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Relationship of 5′-Nucleotidase Activity in Lamb Muscle to Selenium Levels Administered to their Dams.* (29760)

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White muscle disease (WMD) of sheep is a selenium responsive malady common to many areas of the world with an inherent deficiency of selenium in the forage. It has been previously reported(2) that lambs afflicted with this myopathy exhibit a marked elevation of 5′-nucleotidase activity in semitendinosus muscle tissue. Selenium administered to pregnant ewes will protect the subsequent lambs from developing WMD. To determine what the relationship of selenium to 5′-nucleotidase might be, muscle tissue specimens from lambs of both selenium treated and untreated dams were assayed for 5′-nucleotidase.

Methods and materials. The semitendinosus muscle tissue samples to be assayed were selected from lambs 6 weeks of age whose dams had been randomly allocated to experimental treatments as follows:

Lot 1—Control alfalfa hay (Union, Oregon) plus ¼ lb oats

Lot 2—Basal alfalfa hay (Madras, Oregon) plus ¼ lb oats

Lot 3—Basal alfalfa hay plus 0.1 ppm selenium with respect to the total ration in ¼ lb oats

Lot 4—Basal alfalfa hay plus ¼ lb oats plus 30 mg selenium in 10 ml water, given orally as a single dose

Lot 5—Basal alfalfa hay plus ¼ lb oats plus 30 mg selenium in wax parenterally as a single dose.

Selenium was administered as sodium selenite. The control hay (Lot 1) contained about 0.07 ppm, the basal hay 0.02 ppm and the oats 0.01 ppm selenium. The incidence of both gross and microscopic WMD lesions was nil in lambs from all lots except Lot 2, where 7 of 17 lambs developed lesions(1).

The semitendinosus muscle samples were obtained from freshly slaughtered lambs at 6 weeks of age, packaged in polyethylene bags, immediately frozen and stored for approximately 1 year at —20°C before being assayed. Six samples of muscle per treatment group were selected randomly, but equally distributed between sexes, except Lot 2 where afflicted lambs only were chosen.

The enzyme extract was initially prepared by transferring approximately 2 g of frozen muscle to a tissue press (Howard Apparatus Co., Inc., Dover, Mass.) with 5 ml of 0.05 M NaCl - 0.005 M Tris buffer, pH 8.3 and pressed to remove the bulk of the connective tissue. The macerated material less the connective tissue was transferred to a Potter-Elvehjem homogenizer and the residue pressed again with an additional 5 ml NaCl-Tris. The total pressed material was homogenized at 0-5°C in about 10 ml NaCl-Tris for 2 minutes with 1 minute rest time between minutes to prevent frictional heating and the smooth homogenate quantitatively transferred into polyethylene cups. The homogenate was centrifuged at 5,400 × *g* for 15 minutes at 0-5°C and the supernatant diluted to 10 × the weight of the tissue (10% tissue weight equivalent). Five ml of this enzyme extract was dialyzed overnight against two 125 ml portions of the NaCl-Tris. The remainder of the extract designated "Fresh" was retained for immediate use.

The assay procedure used by Schubert *et al* (2) was modified in that relative amounts

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TABLE I. Summary of 5'-Nucleotidase Activities μmoles P_i Released/hr/mg N.

	Fresh		Dialized	
Lot*	Mean	Standard error	Mean	Standard error
1	.40	± .20	2.56	± .18
2	4.33	±1.37	8.90	±2.44
3	.16	± .06	2.18	± .20
4	.12	± .06	2.42	± .10
5	.10	± .05	2.45	± .17

* 6 animals per lot.

of all reagents remained the same, but total amounts in the incubation tubes were reduced by a factor of 5. Two ml of 16% trichloroacetic acid were added directly to the tubes after incubation to stop the reaction. The precipitating protein was centrifuged down at 2500 rpm for 10 minutes and 2 ml of the brei withdrawn for subsequent color development. Phosphate determinations were made by the Fiske-Subbarrow procedure and the nitrogen analyzed by the micro Kjeldahl method. The actual determination of 5'-nucleotidase activity was done by subtracting the p-nitrophenylphosphate (NPP) hydrolysis representing general alkaline phosphatase activity from adenosine monophosphate (AMP) hydrolysis representing general and specific alkaline phosphatase activity. Appropriate enzyme and reagent blanks were also subtracted. Duplicate assays for each muscle sample were run on different days.

Results. Enzyme activity is expressed as micromoles inorganic phosphate released per hour per mg nitrogen in the extract. The general phosphatase activity from NPP hydrolysis is consistently negligible for all samples in the dialyzed preparations, and nitrogen values are consistently lower than those of the fresh extracts. Since dialyzing eliminates uncharacterized debris from the extract, which may or may not affect enzyme activity, it is felt that the dialyzed extract provides a more reliable measure of 5'-nucleotidase activity. The data obtained are summarized in Table I, and the mean level of 5'-nucleotidase in each respective lot from both fresh and dialyzed preps is evident. The individual dystrophic lambs showed a range of activity from about 2 to 7 times the mean of the control group (Lot 1) in the dialyzed extracts and an even greater relative enhancement for the fresh extracts, although the individual activities were always lower than the corresponding dialyzed values. The lambs from selenium treated ewes all reflected a lower mean 5'-nucleotidase activity than the normal control for both fresh and dialyzed preparations. Variation in methods of selenium administration did not produce any differences (P=.05).

Although the time at which 5'-nucleotidase becomes elevated with respect to the progression of the disease has not been definitely established, this research does confirm its enhancement. It is also of interest to note that 5'-nucleotidase activity of the lamb tissue responds to the various selenium treatments given to their dams. Likewise, hydrolytic enzymes of the lysosome are reported higher in certain nutritional muscular dystrophies including the chick(3), which also responds to selenium treatment. These enzymes differ, however, as they have an acid pH optimum(4). The metabolic role of selenium, its interrelationship with this enzyme, and its function in tissue calcification in various other species are being investigated.

Summary. The usual enhancement of 5'-nucleotidase activity in muscle tissue of white muscle diseased lambs is not apparent in muscle of lambs whose dams have received selenium supplements as sodium selenite in the diet orally or parenterally in aqueous or wax solutions.

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