake, 1965; in Pearse, 1968) activities were cut with an Ames Cryostate II and mounted on cover glasses. In the assay of β-glucuronidase and β-N-acetylglucosaminidase activities Brentamin Fast Garnet GBC was used as the post-coupling agent instead of the original reagent (Fast Dark Blue, Chayen et al., 1973; Shannon, 1975). Section thickness was approx. 8 µm. For control purposes liver samples were on each occasion cut and stained simultaneously with muscle samples. The specifity of the reactions were determined by suitable inhibitors and/or performing the incubations without substrate. One liver and ten muscle samples were cut and stained during each laboratory day.

Histological stainings from cryostate sections were made in order to identify degenerating, necrotic and regenerating muscle cells. For this purpose routine hematoxylin-eosin staining was used.

NADH-diaphorase staining was used to classify muscle cells either as red or white, i.e. fibres having high or low oxidative capacity, respectively.

Photomicrography was performed by a Leitz Ortholux II microscope with a Leitz Orthomat camera using colour slide film (Agfachrome professional L 50). Coloured prints were prepared by a Cibachrome A print system (Ciba-Geigy, Switzerland). These prints were photographed for black and white copies.

Results

Acid Hydrolase Activities in m. rectus femoris. The sequence in the changes in the activity of β-N-acetylglucoaminidase or β-glucuronidase during a period following exhaustive exercise was such that the strongest activity in muscle fibres was recorded five and seven days after exhaustion. Activity changes were more prominent in highly oxidative red than in less oxidative white fibres. Inside normal muscle fibres, no β-glycerophosphatase activity was seen by the method used.

Typical for samples which showed β-N-acetylglucosaminidase (Fig. 1) or β-glucuronidase (Fig. 2) activity was the localization of activity granules inside muscle cells. Activity of these two glycosidases was always higher in red fibres as compared to white fibres (Fig. 2; 3 C, F). Activity was also seen in the interstitium originating probably from fibroblasts and occasional mononuclear cells. Activity in connective tissue was usually observed only in animals exhausted

3–7 days earlier. However, simultaneous activity inside fibres exceeded that located in connective tissue. Strongest activity was observed in the junctions between fibres and connective tissue. In such cases most of activity was within the muscle cells in the immediate vicinity of the border (Fig. 1 A).

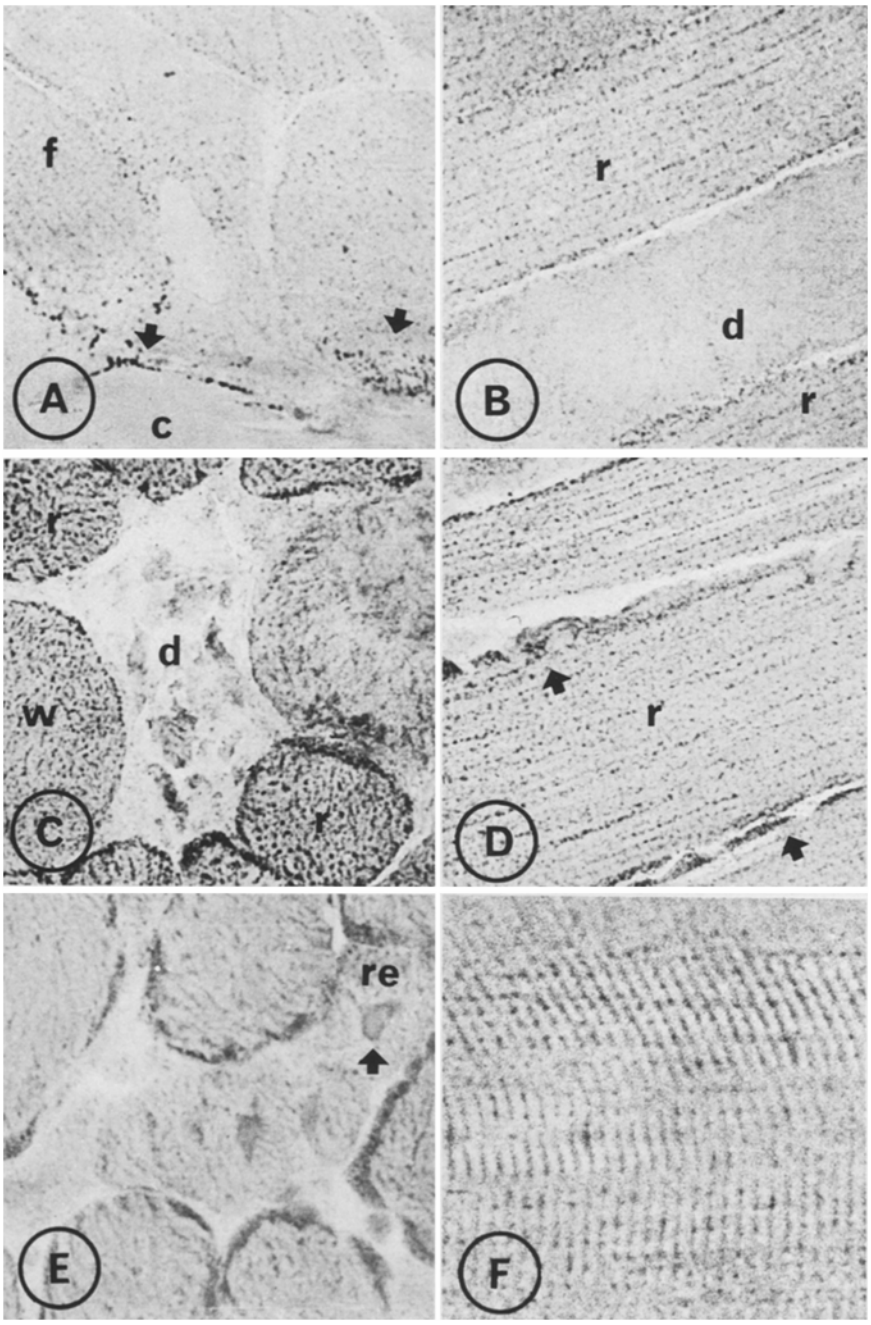
Activity granules of β-glucuronidase and β-N-acetylglucosaminidase in red fibres were more numerous in the perinuclear than in the interfibrillar area (Fig. 1 C, D; 2). In longitudinal sections granules were arranged between myofibrils in a chainlike manner (Fig. 1 B, D, 2). The distribution of activity granules in white fibres was more even and their number less than in red fibres.

β-N-Acetylglucosaminidase. This activity was regularly seen in samples taken from unexhausted control animals. One day after exhaustion β-N-acetylglucosaminidase activity was usually similar to that of controls but two days after exercise an increase in the activity was seen in red fibres. Three days after exhaustion β-N-acetylglucosaminidase activity was clearly increased (Fig. 1 C). The activity in white fibres was much less influenced by exercise than that in red fibres. Activity in connective tissue was not seen in every sample.

A further increase in the number of activity granules of β-N-acetylglucosaminidase was seen in samples taken five or seven days after exhaustion (Fig. 1 D). The localization of activity in longitudinal chains was, five or seven days after exhaustion, similar to that observed three days after exhaustion. The staining intensity and number of granules were even higher. Two weeks after exhaustion β-N-acetylglucosaminidase activity was slight when compared to samples taken 3–7 days after exhaustion. Occasional activity was also seen in connective tissue.

β-Glucuronidase. β-Glucuronidase activity was not seen in every control sample. The activity changes of this enzyme both in the red and in the white fibres and in connective tissue were very similar to those observed in β-N-acetylglucosaminidase activity and they were even more clear (Fig. 2). Accordingly, the highest activity was seen 3–7 days after exhaustion, stronger activity occurring in red

Fig. 1 A–E. The localization and changes of β-N-acetylglucosaminidase activity in mouse skeletal muscle during a period following exhaustive running exercise. **A** β-N-Acetylglucosaminidase staining one day after exhaustion showing strong activity at the border between fibres (*f*) and connective tissue (*c*). Numerous activity granules in the vicinity of the junction (arrow). Oblique section from *m. rectus femoris*. Magnification ×610. **B** A longitudinal section from *m. rectus femoris* of a mouse two days after exhaustive running exercise, β-N-acetylglucosaminidase staining. A degenerating fibre (*d*) is seen between red fibres (*r*). Activity granules are more numerous than one day after exercise. Magnification ×630. **C** A transverse section from *m. rectus femoris* of a mouse three days after exhaustion, β-N-acetylglucosaminidase staining. Smaller, highly oxidative red fibres (*r*) contain more activity than large-sized, slightly oxidative white fibres (*w*). A degenerating cell (*d*) containing slight activity and probably mononuclear cells. Magnification ×580. **D** A longitudinal section from *m. vastus intermedius* of a mouse five days after exhaustive exercise, β-N-acetylglucosaminidase staining. Activity is strongest (arrows) in the perinuclear area of red fibres (*r*) and appear as regular, longitudinal chains between myofibrils. Magnification ×630. **E** A cross-section from *m. rectus femoris* of a mouse seven days after exhaustion, β-N-acetylglucosaminidase staining together with Mayer's hemalumin staining. Regenerating fibres (*re*) with centrally located nuclei (arrow). Magnification ×540. **F** A longitudinal section from *m. rectus femoris* of a mouse seven days after exhaustive running exercise, β-N-acetylglucosaminidase staining. Darker spots are activity granules. Magnification ×1100



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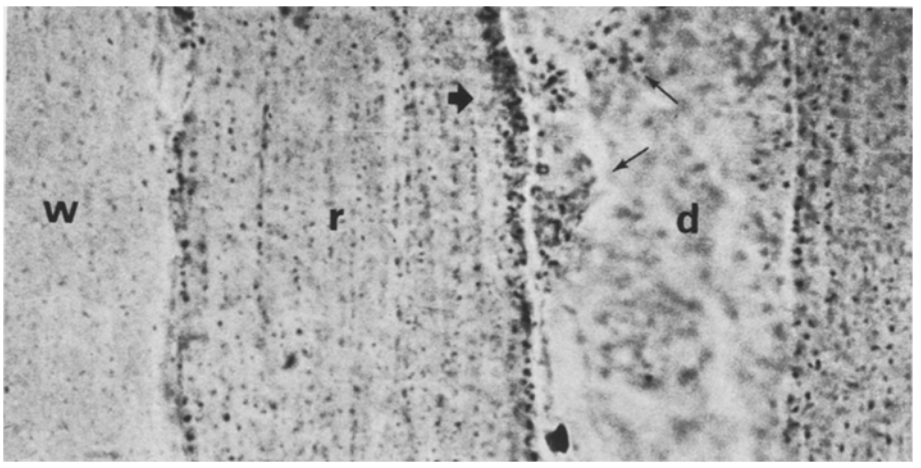

Fig. 2. A representative microphotograph showing β-glucuronidase activity in red fibres (*r*) five days after exhaustive running exercise in *m. rectus femoris* of a mouse. Activity is typically arranged as longitudinal chains and granules are numerous in the perinuclear area of the cells (arrow). White fibre (*w*) shows less activity which is evenly stippled in the interfibrillar and perinuclear areas. A degenerating cell (*d*) shows dense areas of activity granules. These denser spots (small arrow) probably originate from mononuclear cells. Magnification ×1200

fibres and in their perinuclear area than in white fibres or interfibrillar area. Activity in connective tissue was also increased. The arrangement of activity in longitudinal chains resembled that of β-N-acetylglucosaminidase activity (Fig. 2).

Other Muscles. Samples from *m. vastus intermedius* and *m. vastus lateralis* were taken 3, 5, and 7 days after exhaustion in order to investigate the response of acid hydrolase activity to exhaustive loading separately in predominantly red (MVI) and predominantly white (distal head of MVL) muscle.

In control MVI-samples the activity of β-glucuronidase was generally clearly higher than in MRF-samples. Interindividual variation in staining intensity was less in MVI than in MRF. MVI-samples, taken 3, 5, and 7 days after exhaustion, contained higher β-glucuronidase activity than respective control samples. In MVL the activity was low both in control and in post-exhaustion samples and no clear increase in the activity was observed.

A slight increase in β-N-acetylglucosaminidase activity in MVI was observed 3, 5, and 7 days after exertion. This activity in the distal head of MVL was, like that of β-glucuronidase in the same muscle, low and not seemingly influenced by exhaustive exercise.

Histopathological Alterations. In contrast to β-N-acetylglucosaminidase and β-glucuronidase activities that of β-glycerophosphatase was not seen in histologically "healthy" or normal-looking fibres. Instead activity was seen in connective tissue fibroblasts and in evidently phagocyting mononuclear cells observed inside

Table 1. The average number of degenerating together with necrotic and regenerating muscle cells per total cross-sectional area of *m. rectus femoris* of mice during a two-week period following exhaustive running exercise. The cross-sectional area of *m. rectus femoris* averaged approximately 2200 fibres. Number of animals in each experimental group is also given (*N*). Identification of pathological fibres was performed on the basis of hematoxylin-eosin and acid phosphatase stainings

Experimental group	Degenerating + necrotic fibres	Regenerating fibres (central nuclei)	*N*
Controls	1	<1	13
Days after exercise:			
1	1	<1	6
2	39	<1	6
3	24	1	15
5	12	2	12
7	4	23	14
15	<1	14	6

injured fibres. This staining was therefore used, together with hematoxylin-eosin staining, to estimate the strenght of inflammatory reaction.

The number of degenerating or necrotic and regenerating fibres in different experimental groups is given in Table 1. The histological appearance of samples taken one day after exhaustion was usually similar to that of controls. Two days after exercise rather many degenerating fibres together with interstitial oedema in some samples were observed.

The first degenerating fibres clearly classified necrotic were observed in specimens taken three days after exhaustion. A few samples also contained regenerating cells. Mononuclear, eveidently phagocytic, cells were observed in most samples. Their number among samples varied largely.

The inflammatory reaction, estimated on the basis of hematoxylin-eosin and β-glycerophosphatase stainings, was at its strongest the fifth day after exercise. Mononuclear cells were seen mainly inside necrotic fibres (Fig. 3), the number of which had increased when compared to the situation three days after exercise. The number of degenerating cells had simultaneously diminished while that of regenerating cells had strongly increased to such an extent that they could be found in almost all samples (Table 1), mostly surrounded by necrotic material.

The number of regenerating fibres further increased and the number of degenerating and necrotic fibres notably decreased in samples taken seven days after exhaustive exercise. Some samples contained still mononuclear cells. Two weeks after exhaustion inflammatory cells or degenerative reactions were not observed, while regenerative cells were still found in every sample taken from exhausted animals (Table 1). They were, however, clearly fewer than one week after exhaustion.

Histological changes (oedema, inflammatory reaction, fibre necrosis and regeneration) in MRF occurred mainly in its proximal part, which contains far more red fibres than the distal part. Changes in MVI, which is a predominantly red muscle, were reminiscent of those in MRF and inflammatory reaction was even more pronounced in MVI than in the proximal head of MRF. The predominantly white MVL showed no changes in its histological appearance.

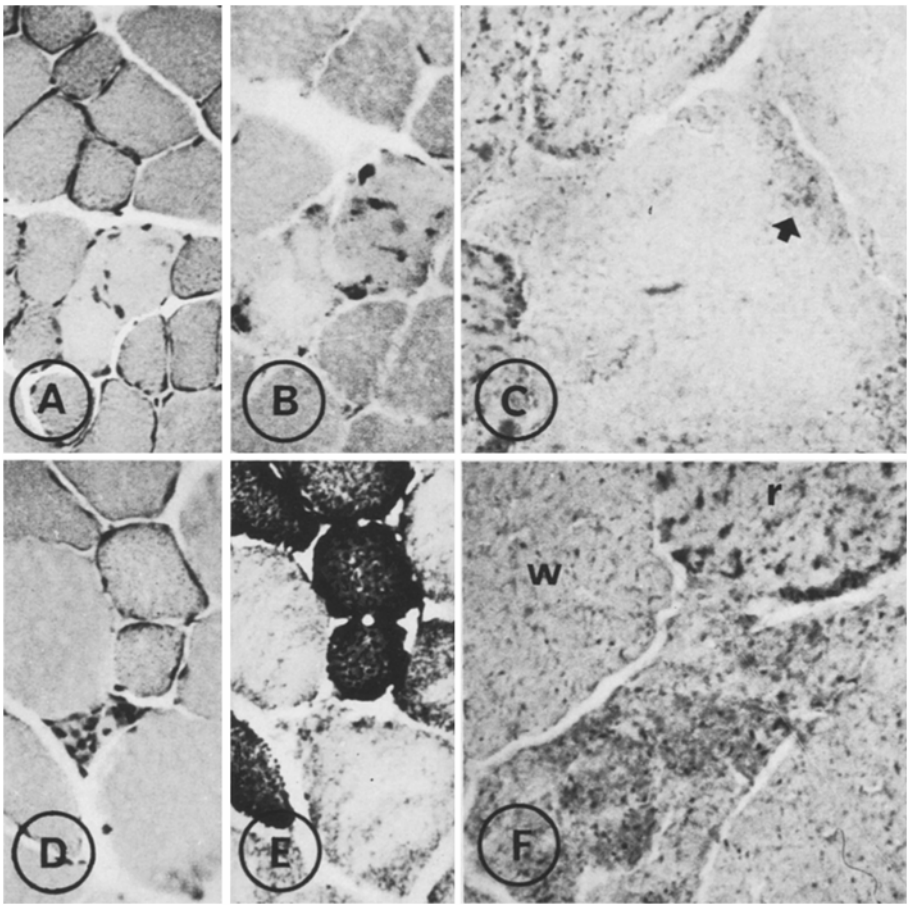

Fig. 3. Examples of fibre degeneration with slight (**A–C**) and abundant (**D–F**) mononuclear cell invasion. **A–C** In hematoxylin-eosin staining (**A**, magnification ×220) two degenerating cells, which contain some acid phosphatase activity (**B**, magnification ×340) originating probably from phagocyting cells, are seen. In β-N-acetylglucosaminidase staining (**C**, magnification ×1200) these two cells contain only slight activity compared to histologically normal-looking fibres in the neighbourhood. Even this slight activity (arrow) might originate from mononuclear cells because in early degeneration no β-N-acetylglucosaminidase or β-glucuronidase activity are seen (*cf.* Fig. 1 B). Sample is taken from *m. rectus femoris* three days after exhaustive exercise. **D–F** In hematoxylin-eosin staining (**D**, magnification ×310) the necrotic fibre is filled with nuclei from phagocyting cells. NADH-diaphorase activity (**E**, magnification ×310) of the fibre is still slight showing that regeneration has not yet commenced. β-N-Acetylglucosaminidase activity (**F**, magnification ×1200) probably originates from mononuclear cells which fill the fibre (cf. Fig. 2). Figure 3 F shows a typical arrangement of β-N-acetylglucosaminidase activity in cross-sections: high activity in perinuclear area of red fibres (*r*) and evenly stippled activity in white (*w*) fibres. Same animal as in Figure 3 A–C

Discussion

The most important finding in the present study was the observation that increases in the activities of β-glucuronidase and β-N-acetylglucosaminidase, found in previous biochemical studies to follow exhaustive physical exercise in skeletal muscles of untrained mice (Vihko et al., 1978a, b), originate principally from muscle fibres per se. Activity in connective tissue as well as activity in infiltrated mononuclear cells seen inside necrotic fibres contribute a smaller proportion of the increase. We have in previous quantitative studies, using muscle homogenates, suggested that acid hydrolase activity of muscle cells per se is not or is only a little influenced by training or heavy brief exercise, while activity in connective tissue might be more sensitive to physical activity (Pilström et al., 1978; Vihko et al., 1978a). This conclusion was partly based on results published by Canonico and Bird (1970), which suggest a bimodal distribution of acid hydrolases in skeletal muscle homogenates. Accordingly, the activity of acid phosphatase (p-nitrophenylphosphatase) in normal skeletal muscle originates (95%) from muscle fibres. Our previous biochemical studies have usually shown no effects of exhaustive exercise, or prolonged training, on p-nitrophenylphosphatase activity (Vihko et al., 1974b; 1978 a, b; Pilström et al., 1978). The present results showed β-glycerophosphatase activity after exhaustion only in connective tissue fibroblasts and mononuclear cells. Acid phosphatase activity was not demonstrated inside normal fibres. The current method may have some influence, because quite recently Lojda and Gutmann (1976) showed acid phosphatase activity in cross-striated fibres of rats using a semipermeable membrane technique and naphtol AS-B1-phosphate as the substrate. Our simultaneous quantitative determinations in part with same experimental animals as in the present study (Vihko et al., 1978b) showed, however, no increase in the activity of p-nitrophenylphosphatase or β-glycerophosphatase. Therefore the contribution of such hydrolase activity, which originates from non-muscle cells, is probably small also during exercise-induced increase in acid hydrolytic capacity.

The gross histological changes in the present investigation were oedema one and two days after exercise together with degenerative fibre alterations which were followed by necrosis and later by regeneration. Elevations in serum activities of some enzymes after physical loading have been described (e.g. Sanders and Bloor, 1975) and it has been stated that such increases are in relation to cells short of necrosis (e.g. Henley et al., 1960). Previous histological studies with heavily exercised animals have indeed shown transient pathological lesions in exercised skeletal muscles. Altland and Highman (1961) and Highman and Altland (1963) report small foci of inflammation and necrosis in muscle fibres of rats exercised in a rotating drum for several hours. Works by van Linge (1962), Schumann (1972) and Hecht et al. (1975) have also shown degenerative histological changes followed by regeneration in experimental animals after heavy physical loading. The histological results of the present study are in good agreement with these previous investigations.

Heavy physical loading causes a shift of water from extracellular to intracellular compartments and changes in electrolyte concentrations in muscles (Körge

and Viru, 1971). Fibre swelling evidently causes changes in the architechture of the sarcolemma and greater molecules than normally can escape from the cell. Interstitial oedema follows the swelling of fibres. According to Highman and Altland (1963) serum enzyme changes are in part non-specific and the specific muscle lesions found after heavy exercise in rats may not be the major factors contributing to the changes in blood enzyme values. This is obvious since serum enzyme activities are at normal level already one day after exercise (Siest and Galteau, 1974), i.e. before the pathological fibre lesions are histologically observeable. Material from degenerating cells is anyway removed by mononuclear cells (see Fig. 3D–F). Alterations in the permeability properties of mitochondria in addition to such changes in the sarcolemma have also been considered as important factors contributing to the increase in serum enzymes (Highman and Altland, 1963).

A single bout of heavy exercise has immediate effects on the ultrastructure of skeletal muscle. Gollnick and King (1969) reported large spaces between adjacent myofibrils and mitochondrial swelling with disruption of cristae. Despite results in which similar changes in exhausted muscle have not been found (Terjung et al., 1972) or in which it has been shown that such changes result in certain fixation procedures but only in exhausted animals (Gale, 1974; Bowers et al., 1974), these studies suggest increased lability of mitochondria in exhausted skeletal muscle.

Decreased oxygen tension and decreased cellular ATP have been suggested as reasons, among others, for the mitochondrial swelling after heavy exercise (Gollnick and King, 1969). Why only some mitochondria are enlargened and swollen might be caused by local differences in the oxygen tension of exercised fibres (Vihko and Arstila, 1974a).

During recovery from experimental ischemia the skeletal muscle activities of β-glucuronidase, β-N-acetylglucosaminidase and p-nitrophenylphosphatase are greatly increased (Shannon et al., 1974; Shannon and Courtice, 1975) in the plantar muscle of rabbits. The similarity of the present β-glucuronidase results with those of Shannon et al. (1974) and β-N-acetylglucosaminidase results with those of Shannon and Courtice (1975) is obvious and striking. The localization of acid hydrolase activity in fibres and the timing of the appearance of degenerating and necrotic fibres together with peaks of enzyme activities at approximately the same time (Vihko et al., 1978b) were similar. Thus the responses of skeletal muscle to experimental ligation ischemia and to heavy physiological stress are to a considerable extent similar but with two main differences, i.e. the effects of ischemia are quantitatively stronger and p-nitrophenylphosphatase activity is also increased.

According to Trump et al. (1974) there are only two, closely interacting mechanisms, which may cause an 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. The metabolic state in skeletal muscle immediately after intensive physical loading and exhaustion (high concentration of lactate, low glycogen, ATP, and creatine phosphate concentrations, decreased pH and low oxygen tension) (e.g. Simonson, 1971) resembles to certain extent the metabolic state during ischemia. Both during intensive physical exer-

cise and during ischemia an increased ADP/ATP ratio stimulates anaerobic glycolysis in skeletal muscle connected with decreased glycogen and increased lactate concentrations. The main differences between the two states are perhaps that during exercise high lactate and low oxygen tension can be reached in a shorter time and physiological recovery is also rapid whereas during ischemia glycogen decrease and lactate accumulation are relatively slow events and oxygen tension may reach minimal levels.

During heavy physical stress some muscle fibres evidently reach the point of no return (e.g. Arstila et al., 1974) and such fibres are irreversibly damaged undergoing thereafter degeneration and necrosis followed by regeneration (Highman and Altland, 1963; Reznik, 1973; Hecht et al., 1975). One reason for this sequence of pathobiological events is most probably hypoxia, which in pathological experiments, like in that used by Shannon and Courtice (1975), is more complete and causes more fibre necrosis than our more physiological experiment. Further, in exhaustion some fibres undergo minor sublethal cell injuries, which manifest themselves as increased lysosomal capacity a few days after exhaustion. The above mentioned mitochondrial changes after heavy physical exercise (Gollnick and King, 1969; Vihko and Arstila, 1974; Gale, 1974; Bowers et al., 1974) might be examples of such sublethal injuries. Red highly oxidative fibres seemed to be more susceptible to strenuous conditions than white fibres as judged on the basis of stronger increases in red compared to white fibres and on the differences between muscles studied. This may depend on their continuous recruitment during the loading (Vihko et al., 1978b). White fibres seem, however, also *post mortem* to resist ultrastructural changes better than red fibres, possibly because of their higher glycolytic and anaerobic capacity (Hirsimäki et al., 1978).

The longitudinal arrangement of activity granules of β-glucuronidase and β-N-acetylglucosaminidase suggest the sarcotubular system for their origin (e.g. Bird, 1975). In atrophic and dystrophic states the number of primary lysosomes in the perinuclear area of fibres increases (Schiaffino and Hanzlikova, 1972; Manolov and Ovtscharoff, 1974; Christie and Stoward, 1977) and lysosomes are also seen between myofibrils in the interfibrillar area. Ultrastructurally the clearest sign of the activation of the lysosomal system in these states is the appearance of autophagic vacuoles in muscle fibres.

The activation of the lysosomal system in degenerative states of muscle result in their participation in the cellular break-down or catabolism. Increased activity of acid hydrolases is considered as a sign of such an activation. We have suggested, in connection with studies of the effects of endurance training on skeletal muscle activities of acid hydrolases, that a more "effective" lysosomal system, evidenced by increased activities of β-glucuronidase, β-N-acetylglucosaminidase, and cathepsin D, would compensate for a higher level of biosynthesis in trained than in untrained muscle (Vihko et al., 1978a, b; Pilström et al., 1978). The moderately increased activities may well represent a more effective catabolism balancing enhanced biosynthesis in maintaining cell homeostasis but the strong activation of the lysosomal system in untrained exhausted muscle is most probably associated with recovery and repair processes after acute, sublethal cell injury. After exhaustive physical loading the lysosomal

system is activated, evidenced in the present study by highly increased acid hydrolytic capacity, in order to remove the damaged structures.

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