

## Collagenase Activity in Tissues of Selenium Responsive Myopathic Lambs<sup>1</sup> (37306)

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(Introduced by Hugo Krueger)

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Previous work from this laboratory (1) has shown that skeletal muscle levels of  $\beta$ -glucuronidase and alkaline and acid phosphatases were significantly higher in crude homogenate preparations and in all of the intracellular fractions of the semitendinosus muscle in white muscle diseased (WMD) compared with normal lambs. The activities of these enzymes have also been reported to increase in muscle where the dystrophy has a genetic origin (2, 3). For example, increased levels of the lysosomal enzymes have been observed in inherited dystrophic muscle of mice (2) and chicks (2, 3). In addition, the proliferation of connective tissue characteristic of human muscular dystrophy is accompanied by appreciably greater 5'-nucleotidase (4) and acid and alkaline phosphatase (5) activity than is normally present.

Factors affecting collagenase activity have been studied (6) and its implication in rheumatoid arthritis and similar disorders in man have been discussed. Since collagenase appears to be a lysosomal enzyme (6) and since the free activity of other lysosomal enzymes (1, 10) have been reported to be elevated in muscle of WMD lambs, perhaps collagenase activity might be elevated in tissues of affected animals. Therefore, it was considered important to assay collagenase in tissues of WMD and normal lambs.

*Materials and Methods.* Prior to assaying for collagenase activity in lamb semitendinosus muscle, heart, liver and kidney, a

pH study was performed to determine a level for maximum enzyme activity. Since it is known that certain collagenolytic enzymes exhibit maximum activity at acid pH values, citrate-phosphate buffer (7) was employed in the range from pH 2.6 to 6.0 with the finding that the use of pH 3.0 buffer yielded the maximum amount of activity.

Substrate collagen was prepared essentially by the method of Anderson (6). Collagen from an extract of bovine Achilles tendon, was obtained from Sigma Chemical Co. and the fibers were ground to a fine powder in a micro-Wiley mill with a 40 mesh screen and then treated as described by Anderson (6). For collagenase, tissue samples of heart, kidney, liver and semitendinosus muscle were taken from 6 wk old lambs at necropsy and stored at  $-10^{\circ}$  until used. The enzyme activity was found to be similar in tissue which had been frozen and stored for a few days with other tissues which had been stored a few months. Four normal heart samples which had been stored frozen for several months yielded a mean enzyme level of 50.5  $\mu\text{g/g}$ , while four others stored only a few days had a value of 36  $\mu\text{g/g}$ . Four tissues from WMD lambs stored several months resulted in a mean value of 96  $\mu\text{g}$  while four different ones frozen a few days gave a collagenase activity measurement of 77  $\mu\text{g}$ . Lambs whose dams were fed low selenium alfalfa hay (about 0.018 ppm Se) and injected with 5 mg selenium, as sodium selenite, 3 times at 1 mo intervals prior to parturition, are referred to as normal lambs while those termed WMD were from uninjected ewes. Blocks of heart and semitendinosus muscle tissue were routinely selected, processed, examined histologically and rated according

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to the severity of tissue damage (8).

The release of hydroxyproline was used as a measure of collagenase activity in the various tissues. The tissue was prepared and incubated essentially according to the procedure of Anderson (6) except that the length of incubation was increased to 18 hr with the inclusion of suitable blanks. The undigested collagen was removed by centrifugation and the supernatant was acidified and autoclaved at 15 psi for 3 hr. The hydrolysates were then neutralized and assayed for free hydroxyproline according to the procedure of Neuman and Logan (9).

**Results and Discussion.** Collagenase activity was significantly greater in heart and semitendinosus muscle of WMD lambs compared to normal tissues (Table I). This appears to be in agreement with previous work (1, 10) with WMD tissues, indicating an increase in lysosomal enzyme activity in muscle of affected lambs. The heart and muscle samples, however, showed a different pattern in relation to severity of damage to the respective tissues. The collagenase activity was significantly increased in lambs with mild WMD lesions, whereas this activity in the heart tissue did not significantly increase until severe lesions were found in this tissue. This is the only lysosomal enzyme in our studies in which the activity has been found to be significantly different in heart tissue between normal and WMD lambs.

TABLE I. Collagenase Activity of Tissue Extracts from Control Lambs and Lambs with Different Levels of White Muscle Pathology ( $\mu\text{g}$  Hydroxyproline Released per g of Tissue).

Tissue	No. of lambs sampled:	
	13	8
	White muscle pathology:	
	Normal	WMD
Muscle	22.9 $\pm$ 1.6 <sup>b</sup>	87.6 $\pm$ 3.3 <sup>a</sup>
Heart	42.2 $\pm$ 6.1	90.1 $\pm$ 13.2 <sup>a</sup>
Liver	51.2 $\pm$ 6.7	55.2 $\pm$ 8.9
Kidney	217.9 $\pm$ 12.1	137.7 $\pm$ 58.7

<sup>a</sup> Values significantly higher than normal group

<sup>b</sup> Value represents mean  $\pm$  standard error of ( $p < .01$ ).

the mean (SEM).

No significant differences were found in the collagenase activity of liver and kidney extracts between normal and WMD lambs (Table I). Of the four tissues examined, the collagenase level was highest in the kidney. The significance of this particularly high activity is not readily apparent, but deserves further investigation.

The inclusion of the detergent Triton X-100 (0.2%) in the homogenizing solution generally yielded an increased activity of the WMD tissues. However, no increase in activity of the normal tissues was found over that when no Triton X-100 was used. Some selected normal tissues homogenized without Triton X resulted in an average value of 24  $\mu\text{g}$  hydroxyproline/g tissue, while the same tissues homogenized with Triton X yielded an average level of 26  $\mu\text{g}/\text{g}$ . Some selected WMD tissues homogenized without the detergent measured 143  $\mu\text{g}/\text{g}$  with an average increase to 216  $\mu\text{g}/\text{g}$  with the inclusion of Triton X.

Evidences that collagenase is a lysosomal enzyme are that its optimum pH activity is 3.0 and that it possesses latent activity as indicated by the inclusion of Triton X-100 in the homogenizing solution. One possible reason for the lack of effect of a detergent on normal tissues whereas a response was obtained with WMD tissues could be that the WMD tissues already have partially damaged lysosomes in which the enzymes are more loosely bound to the lysosomal membrane. Once they are lysed the Triton X can effect a release of enzymes. In the undamaged, intact lysosomes of the normal tissues, the enzymes are perhaps bound more tightly to the cell particles which the Triton X does not have the capacity to disrupt. Another point of view suggests that the increased proliferation of connective tissue, in response to WMD damage, requires increased lysosomal production to allow destruction of connective tissue fibers in order that the damaged muscle may be reconstituted. Collagenase activity would be tied to the necessity of removing connective tissue fibers.

An abnormally high collagenase has been implicated as a possible contributor to rheumatoid arthritis as well as similar dis-

orders (6). The present report indicates that selenium is required to maintain normal low levels of this enzyme in skeletal and cardiac muscle, thus providing evidence for the involvement of selenium in maintaining the integrity of the lysosomal membranes. Whether these results provide any health implications of this element in man is, of course, a matter of speculation.

*Summary.* Collagenase activity, measured in terms of amount of hydroxyproline released, was significantly higher in muscle and heart of white muscle diseased (selenium deficient) lambs than normal (selenium supplemented) lambs. No significant differences were found in the activity of this enzyme in liver and kidney between normal and deficient lambs. The significance of these results are discussed.

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