Alkaline Phosphatase in Normal and Diseased Human Muscle[1] (36704)

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In a recent report, Engel and Cunningham (1) have demonstrated histochemically that positive staining with the alkaline phosphatase reaction is characteristic of a particular type of abnormal fiber in human skeletal muscle. Numerous positive fibers have been found in Duchenne dystrophy, other active dystrophies and in active polymyositis. Such fiber staining is also numerous in denervation atrophy of moderate or severe degree in both adults and children. In contrast, no positive fiber reaction has been found in normal muscle biopsies or in minimally abnormal muscle from persons with various benign disorders.

In view of these histochemical findings we have measured quantitatively the level of alkaline phosphatase in skeletal muscle from normal persons and in patients with various muscular and neuromuscular diseases and have observed a positive correlation between the degree of histopathologic changes in the muscle and its level of enzyme activity.

Materials and Methods. Muscle specimens were biopsies of deltoid, gastrocnemius or quadriceps obtained from 23 patients with various muscle and neuromuscular diseases aged 5–62 years, diagnosed on the basis of clinical, histological and biochemical tests. In some cases multiple biopsies of muscle samples were obtained from the same patient at one surgery. The normal samples of deltoid and quadriceps were obtained at surgery on 7 patients aged 5–63 years, who showed no evidence of muscle disease. In all cases the biopsy samples were frozen as soon as possible (usually within 30 min) and the activity of alkaline phosphatase was measured the following day.

A portion of muscle tissue was routinely examined histologically (2) and rated as "mildly" affected and "severely" affected according to the degree of tissue degeneration. Histopathological classification was based on the type and extension of changes in the muscle fibers, such as atrophy, hypertrophy, increase in the number and centralization of sarcolemmal nuclei, degenerative changes, and increase of interstitial fat and connective tissue.

The muscle (about 0.2 g) was freed from any visible fat and connective tissue and was minced finely with scissors. The minced muscle was homogenized with 9 vol of ice-cold distilled water in a Potter-Elvehjem homogenizer for 2 min with intermittent cooling in ice.

Alkaline phosphatase or orthophosphoric monoester phosphohydrolase (EC 3.1.3.1) was assayed with *p*-nitrophenyl phosphate as described by Dechatelet and Cooper (3). The reaction mixture contained 2 m*M* substrate, 50 m*M* 2-amino-2-methyl-1-propanol buffer (pH 10), 0.33 m*M* $MgCl_2$ and 50 μl of homogenate in a total volume of 1 ml. The mixture was incubated at 37° for 30 min. The reaction was terminated with 4 ml of 0.1 *N* NaOH. The absorbance at 420 nm was determined and referred to *p*-nitrophenol standards treated in the same manner. Controls in which the enzyme was added after incubation were routinely included in each determination.

The noncollagen protein was determined according to a method described before (4). One volume of muscle homogenate was mixed with 9 vol of 0.05 *N* NaOH and kept overnight at room temperature. The protein content of the clear supernate was determined

[1] This investigation was supported by U.S. Public Health Service Grant GM 15759 and by the Muscular Dystrophy Associations of America, Inc.

TABLE I. Alkaline Phosphatase Activity of Human Muscle Homogenates.[a]

Group	No. of muscles analyzed	Degree of tissue degeneration	Alkaline phosphatase (nmoles/mg NCP/hr)
Normal	7	—	21.7 ± 3.13[b]
Muscle diseases	13	Mild	23.7 ± 2.29
Neurogenic diseases	5	Mild	19.2 ± 3.03
Muscle and denervating diseases	10	Severe	90.0 ± 10.03[c]

[a] Individual values are shown in Fig. 1.

[b] Mean ± standard error.

[c] Significantly different from normal values ($p < .001$).

by the method of Lowry *et al.* (5).

Results and Discussion. The level of alkaline phosphatase in skeletal muscle from normal persons and from patients with various muscular and neuromuscular diseases is shown in Table I. The mean activity of alkaline phosphatase in mildly affected muscles from all the patients was not significantly different from that in normal muscles. Its activity was significantly increased in severely affected muscles from patients affected with diseases of various etiologic origin. The increased alkaline phosphatase activity correlated well with the severity of muscle damage. Multiple muscle biopsies, one of which was mildly abnormal and the other was severely abnormal from one patient with Duchenne dystrophy, one with facioscapulohumeral dystrophy, two with active polymyositis, and one with spinal muscular atrophy, showing, respectively, normal and elevated values for alkaline phosphatase clearly illustrated this relationship (Fig. 1). These biochemical data confirm the histochemical study of Engel and Cunningham (1). The data on alkaline phosphatase activity in normal human skeletal muscle was somewhat lower than those found in the skeletal muscle of other species (6).

The enzyme activity in the muscle samples presented in this report was referred to a base of "noncollagen protein" content, which is a simple and widely used reference for comparing enzyme activities in normal and diseased muscle (7). It must be emphasized, however, that a biopsy of diseased muscle may contain a mixture of histologically normal muscle fibers and fibers in various stages of degeneration and regeneration, together with proliferating connective tissue cells, fat cells and macrophages. A change in any or several of these may result in an increased enzyme activity referred to noncollagen protein. Some of these nonmuscle cells are known to contain large amounts of phosphatases which are active at alkaline pH. Histochemical studies of Golarz, Bourne and Richardson (8) demonstrated a greatly increased activity of certain phosphatases particularly of 5-nucleotidase in the proliferating endomysium of the affected muscles in human muscular dystrophy. These observations were later confirmed by quantitative techniques (9). Polymorphonuclear leukocytes are also rich sources of alkaline phosphatase (10). Furthermore, it was shown that the alkaline phosphatase activity of the polymorphonuclear leukocytes increased in inflammatory states (11). These observations suggest that the metabolically active connective tissue cells and polymorphonuclear leukocytes are probably the significant determinant of the alkaline phosphatase level in the severely abnormal muscles. Our results, showing increased activity of alkaline phosphatase only in the degenerated muscles, indicate that this enzyme may play some role in muscle destruction.

Summary. The activity of alkaline phosphatase in skeletal muscle from normal persons and from patients with various myopathies and neuropathies was determined by a quantitative technique. Increased enzyme activity was found only in the severely abnormal muscles in patients affected with diseases of various etiologic origin. The results of this study confirm the original reported histochemical findings about this enzyme and sug-

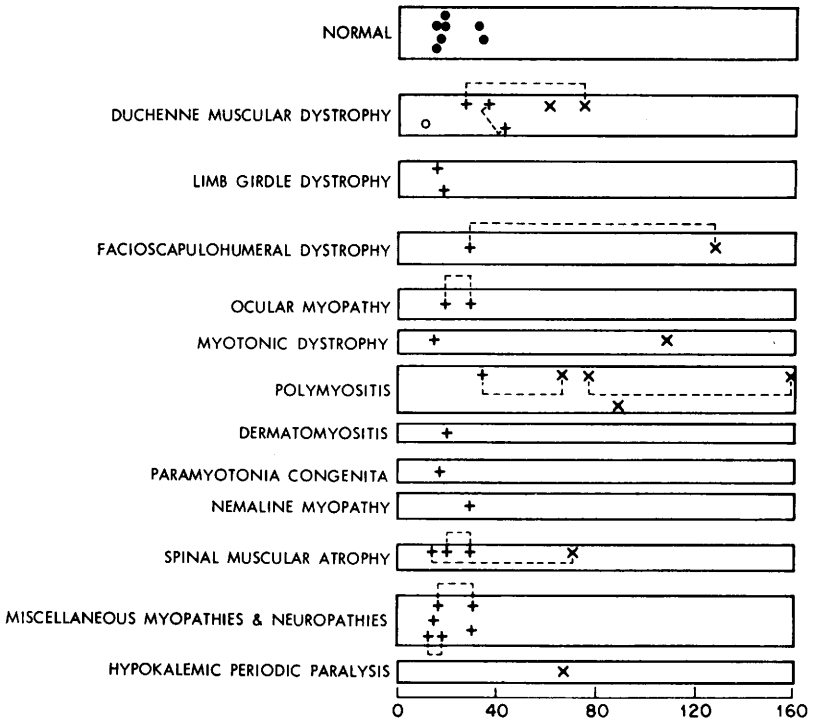

FIG. 1. Alkaline phosphatase activity of human skeletal muscles. Activity is expressed as nanomoles of nitrophenol released per milligram of noncollagen protein per hour. (●) normals; (○) Duchenne dystrophy carrier; (+) mildly affected muscles; (×) severely affected muscles. Symbols joined by tie lines (- - -) represent samples obtained at one operation from different sites on the same patient.

gest that the increased activity of alkaline phosphatase in diseased muscles is secondary to the pathologic changes. It may also be involved in some fashion in the process of muscle destruction.

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Received Apr. 28, 1972. P.S.E.B.M., 1972, Vol. 141.