BIOCHEMISTRY

Activity of Lysosomal Enzymes in White Muscle Disease

Deficiency of selenium and vitamin E in the diet of late-gestation ewes is known to cause white muscle disease (WMD) in lambs[1,2]. It is generally characterized by degenerative changes in skeletal and cardiac muscles. Since the myopathy is prenatally influenced, the development of the disease presumably has a direct relationship with the deprivation of protectors essential for the prevention of nutritional muscular dystrophy.

Lysosomes, a group of sub-cellular particles rich in a variety of acid hydrolases, have already been shown to occur in the dystrophic tissues of vitamin E-deficient rabbits[3] and chicks[4] and in the genetic dystrophy of mice and chickens[5]. Since white muscle disease is a degenerative disease of nutritional origin, I set out to investigate its lysosomal activity so as to make possible a comparison between the biochemical lesions of this disease and those observed in the dystrophies of other species. This report concerns the activities of five typical lysosomal enzymes in the muscle tissues of normal and dystrophic white muscle disease lambs.

Lambs, 4–6 weeks old and showing distinct symptoms of leg weakness were killed and the dystrophic tissues from leg muscles were collected and immediately frozen. Equivalent tissue samples were also collected from normal lambs of similar age. For chemical analysis, a 10 per cent tissue homogenate was made in ice-cold, de-ionized water by mincing the tissue in a Servall 'Omnimixer' for 1 min.

The activity of individual lysosomal enzymes was determined by several assays as follows. Acid phosphatase, β-glucuronidase and cathepsin were determined by the methods of Gianetto and de Duve[6]. β-Galactosidase was assayed by the method of Sellinger *et al.*[7]. Aryl sulphatase was assayed by the method of Roy[8] with 2-hydroxy-5-nitrophenyl sulphate as substrate. The specific activities of the enzymes are expressed in mμmoles substrate hydrolysed/mg nitrogen/min.

Table 1. Lysosomal Enzymes in Leg Muscle of Normal and Dystrophic WMD Lambs

Enzyme	Specific activity* Control	Specific activity* Dystrophic WMD	Increase in lysosomal activity over control
Aryl sulphatase	0·16	5·63	35 ×
β-Glucuronidase	3·01	15·18	5 ×
β-Galactosidase	0·64	1·80	3 ×
Cathepsin	0·14	0·44	3 ×
Acid phosphatase	4·64	9·98	2 ×

* Expressed as mμmoles of substrate hydrolysed per mg nitrogen per min. Values represent average of two lambs in each group.

The specific activities of five typical lysosomal enzymes in muscle tissues of normal and dystrophic white muscle disease lambs are presented in Table 1. The activity of individual lysosomal enzymes in dystrophic tissues was greater than in the controls, and the increases were as follows: aryl sulphatase, 35-fold; β-glucuronidase, 5-fold; β-galactosidase and cathepsin, 3-fold; acid phosphatase, 2-fold. These ratios are similar in magnitude to those observed in dystrophic muscle tissues of vitamin E, selenium and sulphur amino-acid-deficient chicks[4] and vitamin E-deficient rabbits[3]. Increased 5-nucleotidase activity in white muscle disease of lambs[5] may also be associated with the lysosomal complex.

It appears from all these observations that the phenomenon of increased lysosomal activity is directly associated with degradative changes occurring in tissues undergoing dystrophy. A recent time-sequence study[10] of the appearance of nutritional muscular dystrophy in chicks indicated that initiation of the primary damage is usually caused by the deficiency of the tissue protectors and that the activities of the lysosomal enzymes increase as a result of the infiltration of macrophages, the carriers of lysosomes. Although lysosomes may not be the primary initiators of the tissue damage, the greatest part of the hydrolytic breakdown in a tissue undergoing dystrophy is the result of large increases in free and total activities of various lysosomal hydrolases. Recent studies showed that isolated lysosomes are fully capable of digesting most cellular constituents[11]. The triggering mechanism responsible for the involvement of lysosomes in muscular dystrophy still remains obscure.

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Abnormal Isoenzyme Patterns in Human Myopathies

Proteins may exist in different molecular forms possessing the same enzymatic activity and which have been called isoenzymes[1] or isozymes[2]. Lactate dehydrogenase has been extensively studied and appears to consist of five isoenzymes, each of which is a tetrameric association of two sub-units, M and H. It has been shown that the synthesis of either sub-unit may be altered in response to hormonal, environmental or neural changes[3]. Evidence has also been presented which suggests that the synthesis of the two sub-units is controlled by two separate genes[4].

In normal adult human skeletal muscle, the slowest isoenzyme, LDH-5, predominates[5–7]. (By convention[1], the isoenzyme having the highest mobility towards the anode is numbered 1.) In normal foetal skeletal muscle, LDH-5 possesses little or no activity[8,9]. Recently, Emery reported that LDH-5 is also absent in skeletal muscle from victims of Duchenne muscular dystrophy[8], but this gross aberration has not been found in all cases of Duchenne dystrophy[10].

The isoenzyme patterns of NADP-linked isocitrate dehydrogenase (ICDH), malate dehydrogenase (MDH) as well as lactate dehydrogenase (LDH) were investigated electrophoretically in human myopathies to see if any alterations were specific for one disease.

Biopsy samples were removed from the gastrocnemius muscle, cooled in ice and processed immediately. The specimens were dropped in 0·25 M sucrose and blotted to remove blood. A 100-mg sample of tissue was then homogenized briefly in a small glass tissue grinder. Then 0·2 ml. of distilled water were added and the homogenization was continued. The whole procedure was performed in an ice-bath and the homogenization was discontinued frequently to prevent the accumulation of heat. The time occupied in homogenization was 2 min. The homogenate was transferred to a micro-centrifuge tube and was frozen and thawed once, after which it was centrifuged at 20,000g and 0° C for 15 min. The resultant supernatant

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