# Serum β-Acetylglucosaminidase and Arylsulfatase A in Inflammatory Disorders of Muscle and Connective Tissue[1] (36700)

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Polymyositis and dermatomyositis are members of a group of disorders in which muscular weakness is the principal clinical feature and inflammatory or degenerative lesions of the striated muscles are the chief pathological characteristics (1). In recent years there has been considerable interest in the possibility that lysosomal acid hydrolases may play an important role in inflammation (2–5). It is suggested that cells, which include blood polymorphonuclear leukocytes and tissue macrophages, might migrate to the inflammatory sites and there partially discharge their lysosomal enzymes into the extracellular spaces, probably as a result of aggressive endocytosis (6, 7). Increased activities of lysosomal enzymes have been found in inflamed tissues in connective tissue disorders (8–10) and also in experimentally induced inflammation in animals (11). It has also been shown that the beneficial action of anti-inflammatory drugs may result, at least in part, from lysosomal enzyme inhibition (12, 13), stabilization of cell or lysosomal membranes (14, 15), or decreased influx of leukocytes (16). It seemed to us, therefore, to be of interest to measure the activities of lysosomal enzymes in serum from patients with various inflammatory conditions including polymyositis, systemic lupus erythematosus (SLE), and other systemic collagen diseases and to compare them with those present in serum from patients with some noninflammatory conditions such as the muscular dystrophies and some neuromuscular disorders. Two lysosomal indicator enzymes, β-acetylglucosaminidase (17) and arylsulfatase A (18), have been studied. Serum level of creatine kinase (CK) has also been determined as an indicator of skeletal muscle involvement.

*Materials and Methods.* The 160 patients, of both sexes, drawn from the Muscle Disorder Clinic and the Immunosuppressive Clinic at the UCLA Hospital, ranged from 1 to 67 years in age. Thirty-five had polymyositis or dermatomyositis, 65 had muscular dystrophies and other primary myopathies, 15 had neurogenic diseases, 33 had SLE (most with nephritis), 4 had scleroderma (progressive systemic sclerosis), and 8 had various other collagen–vascular disorders. Control sera were obtained from normal volunteers or were drawn from normal siblings of patients with muscular dystrophy who attended the Muscle Disorder Clinic. The 34 males and 49 females in the control group ranged in age from 5 to 58 years. Information was obtained about the duration of the disease, drug therapy (past and present) and the extent and activity of the disease. Routine laboratory data including erythrocyte sedimentation rates (ESR), total leukocyte counts and serum levels of antinuclear antibody titers, bilirubin, albumin, globulin and alkaline phosphatase were obtained in all patients with myositis and connective tissue disorders. At the time of this study most of the patients with SLE were undergoing treatment with the immunosuppressive drug, azathioprine, along with prednisone. Most enzyme determinations were done on the same day the serum was obtained. A few sera were kept frozen at −18° and determinations were made within 1 week and on only once-thawed serum.

β-Acetylglucosaminidase [β-2-acetamido-2-deoxy-D-glucoside acetamidodeoxyglucohydrolase (EC 3.2.1.30)] activity was deter-

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TABLE I. Serum Enzyme Levels in Inflammatory Myopathies, Muscular Dystrophies and Related Disorders.

| Disease | No. of cases | β-Acetylglucosaminidase (nmoles/ml/min) | | | Arylsulfatase A (nmoles/ml/hr) | | | CK (nmoles/ml/ | |
|---|---|---|---|---|---|---|---|---|---|
| | | Range | Mean | %E[a] | Range | Mean | %E | Range | Mea |
| | 83 | 5.7–15.0 | 11.0 | — | 8.4–26.0 | 16.5 | — | 18–60 | 4 |
| yositis | 18 | 6.8–23.5 | 15.3 | 55 | 8.4–34.2 | 22.0 | 39 | 18–910 | 24 |
| tomyositis | 17 | 6.8–34.8 | 16.4 | 41 | 8.4–35.6 | 21.4 | 29 | 3–191 | 4 |
| ıne muscular dystrophy | 20 | 6.0–13.2 | 9.0 | 0 | 8.6–16.8 | 13.2 | 0 | 190–7728 | 345 |
| girdle dystrophy | 5 | 7.2–10.6 | 8.7 | — | 8.4–15.0 | 9.7 | — | 109–344 | 23 |
| muscular dystrophy | 5 | 7.6–15.0 | 12.2 | — | 8.4–26.0 | 16.6 | — | 70–429 | 21 |
| capulohumeral dystrophy | 11 | 7.6–14.6 | 10.8 | 0 | 8.4–19.8 | 14.7 | 0 | 29–3.8 | 11 |
| nic dystrophy | 8 | 8.2–15.0 | 11.5 | 0 | 6.1–26.0 | 13.5 | 0 | 12–114 | 5 |
| laneous myopathies | 20 | 6.0–14.6 | 10.5 | 0 | 6.5–23.8 | 14.9 | 0 | 27–327 | 13 |
| muscular atrophy | 9 | 9.1–16.4 | 11.8 | 11 | 12.9–20.0 | 16.6 | 0 | 54–504 | 18 |
| eal muscular atrophy | 4 | 9.1–16.6 | 12.5 | — | 10.3–19.5 | 14.5 | — | 59–145 | 11 |
| rophic lateral sclerosis | 1 | | 28.8 | | | 27.7 | | | |
| henia gravis with sarcoid myopathy | 1 | | 15.0 | | | 21.3 | | | 14 |

[a] ntage of determinations with values above normal limit.

mined using *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide as substrate in citrate buffer (19). The reaction mixture contained 100 m*M* citrate buffer (pH 4.5), 1 m*M* substrate, and 0.1 ml serum in a total volume of 1 ml. The reaction was terminated, after incubation at 37° for 1 hr, with 4 ml of 0.1 *N* NaOH. The absorbance of 420 nm was determined and referred to *p*-nitrophenol standards treated in the same manner. Arylsulfatase A or arylsulfate sulfohydrolase (EC 3.1.6.1) was assayed with *p*-nitrocatechol sulfate at pH 5.0 as described previously (20). The reaction mixture contained 100 m*M* sodium acetate buffer, 5 m*M* substrate, $2.5 \times 10^{-4}$ *M* sodium pyrophosphate and 5% (w/v) NaCl and 0.5 ml serum. The tubes were incubated at 37° for 1 hr. The reaction was stopped with 1.5 ml of 1 *N* NaOH. The absorbance of 515 nm was determined and referred to *p*-nitrocatechol standards treated in the same manner. Controls in which the serum was added after incubation was kept for each determination. In each enzyme assay procedure the linearity of the assays with time and enzyme concentration was checked. CK [ATP:creatine phosphotransferase (EC 2.7.3.2)] was determined by a previously described method (21) using preweighed reagent vials obtained from Calbiochem in Los Angeles.

*Results. Effect of storage.* The effect of storage on serum β-acetylglucosaminidase and arylsulfatase A was studied. Serum samples frozen at —18° for 1 or more weeks showed almost no changes in β-acetylglucosaminidase activity. The activity of arylsulfatase A declined about 33% in 1 month (data not shown).

*Values in normal individuals.* Serum levels of β-acetylglucosaminidase, arylsulfatase A and CK in normal individuals are shown in Table I. There is little difference in serum β-acetylglucosaminidase and arylsulfatase A values between children and adults. The serum CK level in normal children is slightly higher than that in adults, but the range of values is the same in both groups.

*Values in inflammatory myopathies, muscular dystrophies and related disorders.* The results of serum β-acetylglucosaminidase, arylsulfatase A and CK assays in patients with inflammatory myopathies, muscular dystrophies and other primary myopathies, and neurogenic diseases are shown in Table I. The patients with Duchenne muscular dystrophy, other dystrophies and noninflammatory myopathies all showed serum β-acetylglucosaminidase and arylsulfatase A within the normal range. A majority of the patients with neurogenic disease showed normal values for these acid hydrolases. Two cases showed slightly increased values for β-acetylglucosaminidase: a 50 year old man with peroneal muscular atrophy, and a 48 year old man with spinal muscular atrophy. A 62 year old woman with amyotrophic lateral sclerosis showed greatly elevated values for both enzymes.

Increased activities of serum β-acetylglucosaminidase and arylsulfatase A were found only in the group of patients with inflammatory myopathies. About half of these patients with polymyositis or dermatomyostis showed elevated serum β-acetylglucosaminidase activity. Raised levels of arylsulfatase A were found in 34% of the patients in this group.

High serum CK values were constantly present in patients with Duchenne dystrophy, limb-girdle dystrophy and adult onset muscular dystrophy. Lesser elevated CK values were found in facioscapulohumeral dystrophy and myotonic dystrophy. Only 40% of the patients with myotonic dystrophy showed slightly elevated values. High serum CK activity was also found in 3/4 cases of peroneal muscular atrophy, 6/9 cases of spinal muscular atrophy and 1 case of myasthenia gravis. In the group of patients with inflammatory myopathies (35 cases), elevated serum CK activity was found in 67% of patients with polymyositis and in 22% of patients with dermatomyositis.

The highest value of serum β-acetylglucosaminidase (34.8 nmoles/ml/min) in inflammatory myopathies was found in a 49 year old woman with typical skin rash of dermatomyositis and with no active muscle involvement. This patient had normal serum CK. A rise in serum CK activity has been shown to generally be proportional to the rate and severity of the myopathic process. Seri-

TABLE II. Serum Enzyme Levels in SLE and Other Systemic Collagen Diseases.

| Disease | No. of cases | β-Acetylglucosaminidase (nmoles/ml/min) | | | Arylsulfatase A (nmoles/ml/hr) | | | Creatine kinase (nmoles/ml/min) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Range | Mean | %E[a] | Range | Mean | %E | Range | Mean | %E |
| None | 83 | 5.7–15.0 | 11.0 | — | 8.4–26.0 | 16.5 | — | 18–60 | 43 | — |
| SLE | 33 | 9.8–27.1 | 18.2 | 73 | 8.4–56.4 | 26.7 | 30 | 0–104 | 24 | 6 |
| Progressive systemic sclerosis | 4 | 7.6–22.7 | 12.5 | — | 9.8–38.7 | 23.3 | — | 13–74 | 40 | — |
| Miscellaneous disorders[b] | 8 | 8.2–37.1 | 19.5 | 50 | 8.4–40.0 | 20.7 | 25 | 3–87 | 25 | 12 |

[a] Percentage of determinations with values above normal limit.

[b] Includes these patients: polyarteritis, 1; vasculitis, 3; temporal arteritis, 1; Wegener's granulomatosis, 1; renal hypertension, 1; collagen–vascular disease undefined, 1.

al determinations of serum CK are by far the best laboratory guides to the status of illness as well as to response to treatment in patients with polymyositis (22). It is of interest to note that increased serum β-acetylglucosaminidase activity was found in 3 patients with polymyositis and 6 patients with dermatomyositis who had normal serum CK. Some correlation was found between the serum level of this enzyme and active inflammatory skin lesions and the erythrocyte sedimentation rates in these patients. A 39 year old woman with typical skin rash of dermatomyositis, under treatment with prednisone and methotrexate, had a high serum β-acetygluocosaminidase activity (28.0 nmoles/ml/min) but normal serum CK. The skin lesions almost disappeared, and she gained strength after treatment for 6 months, and her serum β-acethylglucosaminidase returned to normal (13.7 nmoles/ml/min). No correlations were found between the levels of these hydrolytic enzymes and total blood leukocyte counts or the duration of the disease in general.

*Values in SLE and other systemic collagen diseases.* The serum levels of β-acetylglucosaminidase, arylsulfatase A and creatine kinase in patients with SLE or other collagen diseases are shown in Table II. Increased activity of serum β-acetylglucosaminidase was found in 24 out of 33 patients with SLE, in 3 out of 4 patients with polyarteritis or vasculitis (systemic or hypersensitivity). Three patients, one each with temporal arteritis, Wegener's granulomatosus or renal hypertension had normal serum β-acetylglucosaminidase activity. Increased serum arylsulfatase A activity was found in 14 out of 45 patients with SLE or other collagen diseases.

On the other hand, serum creatine kinase was normal in most of the patients. Only an occasional patient with SLE or systemic sclerosis showed serum creatine kinase values slightly higher than normal (Table II).

Raised β-acetylglucosaminidase and less often arylsulfatase A occurred particularly in patients in whom the disease was most active and severe. Thus it correlated, in general, with a high ESR and in patients who had high titers for antinuclear antibody. No cor-

relations were found between the serum level of these hydrolases and total blood leukocyte counts or the duration of the disease. The highest value for β-acetylglucosaminidase (37.1 nmoles/ml/min) was obtained in a 45 year old woman with systemic vasculitis. This patient was undergoing treatment with azathioprine and prednisone, and her serum creatine kinase and leukocyte counts were within the normal range. It must be emphasized, however, that we did not find any strict correlation between the serum level of β-acetylglucosaminidase and the severity of the disease condition. Thus, a 58 year old woman with severe vasculitis in the legs had a normal serum level (8.2 nmoles/ml/min).

*Discussion.* These data on the levels of β-acetylglucosaminidase and arylsulfatase A in normal human serum are in accord with those found by other workers (23, 24). The findings that the levels of these enzymes are normal in Duchenne muscular dystrophy and other dystrophies in which the level of serum CK is abnormally high suggests that skeletal muscle is not the source of increased serum levels of these hydrolases in polymyositis and dermatomyositis where the CK levels are often quite elevated. It should also be noted that normal human skeletal muscle contains a very low level of these hydrolases (25). An increase in the level of serum β-acetylglucosaminidase has been observed in patients with liver disease, particularly acute or chronic hepatitis (26). Marked elevations of β-acetylglucosaminidase and arylsulfatase A along with certain other hydrolases were also found in the serum of patients with mucopolysaccharidoses types I and III (27, 28). The significance of these findings has not been ascertained. These serum elevations may be a reflection of the changes in enzyme activity of tissues generally. β-Acetylglucosaminidase was found to be grossly elevated in the skin (27), liver (29) and brain (30) in type I mucopolysaccharidosis. Normal liver function tests and normal serum level of alkaline phosphatase in our series of patients with polymyositis, dermatomyositis or SLE indicate that the liver is probably not involved. Also we did not find any correlation between the serum level of β-acetylglucosaminidase and the leukocyte counts. It appears, therefore, that the increased level of this enzyme is not due to a release from liver or to a rapid breakdown of circulating leukocytes, which are one of the richest sources of lysosomes (31). The results indicate that increased serum levels of β-acetylglucosaminidase and arylsulfatase A in inflammatory myopathies, SLE and other systemic collagen disorders are somehow related to the etiology or at least to the pathogeneses of these disease conditions. The elevated serum levels are probably the result of an increased release from lysosomes or an abnormal fragility of lysosomes present in the inflamed tissues. These observations are in accord with the hypothesis (4, 6) that lysosomal enzymes may be involved in the pathogenesis of inflammatory disorders. The role of either β-acetylglucosaminidase or arylsulfatase A in inflammation is not known. However, it is possible that β-acetylglucosaminidase, in conjunction with hyaluronidase and β-glucuronidase, may degrade acid mucopolysaccharides (32).

The various types of polymyositis are likely related to the connective tissue disorders, since a number of them show clinical and pathological overlaps, especially with such diseases as scleroderma and rheumatoid arthritis. Some similarities also exist in the skin manifestations and Raynaud's phenomenon (vasculitis) between SLE, scleroderma and polymyositis. The presence of a mild or transitory arthritis is also seen in many cases of polymyositis (33) and SLE. Increased serum β-acetylglucosaminidase activity has also been found in a few patients with active rheumatoid arthritis (unpublished data). These results showing increased serum β-acetylglucosaminidase in all these disease conditions suggests that similar biochemical degradative processes may be operative in this group of disorders.

*Summary.* Increased serum β-acetylglucosaminidase was found in some patients with active polymyositis or dermatomyositis, in a majority of patients with SLE, and in a few patients with local or systemic vasculitis. Arylsulfatase A was also increased, but only in a smaller number of patients. Enzyme

levels were normal in most patients with inactive myositis and always in Duchenne muscular dystrophy, other forms of dystrophy, various other myopathies and the majority of patients with neurogenic atrophies. High serum creatine kinase activity was nearly always present in the dystrophies and in a few patients with neurogenic disease and in one patient with myasthenia gravis.

This study suggests a correlation between lysosomal enzymes in the inflamed tissues and serum β-aetylglucosaminidase activity in certain inflammatory disorders of muscle, connective tissue and blood vessels.

1. Adams, R. D., Denny-Brown, D., and Pearson, C. M., *in* "Diseases of Muscle, A Study of Pathology," 2nd ed. Harper & Row (Hoeber), New York (1962).

2. DeDuve, C., and Wattiax, R., Annu. Rev. Physiol. **28**, 435 (1966).

3. Weissmann, G., and Dingle, J. T., Exp. Cell Res. **25**, 207 (1961).

4. Weissmann, G., Lancet **2**, 1173 (1964).

5. Dingle, J. T., Proc. Roy. Soc. Med. **55**, 109 (1962).

6. Weissmann, G., Annu. Rev. Med. **18**, 97 (1967).

7. Fell, H. G., Ann. Rheum. Dis. **28**, 213 (1969).

8. Luscombe, M., Nature (London) **197**, 1010 (1963).

9. Barland, P., Novikoff, A. B., and Hamerman, D., Tran. Ass. Amer. Physicians **77**, 239 (1964).

10. Hendry, N. G. C., and Carr, A. J., Nature (London) **199**, 392 (1963).

11. Anderson, A. J., Ann. Rheum. Dis. **29**, 307 (1970).

12. Anderson, A. J., Biochem. Pharmacol. **17**, 2253 (1968).

13. Ennis, R. S., Granda, J. L., and Posner, A. L., Arthritis Rheum. **11**, 756 (1964).

14. Weissmann, G., Arthritis Rheum. **9**, 834 (1966).

15. Tanaka, K., and Iizuka, Y., Biochem. Pharmacol. **17**, 2023 (1968).

16. Phelps, P., and McCarty, D. J., Jr., J. Pharmacol. Exp. Ther. **158**, 646 (1964).

17. Sellinger, O. Z., Beaufay, H., Jacques, P., Doyen, A., and DeDuve, C., Biochem J. **74**, 450 (1960).

18. DeReuck, A., and Cameron, M., 'Lysosomes." Little, Brown, Boston (1963).

19. Pugh, D., Leaback, H. D., and Walker, P. G., Biochem. J. **65**, 464 (1957).

20. Baum, H., Dodgson, K. S., and Spencer, B., Clin. Chim. Acta **4**, 453 (1959).

21. Hess, J. W., MacDonald, R. P., Natho, G. J. W., and Murdock, K. J., Clin. Chem. **13**, 994 (1967).

22. Fowler, W. M., Jr., and Pearson, C. M., Arch. Phys. Med. Rehabil. **45**, 125 (1964).

23. O'Brien, J. S., Okada, S., Chen, A., and Fillerup, D. L., N. Engl. J. Med. **283**, 15 (1970).

24. Begum, A., and Ittyerah, T. R., Clin. Chim. Acta **28**, 263 (1970).

25. Pearson, C. M., and Kar, N. C., Abstr., Int. Congr. Muscle Dis., 2nd, Excerpta Med., Int. Congr. Ser. **237**, 64 (1971).

26. Koizumi, T., Mitsutani, N., Kawata, H., Wada, M., and Yoshida, T., Lab. Invest. **13**, 752 (1964).

27. Öckerman, P. A., Clin. Chim. Acta **20**, 1 (1968).

28. Gordon, B. A., and Feliki, N., Clin. Biochem. **3**, 193 (1970).

29. Van Hoof, F., and Hers, H. G., Eur. J. Biochem. **7**, 34 (1968).

30. MacBrinn, M., Okada, S., Wollacott, M., Patel, V., WanHo, M., Tappel, A. L., and O'Brien, J. S., N. Engl. J. Med. **281**, 338 (1969).

31. Straus, W., *in* "Enzyme Cytology" (D. E. Roodyn, ed.), p. 239. Academic Press, New York (1967).

32. Linker, A., Meyer, K., and Weissmann, B., J. Biol. Chem. **213**, 237 (1955).

33. Pearson, C. M., Arthritis Rheum. **2**, 127 (1959).

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