Ineffectiveness of Factor 3-Active Selenium Compounds in Muscular Dystrophy of Rabbits on Vitamin E-Free Diets. (23928)

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It has been been reported(1) that the standard assay for tocopherol, using resorption sterility in the rat, is not influenced by Factor 3†, even when the latter is supplied in large excess. This factor, or Factor 3-active selenium compounds, prevents muscular degeneration in rat,‡ mouse(2) and also delays "white striation" of muscles in the chick (personal communication); all these lesions occur on *Torula* yeast diets deficient in Factor 3 and Vit. E. Recently, results have been described showing, on the other hand, that intramuscular injections of 20 and 100 μg of selenite-selenium had no effect on nutritional muscular dystrophy of Vit. E deficient rabbits(3). We have found that muscular dystrophy in rabbits, when produced by Vit. E deficient soybean meal diet, is not preventable by selenocystine or sodium selenite, both of which are potent sources of Factor 3 activity. By rat assay against liver necrosis the soybean meal was shown to be a relatively good source of Factor 3. Determination of selenium in soybean meal by activation analysis produced values well in agreement with the bioassay.

Methods. Factor 3-activity of selenium compounds used was determined by rat assay against necrotic liver degeneration as described elsewhere(4,5). Selenium levels in soybean meal were measured by activation analysis (production of specific radioisotopes of selenium by neutron bombardment, and subsequent carrier analysis)(6). The basal diet for production of Vit. E deficiency and acute muscular dystrophy in rabbits has been described briefly(7). Its percentage composition is: soybean meal (solvent extracted, 51% protein), 40; cellulose (non-nutritive fiber of General Biochemicals), 10; sucrose, 32; salt mixture (Hubbel, Mendel, Wakeman) with 0.5% added $ZnCO_3$, 5; B-vitamin premix, 5; lard (Vit. E-free of Distillation Products Industries), 6; and cod liver oil, U.S.P. 2. Vitamin premix contributed the following in μg/g diet: thiamine, riboflavin and pyridoxine, 5 each; calcium pantothenate, 25; i-inositol, 200; niacin, 40; methyl 1,4-naphthoquinone, 0.3; folacin, 2; Vit. B_{12}, 0.03; and choline chloride, 2000. No Vit. E was added to the diet. Where desired, Vit. E was supplied to rabbits as 10 mg dl, α-tocopheryl acetate given orally once a week by calibrated dropper as 10% solution in olive oil U.S.P. Two sources of Factor 3-activity have been tested, *i.e.*, sodium selenite and D,L-selenocystine hydrochloride. The selenite was added to basal diet as a premix in sucrose such that addition of 1% of premix furnished 100 μg% (1 ppm) of selenium. Selenocystine was added to the basal diet as a solution such that 5 ml/kg diet furnished 50 μg% (0.5 ppm) of selenium. Male albino rabbits at 4 weeks of age were distributed among 4 treatment groups as follows: basal diet Vit. E-free; basal diet with Vit. E supplementation; Vit. E-free basal diet with Se as sodium selenite; and Vit. E-free basal diet with Se as selenocystine. Each group had 5 animals. Diets and tap water were given *ad libitum*. Animals were housed individually on raised, half-inch screens, and were weighed 3 times each week. Individual growth curves were plotted. The rabbits were checked daily for gross muscle weakness. Muscular dystrophy was noted on a 0-4 scale; grade 1 was the typical "duck waddle"; grade 2, inability to right itself when placed on its side; grade 3, inability to remain upright when placed upright; grade 4, terminal, immobilized condition, with complete and rather

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† Since various compounds of selenium show greatly different biological activity, the term "Factor 3" is used to designate the biologically active, selenium-containing component, or components, present in nutrients and other materials of biological origin.

‡ Schwarz, K., to be published.

TABLE I. Lack of Effect of Factor 3-Active Selenium Compounds on Muscular Dystrophy in Rabbits Fed a Vit. E Deficient Diet (5 Rabbits/Group).

Supplement	Dose level of selenium (μg/100 g diet)	Factor 3 (Fischer units*/100 g diet)	Initial body wt (g)	Avg body wt increments (g) in 10-day periods			Days to clinical muscular dystrophy	Days to death
				0-10	10-20	20-30		
dl-α-tocopheryl acetate (10 mg/wk)			620	+310	+340	+260		
None			620	+300	+280	−190	24.6 (21-31)	37.1 (34-41)
Na selenite	100	730	640	+356	+160	−110	26.6 (21-31)	33.5 (23-39)
D,L-selenocystine HCl	50	345	610	+322	+184	− 23	25.8 (20-36)	34.0 (23-42)

* 1 Fischer unit is amt required daily/animal by Fischer strain of rats to achieve 50% protection against liver necrosis. For details concerning these tests see reference (8).

spastic paralysis. When moribund or dead, dystrophic animals were examined for gross lesions of muscle, liver, heart and other organs. Portions of liver and leg muscles were saved for possible subsequent histologic evaluation.

Results. Rabbits fed the basal diet without Vit. E developed muscular dystrophy in about 3 weeks (Table I). The dystrophy progressed rapidly through various stages and terminated in death an average of 12 days after onset. All 5 animals in the group responded similarly. The 5 animals supplemented with Vit. E grew at a normal rate of over 30 g/day and remained in perfect health. Previous experience has shown that this diet, with Vit. E, maintained rabbits in a normal state for at least a year. Neither the selenite nor the selenocystine supplements to the basal Vit. E-free ration altered the course of dystrophy development in any rabbit fed these diets.

Under our experimental conditions muscular dystrophy in rabbits is caused by Vit. E deficiency alone and not by simultaneous lack of both Factor 3 and Vit. E. Tests for Factor 3 in soybean meal, using the rat assay gave the following results:

% soybean meal in diet	μg % Se supplied by soybean meal	% protection
5	1.5	35
10	3	46
20	6	100

Soybean meal, main component of rabbit diet, thus supplies Factor 3 at levels which appear sufficient to obviate the need for additional supplies of Factor 3-active selenium compounds. The results of bioassays are borne out by activation analysis for selenium: 1 g of soybean meal contains 0.3 μg of the element, *i.e.*, at least 12 μg % of biologically active selenium are present in the basal, 40% soybean meal ration. In the rat necrotic liver degeneration will be prevented by 1.4-1.6 μg % Se in form of α-Factor 3 concentrates(8).

It is obvious from these results that Factor 3-active selenium compounds do not substitute for Vit. E. There also does not appear to be a Vit. E sparing effect, as indicated by rate of development of muscular dystrophy and by time of death. It cannot be concluded, on the other hand, that Factor 3 deficiency would not impair structure and function of muscles, since the diet used for the production of muscular dystrophy contains sufficient amounts of the factor.

Summary. Muscular dystrophy developed within 3 weeks in young rabbits fed a basal Vit. E-free diet which contained soybean meal as the protein source. Sodium selenite, and selenocystine, when added to the basal diet as sources of Factor 3-activity, did not influence the course of the disease. Control rabbits fed the basal diet but supplemented with α-tocopheryl acetate grew at normal rate of over 30 g/day and remained in good health. Factor 3 assays in rats, and also activation analysis for selenium, established that an adequate level of Factor 3 was present in the basal soybean meal diet. Under our condi-

tions, muscular dystrophy is due to lack of tocopherol alone. Factor 3-active selenium compounds do not substitute for Vit. E.

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Urinary and Fecal Excretion of Orally Administered Mg^{28}.* (23929)

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It is well recognized that magnesium is a biologically important ion. Studies of its metabolism, however, have until recently been hampered by technologic difficulties. Within the past several months a synthetic radioactive isotope of magnesium, Mg^{28}, with half-life of 21.8 hr, has become available and has made possible a reevaluation of metabolism of magnesium in human beings. The purpose of this pilot study was to follow the behavior of orally administered magnesium, using Mg^{28} as tracer. Urinary and fecal excretion of the isotope was measured.

Material and methods. Initial observations (Group A) were made in 12 hospitalized adult male convalescents from various diseases. None had any symptoms or signs of gastrointestinal disturbances, and all were on regular hospital diet. Radioactive magnesium (Mg^{28}) as magnesium chloride in an acid solution, was given in water, usually mid-morning. The dose of 10 to 25 μc was contained in 3 to 10 meq of magnesium. All urine passed within next 24 hours was collected in chemically clean glassware. Seven patients were given one dose of Mg^{28}; 4 were given 2 doses a week apart; and the twelfth patient given 3 doses of tracer at weekly intervals. Subsequent studies (Groups B, C and D) were done in 14 healthy medical students. Each student received orally 10 μc of Mg^{28} contained in 3 meq of magnesium. In 7 instances (Groups C and D) 2 gelatin capsules containing a gram of carmine were given at the same time. In addition to specimens of urine and feces collected in chemically clean glassware from all students, several venous blood specimens were obtained from 3 students in Group D. Two students (#11 and 12) were given a second dose of isotope one week after the first. Radioactivity of urine, plasma and feces was determined with a well-type scintillation counter and a scaling circuit. Stool specimens were digested in concentrated sulfuric and nitric acids prior to radioactivity assay. A total of 10,000 counts was made on each sample. All determinations were corrected for physical decay of isotope.

Results. The maximum 24 hour urinary excretion rate for Mg^{28} in hospitalized patients (Group A) was 6.27%; the mean, 2.11 $\pm$ 1.61%. Except in one patient, the second and third determinations of urinary excretion rate gave results closely comparable to the first.

The 24 hour urinary excretion of radioactivity in the first 9 medical students (Group

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