

*Experimentally Induced Muscular Dystrophy.
Use of Antioxidants in the Prevention of Muscular
Dystrophy in Calves*

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MUSCULAR DYSTROPHY can be produced experimentally by a deficiency of certain nutrients (choline, potassium, and selenium) or compounds having antioxidant properties. Because of the availability and economy of antioxidants, the efficacy of two of these compounds and of vitamin E in preventing muscular dystrophy was compared in this test.

The antioxidant activity of vitamin E in the protection of other vitamins has been the subject of many studies since the discovery, by Moore (1940), that α -tocopherol increased the storage of vitamin A in the liver of the rat. He concluded that the increased storage of vitamin A in the presence of tocopherols was due to its protection from oxidation. Hawk et al. (1949) stated that vitamin E is an effective antioxidant for fats because of the presence of a phenolic hydroxyl group in the molecule.

Blaxter et al. (1952) demonstrated that muscular dystrophy in calves could be prevented by the administration of α -tocopherol. Draper and Johnson (1955) demonstrated that diphenyl-para-phenylenediamine (DPPD) could protect lambs from vitamin E deficiency symptoms. Other workers (Proctor et al., 1958; Muth et al., 1958) have reported that selenium will protect calves and lambs against muscular dystrophy.

Creatine excretion increases during dystrophy according to reports of Milhorat and Wolff (1936); Morgulis and Spencer (1936); Knowlton and Hines (1938); Mackenzie and McCollum (1940, 1941); Hoagland, et al. (1945); Butturini (1949); Blaxter et al. (1953); Milman and Milhorat (1953); and Dinning and Fitch (1957).

The objectives of this experiment were to determine: (1) If vitamin E, Vianol, a commercial antioxidant containing DPPD, or DPPD would prevent muscular dystrophy in calves maintained on a milk replacer; (2) If blood creatine (verified by autopsy) is an effective indicator of muscular dystrophy in calves.

Experimental

Twenty-eight two-week-old Hereford calves, male and female, were randomly allotted to seven lots of four animals each. These calves were individually fed a milk replacer (Suckle) and two ounces of either corn oil or cod liver oil twice daily from nipple pails. They were fed one pound of milk replacer per 10 pounds of body weight per day. The calves were housed in pens with dirt floors, overhead shelter, and a concrete runway to allow sunshine, fresh air, and exercise. Wood shavings were used for bedding.

Calves were weighed and blood samples drawn at the beginning of the experiment, at 56 days, and at 90 days. Blood creatine determinations were conducted according to the method of Fister (1950). Histopathological examinations of the heart and skeletal muscles of the animals were made at death.

Each group received the basal diet (Suckle). In addition, each group received the following additives daily: Group 1, four ounces of corn oil; Group 2, four ounces of cod liver oil; Group 3, four ounces of cod liver oil and 340 mg. of *a*-tocopherol; Group 4, four ounces of cod liver oil and 1/10 per cent DPPD; Group 5, four ounces of cod liver oil and 5/10 per cent DPPD; Group 6, four ounces cod liver oil and 1/10 per cent Vianol; Group 7, four ounces of cod liver oil and 1 per cent Vianol. The DPPD and Vianol were added as a per cent of the total dry matter intake.

Data were analyzed by Duncan's Multiple Range Test (1955) to test for significance among treatments.

Results of Discussion

All calves scoured for the first two weeks of the experiment. The Suckle was fortified with chlortetracycline (50 mg. per ton) in an attempt to alleviate this condition. Chlortetracycline did not alleviate the scouring; however, limiting the feed intake eliminated the digestive disturbance. There was a gain in weight in all groups, the most pronounced being in Group 2 (supplemented with *a*-tocopherol).

One calf receiving corn oil died on the 36th day of the test as a result of acute bronchopneumonia. On the 37th day of the experiment one calf in Group 2 was killed accidentally while being fed cod liver oil. One calf in Group 3 died of gastroenteritis on the 50th day of the test.

One case of muscular dystrophy developed in Group 1 (corn-oil-fed calves). This might be expected if the corn oil contained little or no vitamin E activity. Hove and Harris (1947) reported that corn oil that was tocopherol-free was as effective as cod liver oil in producing muscular dystrophy. The corn oil used in this test was not analyzed for its tocopherol content. It was assumed to be low because the histological picture of calves in this group, upon autopsy, was the same as that of the animals that had received cod liver oil. With the exception of this one case, muscular dystrophy was not observed in Groups 1, 3, 5, and 7 where alpha-tocopherol and higher levels of either DPPD or Vianol were used.

Muscular dystrophy occurred in three calves in Group 2, four in Group 4, and four in Group 6 (cod liver oil treated and with low levels of either DPPD or Vianol). The first active case was observed on the 53rd day. Calves that exhibited marked increases in blood creatine levels as well as representative calves with lower blood creatine levels were sacrificed for histopathological examination of the skeletal muscles. Histopathological examination of these calves showed active muscle degeneration. In all cases there was a hyalinization and calcification of the skeletal muscle fibers with a slight foreign body reaction around most of the calcified cells. The cardiac musculature in general showed only slight degeneration. One calf on the 1/10 per cent DPPD treatment (Group 4) exhibited a parenchymatous degeneration and necrosis in the cells of the zona fasciculata and the zona reticularis in the adrenal gland. One other calf in this same lot exhibited a chronic passive congestion of the liver along with central lobular fatty changes and early necrosis.

Dystrophy of the diaphragmatic and intercostal muscles as reported by Blaxter et al. (1953) was not observed in this experiment. Alpha-tocopherol given at a rate of 340 mg. per calf per day, 0.5 per cent DPPD, and 1 per cent Vianol prevented the occurrence of the macroscopic lesions reported by Muth (1955) and Vawter and Records (1947).

At the end of the test, one dystrophic calf was arbitrarily chosen to determine if a-tocopherol therapy would relieve symptoms of muscular weakness. This calf could not stand alone and had not eaten for several days. After receiving 500 mg. of a-tocopherol per day for four days, it was able to stand alone with difficulty. After one week of this treatment, locomotion appeared normal. Treatment with 500 mg. of a-tocopherol per day was continued for 30 days. The response to tocopherol is substantiated by Culik et al. (1951) and Bacigalupo et al. (1952), who reported that 500

mg. of a-tocopherol was needed to correct the deficiency of vitamin E in the blood of dystrophic lambs.

The group means of blood creatine values are reported in Table 1. (Urine samples are difficult to collect from females, hence blood creatine, rather than urinary creatine, was used as an indication of muscular dystrophy.) Analysis of variance of the blood creatine levels shows a highly significant difference ($P < 0.01$) among the treatments and a highly significant difference ($P < 0.01$) among the periods of blood collection (Tables 2 and 3).

Comparison of the means by Duncan's Multiple Range Test (Tables 2 and 3) shows that 1/10 per cent DPPD and 1/10 per cent Vianol failed to prevent an increase in blood creatine levels. Alpha-tocopherol (340 mg. per day), 0.5 per cent DPPD, and 1 per cent Vianol did prevent a significant increase in blood creatine levels and hence prevented the occurrence of muscular dystrophy as verified by histological examination. Data indicate that the protective level of DPPD for calves under the conditions imposed in this test lies between 0.1 and 0.5 per cent of the dry matter intake. Vianol was required in amounts between 0.1 and 1 per cent of the dry matter intake. Alpha-tocopherol, 340 mg. per head per day, protected the calves from muscular dystrophy.

Summary and Conclusions

Twenty-eight two-week-old Hereford calves were randomly allotted to seven groups of four animals each and individually fed until death or for 90 days. The experiment was designed to determine if either vitamin E, Vianol, or DPPD would prevent experimentally induced dystrophy in calves and if blood creatine could be used as an indicator of muscular dystrophy. Data in this experiment indicate that DPPD, Vianol (a commercial compound containing DPPD), or vitamin E, if used in sufficient amounts, will prevent experimentally induced muscular dystrophy. No external symptoms of dystrophy were observed in Groups 3, 5, and 7 where 340 mg. alpha-tocopherol, .5 per cent DPPD, or 1 per cent Vianol were fed. Creatine levels were significantly ($P < .01$) less when 340 mg. a-tocopherol, .5 per cent DPPD, or 1 per cent Vianol per head per day were used. Blood creatine levels were significantly higher ($P < .01$) at 90 days than when the test began. Blood creatine, as verified by histopathological examination, served as a reliable index in indicating the presence of muscular dystrophy under the conditions of this test.

TABLE 1. WHOLE BLOOD CREATINE VALUES EXPRESSED IN MG. %

Lots	Days			Average
	1	56	90	
1 Control ^a corn oil	2.21	3.82	4.38	3.47
2 Control cod liver oil	2.48	3.59	4.80	3.62
3 2 + a-tocopherol	2.51	3.17	3.40	3.03
4 2 + .1% DPPD	2.27	3.10	4.59	3.32
5 2 + .5% DPPD	2.89	3.30	3.34	3.18
6 2 + .1% Vianol	2.44	3.43	5.27	3.71
7 2 + 1 % Vianol	2.20	2.67	3.21	2.69
Average	2.43	3.30	4.14	

^a Suckle, a formulated milk replacer, was used as the control ration.

TABLE 2. DUNCAN'S MULTIPLE RANGE TEST OF SIGNIFICANCE OF BLOOD CREATINE VALUES

	Treatments ^a						
	7	3	1	5	4	2	6
Average blood creatine values, mg. % ^b	2.69	3.02	3.15	3.17	3.28	3.62	3.71

^a See Table 1.

^b P < .01.

TABLE 3. DUNCAN'S MULTIPLE RANGE TEST OF SIGNIFICANCE OF BLOOD CREATINE VALUES

	Period		
	1 Beginning of test	2 56 days	3 90 days
Average blood creatine values, mg. % ^a	2.43	3.30	4.14

^a P < .01.

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