NUTRITIONAL MUSCULAR DYSTROPHY IN CALVES. II. ADDITION OF SELENIUM AND TOCOPHEROL TO A BASAL, DYSTROPHOGENIC DIET CONTAINING COD-LIVER OIL

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SUMMARY

Nutritional muscular dystrophy was produced experimentally in Holstein-Friesian calves. The addition of cod-liver oil to the diet caused an intensification of Zenker's degeneration in the muscles of the tocopherol-deficient calves. One p.p.m. of selenium added to the basal diet did not prevent muscular dystrophy. The addition of 200 mg. of water-dispersible d-alpha-tocopheryl acetate per calf per day completely prevented the development of muscular dystrophy in all calves so treated. All calves which were not fed tocopherol supplements developed Zenker's degeneration of the skeletal and tongue muscles and degeneration of the Purkinje fibers of the heart. Since blood serum magnesium levels declined progressively in all calves in the experiment, it is possible that low blood magnesium levels may work synergistically with low blood tocopherol levels to produce Purkinje fiber degeneration.

In a previous report on nutritional muscular dystrophy in calves (27), the clinical aspects of the disease were outlined and the experimental production of muscular dystrophy in Holstein-Friesian calves was reported. The addition of 10% dried brewer's yeast or 0.85% dicalcium phosphate to the basal dystrophogenic diet did not prevent the occurrence of dystrophic lesions. The results of these experiments (27) and those of Holter, Smith, and Loosli (20), working simultaneously and independently, demonstrated that when a simplified, tocopherol-deficient diet low in unsaturated fats was fed to calves, a long feeding period was required to produce muscular dystrophy.

A more complete review of the literature is contained in the previous paper (27).

The present experiment was conducted to determine if, by the addition of cod-liver oil, the lesions of muscular dystrophy produced with the tocopherol-deficient diet would be hastened or intensified, and if the addition of selenium would prevent Zenker's degeneration under the given conditions.

Agduhr and Stenstrom (1), using an electrocardiogram, detected heart lesions in calves that had been fed cod-liver oil. Barnes, Davis, and McCay (4), however, did not detect changes in the electrocardiogram of calves which were fed good or very poor diets to which cod-liver oil was added. Their data tend to discredit the conclusions of Agduhr and Stenstrom (1). Workers at Cornell University demonstrated that cod-liver oil enhanced the production of muscular dystrophy in guinea pigs, rabbits, sheep, and goats (25). Davis and Maynard (15) found that calves were more resistant to the dystrophy-producing effects of cod-liver oil than were sheep and goats. When they added 0.7 g. of cod-liver oil per kilo-
gram of body weight to the diet of sheep and goats, dystrophy was produced in 90 days. Of six calves fed a level of 0.7 g. of cod-liver oil per kilogram of body weight per day, four developed muscular dystrophy, one at 42 days of age and the other three at 168 to 182 days of age. Davis, Maynard, and McCay (16) were not able to characterize the toxic effect of cod-liver oil.

Blaxter, Wood, and MacDonald (8) demonstrated that 18 ml. of cod-liver oil per day would nullify the action of 50 mg. of dl-alpha-tocopherol per day when the latter was used for the prevention of muscular dystrophy in calves. While cod-liver oil itself contains tocopherols, the effects of the unsaturated fatty acids present nullify the action of the tocopherols present in the cod-liver oil, as well as an appreciable quantity of tocopherols that are already present in the animal body (11, 30).

Schwarz (37, 38) showed that dietary liver necrosis in rats could be prevented by adding cystine and vitamin E to their diets. He also reported that dried brewer’s yeast contained an unidentified factor capable of sparing the amount of vitamin E required for the prevention of liver necrosis. Schwarz called the unidentified compound Factor 3. Schwarz and Foltz (39) reported that selenium in an organic form is an integral part of Factor 3. Inorganic selenium salts, however, were remarkably effective in protecting rats against dietary necrotic liver degeneration. Selenium will not, however, replace tocopherol for some of the deficiency changes which occur in tocopherol deficiency (13, 14).

Poultry diseases which were partially or completely prevented by adding tocopherol to the diet have also been found to be partially or completely prevented by adding selenium to the diet (34, 40).

Recent work with sheep (19, 32, 33) has indicated that a deficiency of selenium in the diet of dams is involved in the production of muscular dystrophy in lambs. The feeding of 1 p.p.m. of selenium to ewes, whose diets contained less than 0.1 p.p.m. selenium, effectively prevented the appearance of dystrophy in their lambs. McLean, Thomson, and Claxton (28) reported a growth response in six out of nine groups of lambs totalling 730, of different breeding and nutritional status when selenium was given by drench or injection.

A report of interest is that of Bunyan, Edwin, and Green (12), which demonstrated that trace elements other than selenium will, to some extent, also protect rats against dietary necrotic liver degeneration. Broberg (9) emphasized the high incidence of copper and cobalt deficiencies occurring in endemic muscular dystrophy areas in Finland. Andersson (2) demonstrated that in muscular dystrophy in rabbits, additional iodide in the diet increased the incidence and severity of Zenker’s degeneration. Blaxter and Sharman (7) found hypomagnesemia to be associated with muscular dystrophy of calves in Scotland.

**EXPERIMENTAL PROCEDURE**

Holstein-Friesian male calves were obtained from the Cornell University dairy herds. The experiment was begun on November 27, 1958, and concluded on April 18, 1959. The length of time the individual animals were on the test is shown in Table 1. The first four calves received were placed on the basal diet, plus
<table>
<thead>
<tr>
<th>Calf No.</th>
<th>Diet</th>
<th>Days on test</th>
<th>Necropsy findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>Basal</td>
<td>112&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Zenker's degeneration and Purkinje fiber degeneration</td>
</tr>
<tr>
<td>18</td>
<td>Basal</td>
<td>101&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Zenker's degeneration and Purkinje fiber degeneration</td>
</tr>
<tr>
<td>19</td>
<td>Basal</td>
<td>34</td>
<td>Died. <em>Escherichia coli</em> septicemia</td>
</tr>
<tr>
<td>20</td>
<td>Basal plus selenium</td>
<td>101&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Zenker's degeneration and Purkinje fiber degeneration</td>
</tr>
<tr>
<td>21</td>
<td>Basal plus selenium</td>
<td>97</td>
<td>Zenker's degeneration and Purkinje fiber degeneration</td>
</tr>
<tr>
<td>22</td>
<td>Basal plus selenium</td>
<td>97</td>
<td>Zenker's degeneration and Purkinje fiber degeneration</td>
</tr>
<tr>
<td>23</td>
<td>Basal plus selenium</td>
<td>95</td>
<td>Zenker's degeneration and Purkinje fiber degeneration</td>
</tr>
<tr>
<td>24</td>
<td>Basal plus selenium</td>
<td>15</td>
<td>Died. <em>E. coli</em> septicemia</td>
</tr>
<tr>
<td>25</td>
<td>Basal plus selenium</td>
<td>35</td>
<td>Died. <em>E. coli</em> septicemia</td>
</tr>
<tr>
<td>26</td>
<td>Basal plus selenium and tocopherol</td>
<td>94</td>
<td>No pathologic lesions</td>
</tr>
<tr>
<td>27</td>
<td>Basal plus selenium and tocopherol</td>
<td>94</td>
<td>No pathologic lesions</td>
</tr>
<tr>
<td>28</td>
<td>Basal plus tocopherol</td>
<td>30</td>
<td>Died. <em>E. coli</em> septicemia</td>
</tr>
<tr>
<td>29</td>
<td>Basal plus tocopherol</td>
<td>99</td>
<td>No pathologic lesions</td>
</tr>
</tbody>
</table>

<sup>a</sup> Cod-liver oil (30 ml.) fed daily to all calves.
<sup>b</sup> Biopsy of *semitendinosus* muscle positive for Zenker's degeneration on 96th day of test.
<sup>c</sup> Very mild Zenker's degeneration on histologic section.
cod-liver oil plus selenium, and the next two were allotted to the basal diet plus cod-liver oil and tocopherol, and the following two to the basal diet, plus cod-liver oil, tocopherol, and selenium. In addition, one of the selenium-treated calves which died was replaced.

Immediately following birth, the calves were housed in individual pens containing about 18 sq. ft. of floor space. The pens were divided by partitions 4 ft. high. Wood shavings were used as bedding.

As in the previous experiments (27), the calves were not allowed to suckle the dams. For the first two days, skinned colostrum was fed at the rate of 5% of body weight per day, divided into two equal feedings. Vitamin A (80,000 I.U.) and vitamin D (12,000 I.U.) were given in capsule form with the first feeding of skimmed colostrum. All of the calves received 30 ml. of cod-liver oil per day with the morning feed. This supplied at least 25,500 I.U. of vitamin A and 2,550 I.U. of vitamin D per day.

The treatment groups were (a) basal diet, (b) basal diet plus selenium 1 p.p.m., basal diet plus selenium, 1 p.p.m., and tocopherol, 200 I.U. water-dispersible d-alpha-tocopheryl acetate, and basal diet plus tocopherol, 200 I.U. water-dispersible d-alpha-tocopheryl acetate.

The basal diet was diluted to 12.5% solids and fed initially at 6% of body weight per day; this was increased gradually to 12% of body weight per day. The basal diet consisted of: Skimmilk (dried), 70.00; dextrose, 4.875; lard (stripped), 25.00; Tween 60, 0.125; mineral mixture, 1.75 ml. per 100 g. of diet. The mineral mixture contained in milligrams per milliliter: MnSO₄ • H₂O, 0.5; CuSO₄ • 5H₂O, 0.5; CoSO₄, 0.1; Fe(NH₄)₆(C₆H₅O₇)₂, 20.0; and MgSO₄, 60.0.

The procedure for collecting the blood samples and the analytical methods employed were the same as in the previous paper (27). Calves were bled before being placed on test, at 30-day intervals, and immediately before necropsy. In addition, serum magnesium levels were determined by the Titan-yellow method (23).

The selenium content of the basal diet was determined by activation analysis³ and the magnesium content of the basal diet was determined using the method of Barchra, Daver, and Sobel (3).

The tocopherol content of the cod-liver oil, shavings, and basal diet was determined by the method of Bro-Rasmussen and Hjärde (10).

The skimmilk used in the basal diet, and the basal diet, contained 57.6 and 161.6 mg. of magnesium per 100 g., respectively. The basal diet contained 0.3 p.p.m. selenium and 0.03 mg. of alpha plus zeta tocopherols per 100 g. The wood shavings contained 0.33 and 0.19 mg. of alpha plus zeta, and other tocopherols per 100 g., respectively, and the cod-liver oil contained 7.28 mg. of alpha tocopherol per 100 g.

The tocopherol content of the wood shavings was not high. While the calves did consume some of these shavings, the fact that the cellulose in wood shavings

³ Nutritional Biochemicals, Cleveland, Ohio.
³ Made by Activation Analysis Group, Oak Ridge National Laboratory, Oak Ridge, Tennessee.
NUTRITIONAL MUSCULAR DYSTROPHY. II.

TABLE 2
Mean blood constituents and cell counts

<table>
<thead>
<tr>
<th>Measure</th>
<th>Basal</th>
<th>Se</th>
<th>Se + Vit. E</th>
<th>Vit. E</th>
</tr>
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<tbody>
<tr>
<td>Plasma tocopherol (γ/100 ml.)</td>
<td>104</td>
<td>112</td>
<td>687</td>
<td>544</td>
</tr>
<tr>
<td>Serum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphorus (mg/100 ml.)</td>
<td>6.7</td>
<td>7.5</td>
<td>7.7</td>
<td>6.8</td>
</tr>
<tr>
<td>Calcium (mg/100 ml.)</td>
<td>11.4</td>
<td>11.4</td>
<td>10.9</td>
<td>11.0</td>
</tr>
<tr>
<td>Magnesium (mg/100 ml.)</td>
<td>1.5</td>
<td>2.1</td>
<td>1.6</td>
<td>1.4</td>
</tr>
<tr>
<td>Sodium (meq/l)</td>
<td>141</td>
<td>142</td>
<td>144</td>
<td>143</td>
</tr>
<tr>
<td>Potassium (meq/l)</td>
<td>5.4</td>
<td>5.8</td>
<td>5.8</td>
<td>5.5</td>
</tr>
<tr>
<td>Chloride (meq/l)</td>
<td>104</td>
<td>103</td>
<td>101</td>
<td>99</td>
</tr>
<tr>
<td>Hemoglobin (g/100 ml.)</td>
<td>12.9</td>
<td>11.7</td>
<td>12.2</td>
<td></td>
</tr>
<tr>
<td>White cell count (M/Cmm)</td>
<td>9.24</td>
<td>9.16</td>
<td>10.40</td>
<td>7.04</td>
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<tr>
<td>Differential cell count (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>41</td>
<td>45</td>
<td>53</td>
<td>61</td>
</tr>
<tr>
<td>Monocytes</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Granulocytes</td>
<td>47</td>
<td>51</td>
<td>45</td>
<td>36</td>
</tr>
</tbody>
</table>

is relatively unaffected by rumen microorganisms (24, 31) casts doubt as to the significance of the tocopherol levels in wood shavings. Whether the tocopherols present in wood shavings are absorbed from the rumen is, however, not known.

RESULTS

The experimental findings are summarized in Tables 1 and 2. These data show that the plasma tocopherol levels were low in all calves fed the basal diet or the basal diet plus selenium. In calves receiving tocopherol supplementation, however, the blood plasma tocopherol levels were high. Calves given no tocopherol supplementation averaged 108 γ of total tocopherol per 100 ml. of plasma, while the calves fed supplementary tocopherol averaged 639 γ per 100 ml., a highly significant difference (P < 0.01). The statistical analyses on these disproportionate subclass numbers were based on the method of Snedecor (41) for a 2² factorial design. Serum electrolyte values were normal in all cases, with the exception of magnesium, which fell as the trial progressed. Calves fed the basal diet plus cod-liver oil had serum magnesium levels of 1.5 mg. per 100 ml.; the calves fed the basal diet plus cod-liver oil and selenium had serum mg. levels of 2.1 mg. per 100 ml.; the calves which were fed supplementary tocopherol and selenium had serum mg. levels of 1.6 mg. per 100 ml.; and the calves supplemented with tocopherol had serum mg. levels of 1.4 mg. per 100 ml. These values are low in comparison with normal values of 2.0 to 2.5 mg. of mg. per 100 ml. of serum, as reported by Huffman et al. (21) and Blaxter, Rook, and MacDonald (6). No statistically significant differences between the blood magnesium levels on the various diets were obtained. The difference between the initial blood levels of magnesium and the final blood levels was highly significant (P < 0.01).

Hemoglobin levels, white cell counts, and differential counts were within accepted normal ranges in all calves and no statistically significant differences were found.

Because of the environmental conditions of dampness and drafts, and because the calves were assembled from several farms, white scours (Escherichia
E. coli septicemia) was a problem in these calves. In Table 1 it can be seen that in the calves that completed the treatment period, all of those that did not receive tocopherol supplementation developed Zenker’s degeneration of the skeletal muscles and Purkinje fiber degeneration of the heart. Calves 19 (basal diet), 24, 25 (basal diet plus selenium), and 28 (basal diet plus tocopherol) died from E. coli septicemia. Calf 28, which received tocopherol supplementation, died at 30 days of age after a prolonged period of diarrhea. On histologic section, this calf showed a mild Zenker’s degeneration of the skeletal muscles. Parrish (35) has pointed out that whenever calves were observed to have a severe diarrhetic condition, concentrations of serum tocopherols decreased.

DISCUSSION

Under the conditions of this experiment the addition of 1 p.p.m. selenium did not prevent muscular dystrophy in calves which were fed a tocopherol-deficient basal diet supplemented with 30 ml. of cod-liver oil per day.

All of the calves which received the basal diet or the basal diet plus 1 p.p.m. selenium, at the end of the experimental period, had Zenker’s degeneration of the skeletal muscles, and degeneration of the Purkinje fibers of the heart. None of the calves which received the tocopherol supplementation had Zenker’s degeneration or Purkinje fiber lesions at the conclusion of the experiment. In this experiment 1 p.p.m. selenium, when added to the diet, was not able to replace tocopherol in the prevention of dystrophy. The basal diet, however, contained 0.3 p.p.m. selenium.

The addition of cod-liver oil to the diet produced much more extensive dystrophic lesions than had previously been observed in experimental cases (27). In these experiments the initial plasma tocopherol values were similar; the experiments were conducted during the same time of the year, i.e., late fall and winter, and housing conditions were comparable. The calves showed no clinical signs of muscular dystrophy, with the exception that Calf 17 was recumbent more than the other calves on experiment and held its head slightly to the side during the last week. The latter sign was not observed until seven days after a biopsy of the semitendinosus muscle was positive for Zenker’s degeneration. During the last 2 wk. on test, Calf 17 was drinking 9.1 liters of reconstituted diet per day and was gaining 0.64 kg. per day. On necropsy this calf had extremely severe Zenker’s degeneration, involving almost all of the skeletal muscles of the body. Because calves can be so severely affected with Zenker’s degeneration but be eating and growing normally, it is understandable how sudden exercise in the spring aggravates or precipitates Zenker’s degeneration. The calves here were in small pens and were not allowed out of them except for a few minutes during cleaning.

Serum magnesium levels were low in all calves in the experiment. The first samples were within the normal range, but subsequent samples showed a marked decline. Magnesium levels in other calves receiving the same diet as these calves but not fed cod-liver oil did not show this decline during a 93-day feeding period.
Dehori et al. (17) found that the addition of cod-liver oil to the diet of calves caused a decrease in blood serum magnesium and carotenoids.

Blaxter, Rook, and MacDonald (6) estimated the requirements of calves to be 16 to 18 mg. of magnesium per 100 ml. of diet. Since Blaxter et al. (6) did not state the intake of their diet, direct comparisons with the present studies are not possible. On analysis the basal diet fed in the trials reported herein contained 20.2 mg. of magnesium per 100 ml. That this level of magnesium did not prevent low serum magnesium levels may have been because the magnesium was being eliminated as magnesium soaps when combined with undigested fat (36). Duncan, Huffman, and Robinson (18) and Huffman et al. (21) showed that magnesium salts were not so well utilized as when the magnesium was present in natural feeds. Two-thirds of the magnesium in the diet fed herein was obtained from magnesium salts. Thomas and Okamoto (43) fed calves whole milk at 10 to 12% of body weight and found a decline in blood magnesium levels at 48 to 131 days of age. When magnesium in amounts equal to that in milk was added as magnesium sulfate, grain, or alfalfa hay, these workers found that the magnesium in grain and hay was utilized better than the magnesium in MgSO₄. The differences in utilization were not significant and blood serum magnesium levels declined on all treatments.

All of the calves which developed Zenker’s degeneration of the skeletal and tongue muscles also showed degeneration of the Purkinje fibers of the heart. These findings are more fully described in a separate publication outlining the pathology of the experimental cases (22). The report by Moore, Hallman, and Sholl (29), describing the pathology of the calves from the experiments on magnesium deficiency of Duncan, Huffman, and Robinson (18), showed that in ten instances where portions of Purkinje fibers were present in heart sections, six showed extensive degenerative changes of these fibers. These workers also found hyaline degeneration of the skeletal musculature of the calves and it is likely that a concomitant tocopherol deficiency was also present (6). Blaxter, Rook, and MacDonald (6) fortified a magnesium-deficient diet with tocopherol. Calves fed this diet developed hypomagnesemic tetany, but on necropsy showed no degeneration of the Purkinje fibers.

Since the appearance of clinical signs of muscular dystrophy has not been a sufficiently critical criterion for determining the onset of Zenker’s degeneration in the experimental calves, more exacting biochemical or other tests must be utilized. Such tests as the analysis for the release of serum glutamic oxalacetic transaminase during muscular dystrophy (42) are being evaluated as suitable criteria.

ACKNOWLEDGMENTS

The help of Dr. Stanley Ames, Distillation Products Industries, Rochester, N. Y., in supplying the tocopherol and stripped lard, and for the analysis of feed and wood shavings for tocopherol, is gratefully acknowledged.

REFERENCES


(41) SNEDECOR, G. W. Statistical Methods. 5th ed. (Section 12.14.) 1956.
