Effectiveness of Selenium in Prevention of Nutritional Muscular-Dystrophy in the Chick.* (27088)

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Selenium, at levels of less than 0.1 ppm, either as the inorganic selenite or in various organo-selenium compounds, has been shown completely to prevent dietary necrotic liver degeneration in rats(1,2) and exudative diathesis in Vit. E-deficient chicks(3,4,5,6,7). Inorganic selenium compounds also have been shown to prevent "stiff lamb disease" and "white muscle disease," common names for a certain type of muscular dystrophy in lambs and calves(8,9,10,11,12).

Early studies on the effects of selenium upon muscular dystrophy in chicks(13) and (7) showed that addition of selenium as sodium selenite at levels of approximately 1-5 ppm had some effect in reducing the incidence of muscular dystrophy in chicks receiving a Vit. E-deficient diet low in methionine and cystine but that selenium was ineffective in completely preventing dystrophy in chicks under these conditions.

In subsequent studies in this laboratory it has been found that the effectiveness of selenium in prevention of muscular dystrophy in chicks appears to be influenced by the amount of Vit. E present in the chicks at the time they are hatched. Thus it was found that when the chicks used as experimental animals were obtained from a flock of hens maintained on a diet very low in Vit. E and the chicks were hatched at the end of the depletion period, selenium had no beneficial effect upon the very high incidence of muscular dystrophy which occurred in these severely Vit. E-deficient chicks.

It was the object of the present experiments, therefore, to determine whether or not a synergistic effect exists between Vit. E and selenium in prevention of muscular dystrophy in the chick.

Materials and methods. The chicks used in these experiments were Vantress x White Plymouth Rock crossbred chicks obtained from a flock of hens maintained on a diet low in Vit. E. The chicks were placed on experiment at one day of age and maintained in thermostatically-controlled, electrically-heated battery brooders with wire mesh floors. Feed and water were supplied *ad libitum*. Both male and female chicks were used and the chicks were distributed at random according to sex, among the various treatments. The basal diet used in these experiments contained the following, expressed as per cent: Casein, 15; gelatin, 10; glucose, 61.98; cellulose, 3; stripped lard, 4; mineral mixture, 5.54; vitamin mixture, 0.48. The vitamin mixture supplied the following per kg of diet: Thiamine, 10 mg; riboflavin, 10 mg; pyridoxine HCl, 4.5 mg; niacin, 50 mg; inositol, 250 mg; folic acid, 4 mg; calcium pantothenate, 20 mg; biotin, 0.20 mg; menadione, 0.50 mg; choline chloride (70%), 2200 mg; B_{12}, 20 μg; 5600 IU Vit. A; 4410 ICU Vit. D_3 and 220 mg diphenyl-p-phenylenediamine (DPPD). The mineral mixture supplied the following in g/kg of diet: $CaHPO_4$, 21.51; $CaCO_3$, 14.92; KH_2PO_4, 8.67; NaCl, 6.0; $MnCl_2 \cdot 4H_2O$, 0.463; $FePO_4 \cdot 4H_2O$, 0.265; MgO, 0.68; KI, 0.0026; $Cu(C_2H_5O_2)_2$, 0.0119; ZnO, 0.06; $CoCl_2 \cdot 6H_2O$, 0.0017; $KHCO_3$, 2.10, $NaMoO_4 \cdot 2H_2O$, 0.0083.

Observations of muscular dystrophy were made at weekly intervals and confirmed by autopsy at the end of the 4-week experimental period. Muscular dystrophy in the chick is characterized by the appearance of grossly visible striations appearing especially in the fiber bundles of the superficial pectoralis and deep pectoralis (breast) muscles of the chicks. Body weights and feed consumption also were recorded at weekly intervals. The experimental plan of each experiment was similar in that in both experiments

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TABLE I. Effect of d-alpha tocopheryl Acetate and Selenium on Muscular Dystrophy in the Chick.

Treatment	Exp. 1		Exp. 2	
	4 wk wt, g	Musc. dyst.	4 wk wt, g	Musc. dyst.
Basal diet	361	16/16*	—	—
d-alpha tocopheryl acetate (Vit. E), 2.5 mg/kg	377	16/16	306	8/9
Vit. E, 5.0 mg/kg	383	9/16	294	7/9
" 10.0 "	393	0/16	341	0/9
Selenium (Se)† 1.0 mg/kg	353	10/16	254	4/9
Vit. E, 2.5 mg/kg + Se, 1.0 mg/kg	358	0/16	316	0/9
Vit. E, 5.0 mg/kg + Se, 1.0 mg/kg	376	0/16	363	0/9
Vit. E, 10.0 mg/kg + Se, 1.0 mg/kg	342	0/16	—	—

* No. of chicks showing symptoms (numerator) over No. of chicks examined (denominator).
† As sodium selenite.

graded levels of 2.5, 5.0 and 10 mg of d-alpha tocopheryl acetate per kg were fed alone as was 1.0 mg of selenium as sodium selenite per kg of diet. In further treatments the 2.5 and 5.0 mg levels of d-alpha tocopheryl acetate were fed together with one milligram of selenium per kg of diet.

Results. The results of both experiments are presented in Table I. These results show that addition of 2.5 and 5.0 mg of d-alpha tocopheryl acetate per kg of diet failed to prevent muscular dystrophy but that dystrophy was prevented by supplementing the diet with 10 mg of Vit. E per kg of diet. The addition of one milligram of selenium as sodium selenite was completely ineffective in preventing muscular dystrophy but produced complete prevention of muscular dystrophy when fed in combination with either 2.5 or 5.0 mg of Vit. E.

The chicks used in Exp. 2 were hatched from hens depleted of Vit. E for a more prolonged period than was the case for the chicks used in Exp. 1. This may account for the somewhat lower growth shown by the chicks in Exp. 2, especially in those lots receiving suboptimal levels of Vit. E.

Discussion. These results show a synergistic effect between Vit. E and selenium in prevention of muscular dystrophy in the chick. Since the basal diets used in producing stiff lamb disease and white muscle disease in lambs and calves consisted of practical feedstuffs, these diets undoubtedly contained appreciable amounts of Vit. E. Thus, the prevention of stiff lamb disease and white muscle disease in lambs and calves by inorganic selenium compounds has always been carried out in the presence of low levels of dietary Vit. E. On the other hand, the previous studies with chicks on the use of selenium for prevention of muscular dystrophy were done with highly purified diets containing negligible traces of Vit. E. The results of the present experiment indicate, therefore, that selenium compounds are as effective in prevention of muscular dystrophy in the chick as they are in prevention of dystrophy in the lamb or calf.

Further evidence that Vit. E is required in addition to selenium in prevention of muscular dystrophy in calves was presented by Maplesden and Loosli(14) who showed that Vit. E, not selenium, was required for prevention of nutritional muscular dystrophy in calves rendered dystrophic by feeding a diet containing cod liver oil. These results as well as those presented here indicate therefore that both selenium and Vit. E are concerned in prevention of nutritional muscular dystrophy in chicks and calves.

Summary. Results have been presented which show that whereas supplementation of a dystrophy-producing diet of severely Vit. E-depleted chicks with either 2.5 mg of d-alpha tocopheryl acetate or 1.0 mg of selenium as sodium selenite per kilogram of diet alone has no effect upon dystrophy, supplementation with these amounts of selenium and Vit. E together completely prevents muscular dystrophy. These results indicate, therefore, that both selenium and Vit. E are concerned in prevention of nutritional muscular dystrophy in the chick.

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Effect of a Single Application of Croton Oil in Skin Carcinogenesis.* (27089)

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It was previously demonstrated that when various dosages of 9,10-dimethyl-1,2-benzanthracene dissolved in acetone were applied to the skin of Swiss mice as single applications, it required 200 μg for effective carcinogenic action. Doses of 20 and 50 μg were without effect and a dose of 100 μg gave rise to but a few benign tumors. A dose of 200 μg gave rise to a high incidence of tumors, both benign and malignant. Above a dose of 200 μg, no additional response was noted(1,2). One reason that was suggested for this dose response behavior is that the injury inflicted by the doses of 200 μg and over of this necrotizing carcinogen plays a role. Injury has been shown by several investigators to play a secondary role in carcinogenesis in the skin(3,4,5,6) and analysis of such experiments has provided the experimental basis for considering this process as a 2-stage phenomenon(7,8,9). It might therefore be thought that at the 200 μg level an additional factor associated with increased injury comes into play and that this provides a promoting stimulus enabling the carcinogen to manifest its full activity. It appeared in our original study that injury might play such a role; however, injury to the skin is extremely difficult to qualify and the current experiment was designed to test the hypothesis. Croton oil has been studied in many experiments as a promoting agent in skin carcinogenesis(2,1,11); it is well known to have an injurious effect on many tissues although the mechanism of action in promotion is still obscure. In the previous studies with croton oil, the agent has always been applied repeatedly. In the present investigation, groups of mice were given single applications of either 100 or 200 μg of DMBA alone or combined with croton oil.

Materials and methods. The mice used were Swiss females, 7 to 9 weeks old at the beginning of experiment, originating from mice obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine and bred in this department since 1951. The mice were housed in plastic cages on wood shavings, fed Rockland mouse diet and given water *ad libitum*. The carcinogen 9,10-dimethyl-1,2-benzanthracene (DMBA) was obtained from Eastman Organic Chemicals, then purified by chromatography on fluorisil in this laboratory. Croton oil was obtained from Boots Ltd., England.

Both the croton oil and the DMBA were applied dissolved in fluorescence free acetone, using a calibrated micropipette. The solutions were applied to the interscapular region of the mice using an area approximately 1.5 per 1.5 cm shaved free of hair.

Mice were checked each week and all the

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