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Acid Hydrolase Activity in Red and White Skeletal Muscle of Mice During a Two-Week Period Following Exhausting Exercise*

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Abstract. The activities of β-glucuronidase, β-N-acetylglucosaminidase, arylsulphatase, ribonuclease, *p*-nitrophenylphosphatase, and malate dehydrogenase together with protein content were assayed from representative mixed (*m. rectus femoris*), predominantly red (proximal heads of *m. vastus lateralis*, *m. v. medius* and *m. v. intermedius*), and predominantly white (distal head of *m. vastus lateralis*) muscle homogenates of mice during a two-week period following one single exposure to exhausting intermittent running on a treadmill. The activities of cathepsin D and β-glycerophosphatase were assayed from mixed muscle only. In all three muscle types, particularly in red muscle, the activities of β-glucuronidase, β-N-acetylglucosaminidase, arylsulphatase, and ribonuclease progressively increased between one to five days after the exercise; thereafter the activities began to decrease, being near the control values 15 days after the exercise. In mixed muscle, cathepsin D activity increased. No corresponding changes were observed in the activities of acid phosphatases.

The time course of the activity changes closely resembled that earlier found to be caused by ischaemia in rabbit muscles. It is tentatively concluded that the two treatments, exhaustive exercise and temporary ischaemia, cause similar cell injuries, and that the lysosomal system involved seems to function similarly in the post-stress recovery of the fibres from these injuries.

Key words: Muscle enzymology – Red/white skeletal muscle – Exhaustive exercise – Acid hydrolase – Cell injury – Mouse.

Introduction

Skeletal muscle contains acid hydrolases and the activities are sedimentable, latent, and normally low [4,19]. Cell fractionation studies indicate that the activities in muscle homogenates originate from two populations of lysosomes: one from fibres per se, the other from connective tissue and macrophages [4]. These results have been confirmed by histochemical data showing that several acid hydrolases were present in fibres in addition to interstitial cells [11,24].

Acid hydrolases of skeletal muscle increase in certain physiological and pathological states in which the break-down or catabolism of the muscle is increased (e.g. [32]). Examples of states of increased skeletal muscle activity of acid hydrolases include some dystrophies (e.g. [32]) and several atrophies, like those produced by denervation (e.g. [13,19]), tenotomy [19], immobilization [14], starvation [3,4] and certain other nutritional deficiences (e.g. [32]). Hypoxic states, both acute like experimental ischaemia [24], and chronic like obstructive arteriopathy [5] induce increases in the activities.

Increased physical activity also affects the acid hydrolytic capacity of skeletal muscle. Regular endurance training increases the activities of certain acid hydrolases [18,29]. Preliminary observations have also shown that one single exhaustive bout of exercise increases β-glucuronidase activity three days after loading [29].

The present investigation was conducted to evaluate the role of the lysosomal system, estimated as the activity of selected acid hydrolases, during the recovery period of skeletal muscle after heavy exhaustive exercise.

Materials and Methods

Animal Care. Altogether 172 young (10–11 weeks old) male untrained NMRI-mice were used in the experiments. The animals lived under normal cage conditions, 8–10 mice to each cage (Scanbur Type IV, Denmark) with free access to solid food pellets (R3, Astra Ewos, Sweden) and tap water. Temperature (22–23° C), humidity (40%) and daylight rhythm (darkness 6.00 p.m.–6.00 a.m.) were kept constant.

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Exhausting Exercise. The mice were exhausted by running on a motor driven treadmill using an intermittent type of loading programme. Animals for the exhaustive run were randomly selected from a large sample of mice of a certain age. They were familiarized with running on the treadmill by a slow exercise (18 m/min) on a horizontal track for 30 min, 6–8 mice exercising at a time. Running was, when necessary, motivated by light electrical shocks from electrodes located in the escape gates of each of the four tracks of the treadmill. During the first 30 min animals unable to run (approx. 5 % of all) were removed. After 30 min the speed was increased to 25 m/min for 5 min and then to 28 and 31 m/min at 5 min intervals. Thereafter the speed was decreased to 18 m/min for 10 min. This exercise programme was then repeated five times resulting in making up a total loading duration of 145 min.

Some mice were unable to follow the highest (31 m/min) running speed, usually during the three last repetitions of the programme. Such animals were allowed to rest during these periods and put again to run on the track at slower speeds. Other mice easily performed the complete programme. The loading of these animals was continued at the highest or at an even higher speed until their exhaustion. In some cases this took 30 min showing the wide variation in the running capacity of the mouse strain from our laboratory. Whether the animals had reached exhaustion or not was decided on the basis of their behaviour on the running track and afterwards in the cage. (Exhausted animals did not perform any voluntary movements in their cages during the first minutes after running.) Before being killed exhausted animals were housed like non-exercised controls.

The loading was usually started at 8.00 a.m. during the active period of the animals. The study was divided into five phases during each of which a mean of 6–8 mice from the group of non-exhausted controls and the same number of mice exhausted 1, 2, 3, 5, 7 or 15 days earlier were killed. In addition to the non-exhausted controls, 3–5 exhausted groups from different time groups (i.e. 1, 2, 3, etc. days) after exhaustion were examined during each study phase. In all 172 mice were used in the experiments. The largest individual groups were controls (C) and groups exhausted 3, 5, and 7 days before killing (denoted E3, E5, E7, respectively). The mean weight (± S.D.) of control group was 40.6 ± 2.5 g and varied from 38.2 ± 2.7 to 39.9 ± 2.4 g in the different time groups without significant differences between the groups.

Muscle Samples and Tissue Preparation. Animals were exhausted so that the different groups could be killed and samples prepared during successive days (within 3–4 days during each phase). During the first experimental phase the left *m. rectus femoris* (MRF) of each mouse was investigated. MRF is a mixed muscle, which is composed mainly of white fibres. During the later study phases the red and white parts of *m. quadriceps femoris* (MQF) were also studied in addition to MRF. The predominantly white part of MQF was composed of the distal head of *m. vastus lateralis* (MVL) and the predominantly red sample from proximal heads of MVL and *m. vastus medius* (MVM) together with red fibres from *m. vastus intermedius* (MVI). The white and red samples were not exclusively composed of white or red muscle fibres but always also included the other fibre type. The samples were, however, regarding the preparation procedure, as white or as red as possible. *M. rectus femoris* of control mice weighed 105.1 ± 10.3 mg and no significant changes were observed in its weight during 1 to 15 days after exhaustion. Approximately equal amount of either red or white muscle was taken from each experimental animal. The mean weights of white samples varied from 30.3 ± 1.0 to 31.1 ± 1.5 mg and those of red samples from 25.1 ± 0.9 to 25.6 ± 0.7 mg in different groups.

When the animals were killed, the skin was rapidly removed. The muscle sample under study was quickly 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). The homogenate was diluted to 3 %. MRF was homogenized in an all-glass Potter-Elvehjem homogenizer (670 rpm) and red and white samples with an all-glass microhomogenizer operated manually. Homogenates were stored at −20° C until analyzed within 2 weeks.

Before acid hydrolase activity assays the homogenate was allowed to melt in an ice-bath and made up to 0.1 % in respect to a Triton X-100 concentration. Activity determinations were performed at random so that all types of muscle samples from each time group were normally assayed daily. Eighteen homogenates corresponding to 6 animals were analyzed each day.

Assay of Acid Hydrolase Activity. The activities of β-glucuronidase (EC 3.2.1.31), β-N-acetylglucosaminidase (EC 3.2.1.30), arylsulphatase (EC 3.1.6.1), ribonuclease (EC 2.7.7.16) and *p*-nitrophenylphosphatase (EC. 3.1.3.2) were assayed, essentially as described by Barrett [2], both from MRF and the white and red parts of MQF. Cathepsin D (EC 3.4.23.5) and β-glycerophosphatase (EC 3.1.3.2) activities were assayed from MRF [2]. The number of observations for each variable in the different time groups is given in Tables 1 and 2. All assays were made in duplicate, and the method error was 2–4 % in all determinations.

Storage at −20° C decreased the activities of acid phosphatases. The activity of β-glycerophosphatase decreased 25 % even during the first 24 h storage and continued decreasing over two weeks to approx. 50 % of the original activity. The activity of *p*-nitrophenylphosphatase decreased evenly and more slowly. The activities of β-glucuronidase, β-N-acetylglucosaminidase, arylsulphatase and ribonuclease were stable over 3 weeks storage at −20° C. The comparability between the different time groups of phosphatase activity results was controlled by randomizing time of storage.

In order to demonstrate the difference in the oxidative capacity of red, white and mixed muscle samples, malate dehydrogenase activity (EC 1.1.1.37) was assayed according to Ochoa [16]. The protein content of the samples was determined according to Lowry et al. [12] after incubating 0.25 ml homogenate with 1.0 ml 1 N NaOH at 40° C for 60 min and using bovine serum albumin for the standard curve.

Statistical Methods. Standard procedures were used to calculate means and standard deviations (S.D.). The significance of the difference in the means between groups were tested by Student's *t*-test. Correlations between hydrolase activities were calculated by the least square method.

Results

Differences in Enzyme Activities Between Red and White Muscle Samples. Enzyme activities in the red and white parts of *m. quadriceps femoris* from control mice are shown in Table 2. The activities of acid hydrolases were significantly higher ($P < 0.001$) in predominantly red than in predominantly white tissue. The difference was relatively largest in the activity of the two glycosidases and arylsulphatase. Oxidative capacity, estimated as the activity of malate dehydrogenase, was almost three times higher in the red than in the white samples. Protein content was similar in both muscle types.

Effect of Exhausting Exercise on Acid Hydrolase Activity in M. rectus femoris. The activities of acid hydrolases in mouse *m. rectus femoris* during the days following exhausting exercise are given in Table 1. The activities of β-glucuronidase, β-N-acetylglucosaminidase, arylsulphatase, and ribonuclease rose very considerably

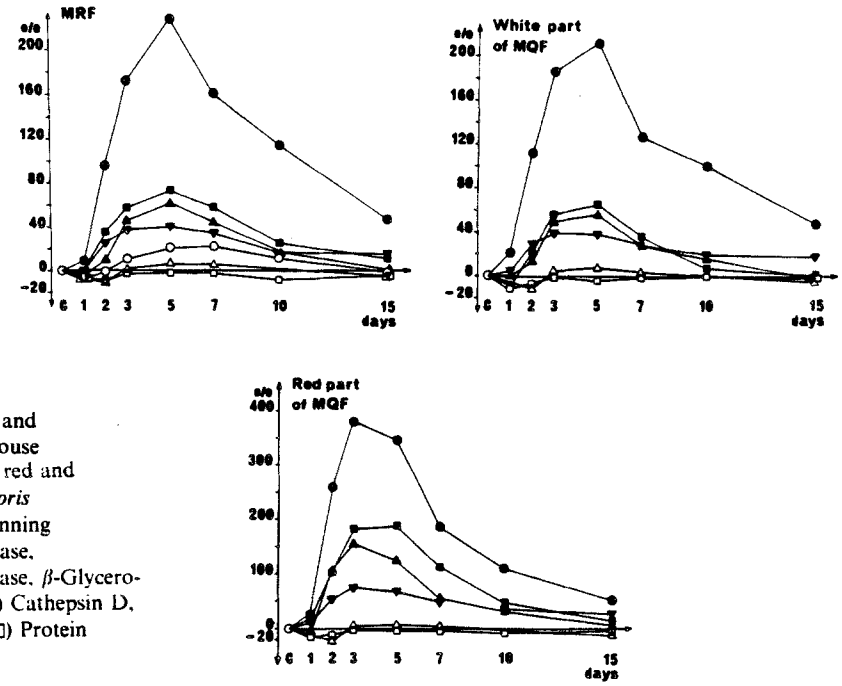

Fig. 1
Relative changes in activities of acid hydrolases and malate dehydrogenase and protein content in mouse *m. rectus femoris (MRF)* and in predominantly red and predominantly white parts of *m. quadriceps femoris (MQF)* during a period following exhaustive running exercise. Symbols of enzymes: (●) β-Glucuronidase, (▲) β-N-Acetylglucosaminidase, (■) Arylsulphatase, β-Glycerophosphatase, (△) *p*-Nitrophenylphosphatase, (○) Cathepsin D, (▼) Ribonuclease and Malate dehydrogenase, (□) Protein

during the days following heavy exertion (Fig. 1). The activities of these enzymes increased already two days after exhaustion. The highest activities were observed 5 days after the exercise. Thereafter the activities slowly decreased. The activities of β-glucuronidase and ribonuclease were still higher than in the controls 15 days after the exhaustive exercise. The activity of cathepsin D increased less than the above activities and the highest activity was observed seven days after exercise. The peaks of the activities of all these hydrolases occurred between 3 and 7 days after exhaustion (Fig. 1, Table 1).

The activities of *p*-nitrophenylphosphatase and β-glycerophosphatase referred per muscle fresh weight did not increase after the exercise. Changes in their activities were similar to those in protein content (Fig. 1). For 2 days after loading the protein concentration in *m. rectus femoris* was significantly lower than in the control samples.

Effect of Exhausting Exercise on Acid Hydrolase Activity in Red and White Parts of M. quadriceps femoris. Activities of β-glucuronidase, β-N-acetylglucosaminidase, arylsulphatase, and ribonuclease increased both in the red and white muscle samples during the days following exhausting exercise (Table 2, Fig. 1). The increases were more prominent in the red muscle. In addition, the highest activities of β-glucuronidase, β-N-acetylglucosaminidase and ribonuclease were noticed already 3 days after exercise in the red and 5 days after exercise in the white muscle. Acid hydrolase activities commenced to decrease 5 days after loading (Fig. 1). β-Glucuronidase and ribonuclease activities were, during the whole follow-up period, higher in the exhausted animals than in the controls (Table 2).

The activity of *p*-nitrophenylphosphatase decreased for 2 days after the loading in both red and the white muscle. Simultaneously protein concentration decreased in the both muscle types. *p*-Nitrophenylphosphatase activity was higher than in controls 5 days after exercise. This increase was significant only in the white muscle. In malate dehydrogenase activity no major changes were observed (Table 2).

Correlations Between Acid Hydrolase Activities in Different Muscle Types. The correlations of β-glucuronidase, β-N-acetylglucosminidase, arylsulphatase, ribonuclease, and cathepsin D activities in *m. rectus femoris* and separately in red and white parts of *m. quadriceps femoris* were highly significant. The correlation coefficients were highest (*r* values from 0.843 to 0.970) in the red muscle in which the range and variation in activities were also largest. The most marked correlations were observed between β-glucuronidase and β-N-acetylglucosaminidase (*r* values: red 0.970, white 0.860 and mixed muscle 0.938). Correlations between activities of close functional similarity (e.g. β-glucuronidase and β-N-acetylglucosaminidase) were generally stronger than between activities representing less related pathways of break-down (e.g. cathepsin D and β-glucuronidase).

Table 1. Activities of acid hydrolases and malate dehydrogenase in *m. rectus femoris* of mice following an acute, exhaustive running exercise (E1, E2, etc. days after exhaustion). Mean ± S.D. Numbers in parentheses below the means give the number of observations in each case

Variable	Group							
	controls	time after exhaustion, days						
		E1	E2	E3	E5	E7	E10	E15
β-Glucuronidase[a]	8.2 ± 1.0 (34)	8.9 ± 0.7*[b] (15)	16.0 ± 5.2*** (16)	22.3 ± 14.7*** (26)	26.9 ± 18.2*** (29)	21.4 ± 13.5*** (31)	17.6 ± 7.1*** (10)	12.1 ± 3.3*** (11)
β-N-Acetylglucosaminidase	243 ± 32 (34)	231 ± 32 (15)	267 ± 46* (16)	353 ± 159*** (26)	393 ± 207*** (29)	350 ± 136*** (31)	286 ± 66* (10)	243 ± 26 (11)
Arylsulphatase	27.3 ± 4.0 (33)	26.4 ± 3.5 (13)	37.0 ± 9.7*** (16)	43.1 ± 17.9*** (25)	47.3 ± 19.4*** (28)	43.0 ± 18.0*** (30)	34.2 ± 8.9** (10)	30.3 ± 6.8 (11)
Ribonuclease	251 ± 28 (34)	251 ± 37 (15)	316 ± 57*** (16)	346 ± 60*** (26)	353 ± 83*** (29)	337 ± 66*** (31)	292 ± 35*** (10)	290 ± 24*** (11)
Cathepsin D	143 ± 12 (34)	133 ± 10** (15)	142 ± 13 (16)	159 ± 25** (26)	173 ± 38*** (29)	175 ± 38*** (31)	159 ± 24* (10)	140 ± 15 (11)
p-Nitrophenylphosphatase	1176 ± 125 (29)	1096 ± 107 (8)	1058 ± 47* (8)	1203 ± 99 (21)	1243 ± 148 (21)	1239 ± 153 (28)	–	1117 ± 77 (8)
β-Glycerophosphatase	99 ± 21 (29)	89 ± 7 (8)	82 ± 17* (8)	101 ± 20 (21)	100 ± 20 (18)	101 ± 22 (25)	–	96 ± 24 (8)
Malate dehydrogenase	143 ± 28 (34)	–	144 ± 34 (16)	139 ± 31 (26)	147 ± 40 (29)	132 ± 34 (31)	138 ± 28 (10)	132 ± 17 (11)

[a] Activities of acid hydrolases are expressed as pmol substrate hydrolyzed/min/mg fresh muscle at 37° C for β-glucuronidase, β-N-acetylglucosaminidase, arylsulphatase, β-glycerophosphatase, and *p*-nitrophenylphosphatase, for cathepsin D as pmol tyrosine equivalents solubilized at 37° C/min/mg fresh muscle, for ribonuclease as pmol nucleotides solubilized at 37° C/min/mg fresh muscle and for malate dehydrogenase as nmol NADH oxidized/min/mg fresh muscle at 25° C.

[b] Significances: *** = $P < 0.001$, ** = $P < 0.01$ and * = $P < 0.05$

Table 2. Activities of acid hydrolases and malate dehydrogenase in red and white parts of *m. quadriceps femoris* of mice following an acute exhaustive exercise (E1, E2, etc. days after exhaustion). Activity units and statistical signs as in Table 1. Figures in parentheses below the means give the number of observations in each case. Mean ± S.D.

Variable		Group							
		controls	time after exhaustion						
			E1	E2	E3	E5	E7	E10	E15
β-Glucuronidase	white	7.2 ± 1.0	8.8 ± 1.4**	15.3 ± 4.6***	20.7 ± 8.1***	22.5 ± 7.7***	16.4 ± 6.6***	14.5 ± 5.1***	10.7 ± 2.4***
	red	12.0 ± 1.6 (26)	15.2 ± 3.0** (7)	43.2 ± 16.0*** (16)	57.7 ± 23.7*** (20)	53.7 ± 23.6*** (23)	34.5 ± 15.9*** (25)	25.3 ± 7.0*** (10)	18.3 ± 3.0*** (11)
β-N-Acetylglucosaminidase	white	209 ± 33	211 ± 34	238 ± 41*	313 ± 85***	326 ± 87***	268 ± 63***	244 ± 53*	205 ± 25
	red	341 ± 47 (24)	325 ± 63 (7)	712 ± 248*** (16)	873 ± 306*** (17)	769 ± 267*** (22)	531 ± 188*** (23)	452 ± 118* (10)	363 ± 55 (11)
Arylsulphatase	white	25.4 ± 4.9	22.9 ± 3.7	31.0 ± 8.1*	39.8 ± 9.3***	42.0 ± 9.8***	34.5 ± 10.6***	27.3 ± 4.1	25.5 ± 5.8
	red	38.8 ± 6.8 (25)	44.3 ± 15.2 (5)	79.2 ± 25.6*** (16)	109.6 ± 35.5*** (19)	111.7 ± 29.9*** (22)	82.9 ± 35.8*** (24)	58.2 ± 16.2*** (10)	44.7 ± 10.0* (11)
Ribonuclease	white	244 ± 36	254 ± 18	317 ± 64***	341 ± 59***	336 ± 50***	314 ± 46***	290 ± 31**	288 ± 32**
	red	299 ± 44 (26)	332 ± 57 (7)	462 ± 89*** (16)	522 ± 68*** (20)	506 ± 63*** (23)	447 ± 95*** (25)	410 ± 67*** (10)	383 ± 58*** (11)
p-Nitrophenylphosphatase	white	1216 ± 82	–	1087 ± 76***	1276 ± 121	1308 ± 102**	1261 ± 119	–	1167 ± 54
	red	1555 ± 164 (21)	–	1196 ± 104*** (8)	1626 ± 222 (15)	1675 ± 168* (15)	1609 ± 207 (22)	–	1396 ± 189* (8)
Malate dehydrogenase	white	114 ± 24	–	111 ± 17	119 ± 26	126 ± 37	118 ± 37	116 ± 33	111 ± 19
	red	299 ± 59 (26)	–	255 ± 57* (16)	261 ± 43* (20)	279 ± 68 (23)	261 ± 60* (25)	297 ± 74 (10)	261 ± 27 (11)

Discussion

The samples of mouse red skeletal muscle contained higher activities of all the seven acid hydrolases tested than the corresponding white muscle samples. Similar differences in some acid hydrolases have earlier been reported for the muscles of rats [11] and guinea pigs [17], and they are in agreement with our recent histochemical data on mouse muscles [30]. The muscle type difference was more pronounced in the activities of the glycosidases and arylsulphatase than in cathepsin or, especially, in ribonuclease and the phosphatases. The muscle type difference may indicate functional differences between the various hydrolase groups in the two fibre types.

Total activities of acid hydrolases in skeletal muscle originate from a heterogeneous cell population. Activities of β-glucuronidase, β-N-acetylglucosaminidase, and β-galactosidase are histochemically higher in red than in white fibres [11,30]. High levels of activity have also been observed locally in some interstitial cells (probably fibroblasts and other mononuclear cells). However, muscle fibres most probably contribute the bulk of the acid hydrolase activities in the samples [4,30]. The histochemical location of ribonuclease or cathepsin D is not known. Some biochemical evidence suggests that cathepsin D activity would originate principally in muscle fibres [4,13]. Skeletal muscle acid hydrolase activities are partly lysosomal in origin [4,19]. However, fractionation and EM-histochemical studies suggest that some acid hydrolase activity is localized in the sarcoplasmic reticulum [22,25].

Acid hydrolytic capacity increased during the period following exhausting exercise. Changes in the activity of acid phosphatases, however, were different from those of the other acid hydrolases. Both β-glycerophosphatase and *p*-nitrophenylphosphatase activities decreased, like protein content, during two days after exertion. These decreases can be explained by oedema, which occurs in heavily exercised muscles [7,10]. In the simultaneous histochemical study interstitial oedema was also observed [30]. Between 3 and 15 days after exhaustion no major changes in acid phosphatase activities were observed. If the increased acid hydrolase activities after exhaustion originate from lysosome-like particles or from structures functionally similar to lysosomes, this lysosomal system, in its activated form, contains the five enzymes but not the two acid phosphatases.

The results of the simultaneous histochemical study suggest that the bulk of the increases in the activity of β-glucuronidase and β-N-acetylglucosaminidase occurred in the muscle fibres, although these activities also increased in connective tissue and were high in mononuclear cells found in the muscle 2–5 days after exercise [30]. Therefore, a part of the increased total activities originate from the interstitial tissue. The fact that no major increases in acid phosphatases were observed, indicates a minor role of e.g. inflammatory cells, which contained high activities of β-glycerophosphatase [30].

The sharp exhaustion-induced increase of acid hydrolytic capacity followed by a slower decrease resembles changes caused by temporary ligation ischaemia in rabbit skeletal muscle [24]. In denervation (e.g. [19]), immobilization [14], and starvation (e.g. [3,4]) increases in activity are slower and the changes less prominent. Compared to transient experimental ischaemia there is a quantitative difference in the response of muscle tissue to exhausting exercise. Shannon et al. [24] report an 11-fold increase in β-glucuronidase activity, a significant increase in *p*-nitrophenylphosphatase activity and a strong inflammatory reaction in rabbit plantar muscle one week after 6 h ischaemia.

The highest increase in β-glucuronidase activity in red muscle was about five- and in white muscle three-fold. In specific activity units the highest increase in red muscle (45.7) was three-fold larger than that in white muscle (15.3). The intrinsic heterogeneity of the muscle samples, resulting from the presence of the other fibre type, probably underestimates the true difference between the fibre types. The response of acid hydrolytic capacity in the white part of *m. vastus lateralis* was almost identical to that found in *m. rectus femoris*. The high increase of acid hydrolytic capacity in red fibres may reflect their particular involvement during exercise or their particular sensitivity to disturbing agents, such as denervation [6], vitamin E deficiency [21], and ischaemia [15].

Slow and fast motoneurones are recruited differently during exercise. Motoneurones, which innervate units composed of red fibres, are stimulated at the beginning of the loading [8]. With an increasing level of tension, units composed of white fibres are also involved. The graded loading used to exhaust the experimental mice, probably provided for a continuous recruitment of red fibres whereas white fibres contracted regularly only at the highest speeds and even then not necessarily with the highest possible impulse frequency. Oxidative red fibres were thus probably exposed to interchange of aerobic and anaerobic conditions and this may have induced the increase of the acid hydrolases.

The observed increases in acid hydrolase activities are most probably due to de novo synthesis of enzyme protein, because changes in e.g. the latency, chemical properties, pH optima etc. of enzymes are improbable [19,32]. The relatively rapid decrease in the activities, observed particularly in red muscle, may show a fast

turnover of acid hydrolases. The halflife of β-glucuronidase in fibroblasts is 4–5 days [31]. Gross estimates of the turnover rates of the glycosidases and arylsulphatase from our data give similar values.

Acid hydrolase activities correlated significantly in all three muscle types. Intercorrelated activity changes support the concept of the synthetic origin of increased acid hydrolase activities. Synchronized activation is essential for the effective functioning of the lysosomal system. The highest correlations were observed between activities of functional similarity (i.e. β-glucuronidase, β-N-acetylglucosaminidase, arylsulphatase) indicating, perhaps, that the acid hydrolytic capacity is „activated" according to the material to be hydrolyzed (i.e. mucopolysaccharides and glycoproteins). The increase of cathepsin D activity after the glycosidases may show that the protein chains are hydrolyzed after polysaccharide moieties. The strong increase in certain acid hydrolase activities after exhaustive exercise suggests a specific induction of protein synthesis.

The marked increase in acid hydrolytic capacity shows disturbed homeostasis in muscle fibres [27, 32]. It also shows the importance of the lysosomal system during recovery and repair processes after exhaustion. The concentrations of several hormones in blood plasma and urine are transiently changed after physical loading. The role of these hormones in the activation of the skeletal muscle lysosomal system is not known. The activation of the lysosomal system in denervation occurs more slowly than the exhaustion-induced increase in acid hydrolytic capacity [19]. In the experimental ischaemia, the lysosomal system is activated in the ligated but not in contralateral leg [24]. Hormonal and neural regulation may therefore be of less importance in the case of exhausting exercise and it is more probable that a myogenic factor or factors activate the lysosomal system.

The lysosomal system is activated by various types of sublethal cell injury (e.g. [1]). Cell death and damage often occur in relation to available energy (e.g. [1, 27]). The functions of ion pumps both in the sarcolemma and in the sarcoplasmic reticulum [27, 33] are disturbed due to lack of ATP also in the cases of ischaemia and exhausting exercise. Disturbances in calcium equilibrium may cause fibre necrosis [26, 33, 34]. Accumulation of calcium ions in the sarcoplasm is accompanied by a simultaneous reduction in the capacity of sarcoplasmic reticulum to bind calcium [23]. Extra calcium is accumulated in mitochondria and nuclei [33, 34]. Prolonged calcium accumulation first causes functional and later structural changes in mitochondria [26, 33]. Lack of energy is followed by fibre necrosis. In addition, high calcium concentration, per se, disrupts membranes and activates a myofibrillar protease [20, 27]. Disturbed calcium equilibrium might thus be a possible cause, among others, for fibre injuries.

Fibres are affected during exhaustive loading to a varying extent [30]. In sublethally affected fibres all mitochondria or supramolecular structures are not damaged to the same extent, since e.g. their localization may be favourable [9, 28]. Such structures remain intact and maintain cellular functions. The removal, by autophagy, of injured structures is a precondition for re-attaining cellular homeostasis in skeletal muscle.

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