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SYNERGISTIC EFFECTS BETWEEN ANTIOXIDANTS AND SELENIUM OR VITAMIN E*

JOHN G. BIERI

Laboratory of Nutrition and Endocrinology, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Md. U.S.A.

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Abstract—Several antioxidants when fed to chicks at relatively high levels in an experimental diet were ineffective in preventing the exudates and mortality from a combined deficiency of selenium and vitamin E. Some of the compounds were toxic, as evidenced by sudden death or subcutaneous hemorrhages. When amounts of either selenite or α-tocopheryl acetate, which individually had little or no effect on symptoms, were given with the antioxidants signs of deficiency and mortality were prevented, and toxicity also was eliminated.

VARIOUS commercial antioxidants when added to diets lacking vitamin E prevent the signs of deficiency which occur in some species. In the chick, three distinct syndromes of vitamin E deficiency have been reported by Machlin *et al.*[1] to respond to antioxidants. One of these syndromes, exudative diathesis, can be prevented by either vitamin E or biologically active selenium.[2] In contrast to the results of Machlin *et al.*, Scott[3] reported that the amount (0·05%) of the antioxidant, ethoxyquin,† necessary to prevent exudates in chicks approached the toxic level. Similar experiments in our laboratory had not revealed toxic effects from this compound. Since we were using a diet containing isolated soy protein, as did Machlin *et al.*,[1] whereas the diet of Scott[3] contained Torula yeast, it appeared that differences in the two protein sources could account for the discrepancy in results. The experiments reported here show that this was indeed the case, and that trace amounts of biologically active selenium in soy protein are probably responsible for the differences. Dietary levels of selenium *below* those required to prevent vitamin E deficiency signs effectively eliminate the toxicity of a variety of antioxidants.

METHODS AND MATERIALS

Chicks and diets

Male day-old chicks from a commercial hatchery‡ were kept in groups of eight in electrically heated brooders with raised wire flooring. Food and water were given *ad libitum*. Birds were inspected daily, beginning on the twelfth day, for exudates over the breast or neck. The two experimental diets are described in Table 1 and differed primarily in their protein source. Diet C34 had Torula yeast; diet C66MT contained

* An abstract of this work has appeared in *Fed. Proc.* **22**, 318 (1963).

† 1,2-Dihydro-6-ethoxy-2,2,4-trimethylquinoline, "Santoquin," Monsanto Chemical Co., St. Louis, Mo.

‡ Arbor Acre Farms, Inc., Glastonbury, Conn.

a mixture of yeast and soybean protein. Ethoxyquin and vitamin E (*dl*-α-tocopheryl acetate) were added in ethanol solution, and the other antioxidants were added as the powdered chemicals. Selenium, as sodium selenite, was made up as a pre-mix in powdered sucrose.

TABLE 1. PERCENTAGE COMPOSITION OF DIETS DEFICIENT IN VITAMIN E AND SELENIUM

Ingredient	Diet C34	Diet C66MT
Torula yeast*	60	20
Soybean protein†		30
"Stripped" lard‡	4	4
Salt mixture	6	6
Vitamin mixture	0·2	0·2
L-Cystine		0·3
DL-Methionine	0·3	
Glycine	1	
Glucose	28·5	39·5

* Feed grade, Lake States Yeast Corp., Rhinelander, Wis.
† Assay protein C-1, Archer Daniels Midland Co., Cincinnati, Ohio.
‡ Distillation Products Industries, Rochester, N.Y.

Chicks that died in the first week of an experiment were not included in calculating the incidence of mortality. Experiments were terminated after 28 days except where otherwise noted.

RESULTS

The incidence of exudates and of mortality in chicks fed the two basal diets with or without ethoxyquin is shown in Table 2. Both diets when unsupplemented produced

TABLE 2. EFFECT OF ETHOXYQUIN (EQ) IN DIETS CONTAINING TORULA YEAST OR SOY PROTEIN*

Diet	Exudates		Mortality	
	(Incidence†)	(%)	(Incidence‡)	(%)
C66MT (soy protein)	22/23	96	1/23	4
C66MT + 0·05% EQ	6/24	25	3/24	13
C34 (Torula yeast)	24/24	100	22/24	92
C34 + 0·05% EQ	11/24	46	21/24	88

* Results from 3 trials.
† Number of chicks with symptoms/number of chicks started.
‡ Number of deaths/number of chicks started.

exudates in essentially all chicks, evidence that the soy protein and Torula yeast were deficient in vitamin E and biologically active selenium. Whereas almost all birds fed the Torula yeast diet died, mortality with the soy protein diet was negligible. With 0·05% of ethoxyquin, the incidence of exudates was reduced one half to one fourth

that of the basal diets. Of special importance is the observation that with the Torula yeast diet one half the chicks that died *did not have exudates*. Death in chicks without exudates was sudden in onset, with the only suggestion of abnormality prior to death being a slight reduction in activity, such as their reaction to handling. The birds ate well and were of normal weight prior to death. Autopsy failed to reveal the cause of death. The low mortality in chicks fed ethoxyquin in the soy protein diet occurred exclusively in chicks afflicted with exudates.

Effect of low levels of selenium and vitamin E

The commercial isolated soy protein used has been reported[4] to have a higher selenium content than has Torula yeast. It appeared that this difference may have been responsible for the lower incidence of exudates and the prevention of mortality when ethoxyquin was fed in the soy protein diet. It should be pointed out that different chemical forms of selenium have widely varying biological activities with respect to prevention of vitamin E deficiency symptoms,[5] so that chemical analyses for selenium do not indicate the biological effectiveness of a natural material. In Table 3 it can be

TABLE 3. EFFECT OF ADDING VARYING LEVELS OF SELENIUM OR VITAMIN E TO THE TORULA YEAST DIET (C34) ON THE INCIDENCE OF EXUDATES AND MORTALITY

Additions to diet	No. of experiments	Exudates		Mortality	
		(Incidence*)	(%)	(Incidence)	(%)
Selenium (ppm)					
0	9	71/71	100	69/71	97
0·0125	3	24/24	100	20/24	83
0·025	8	62/64	97	23/64	36
0·05	5	10/40	25	4/40	10
0·1	6	0 /46	0	0/46	0
Vitamin E (mg/kg)					
10	5	37/40	93	33/40	83
20	2	14/16	88	10/16	63
25	2	0/15	0	0/15	0
100	5	0/39	0	0/39	0

* See footnotes to Table 2.

seen that 0·05 ppm of selenium in the Torula yeast diet was required to give significant protection against exudates and mortality; the lower levels of 0·025 and 0·0125 ppm did not prevent exudates but effectively reduced mortality.

With vitamin E, 10 or 20 mg/kg of diet did not influence the development of symptoms appreciably, but higher levels were completely active.

Effect of selenium and vitamin E on the toxicity of ethoxyquin

The data in Table 4 show that a very low dietary level of selenium, 0·025 ppm, which by itself was without effect on the incidence of exudates but which had a moderate effect on mortality (group 9), when included with 0·05% ethoxyquin almost completely prevented exudates and reduced mortality to 17% (group 3). A higher level of selenium, 0·05 ppm, also was not completely effective by itself (group 10) but together with ethoxyquin gave essentially complete protection and survival (group 4).

With vitamin E, both 10 and 20 mg/kg were ineffective levels alone (groups 12 and 13), but with ethoxyquin all exudates were prevented, and mortality was very low (groups 6 and 7).

*Effect of selenium and vitamin E on the toxicity of DPPD**

Another antioxidant that has been shown to substitute for vitamin E in preventing deficiency symptoms is DPPD.[6] Considerably higher dietary levels than those used

TABLE 4. EFFECT OF SELENIUM AND VITAMIN E ON THE TOXICITY OF ETHOXYQUIN (EQ)

Group	No. of experiments	Diet supplement*	Exudates (Incidence†)	Exudates (%)	Mortality (Incidence)	Mortality (%)	Av. wt., survivors (g)
1	6	None	47/47	100	46/47	98	
2	6	0·05% EQ	19/48	40	35/48	73	382
3	6	0·05% EQ + 0·025 ppm Se	1/48	2	8/48	17	457
4	4	0·05% EQ + 0·05 ppm Se	1/32	3	0/32	0	455
5	4	0·05% EQ + 0·1 ppm Se	0/32	0	0/32	0	459
6	2	0·05% EQ + 10 mg vit. E/kg	0/16	0	2/16	13	419
7	2	0·05% EQ + 20 mg vit. E/kg	0/16	0	0/16	0	512
8	3	0·05% EQ + 100 mg vit. E/kg	0/24	0	0/24	0	398
9	6	0·025 ppm Se	47/48	98	20/48	42	317
10	4	0·05 ppm Se	9/32	28	3/32	9	466
11	3	0·1 ppm Se	0/23	0	0/23	0	471
12	2	10 mg vit. E/kg	13/15	87	12/15	80	379
13	2	20 mg vit. E/kg	7/16	43	3/16	19	438
14	3	100 mg vit. E/kg	0/23	0	0/23	0	445

*Basal diet C34 (Torula yeast).
† See footnotes to Table 2.

TABLE 5. EFFECT OF VITAMIN E AND SELENIUM ON THE TOXICITY OF DPPD*

Group	Diet Supplement	Exudates (Incidence†)	Exudates (%)	Mortality (Incidence)	Mortality (%)	Av. wt., survivors (g)
1	None	24/24	100	19/24	79	198
2	0·25% DPPD	12/24	50	14/24	58	260
3	0·25% DPPD + 0·025 ppm Se	4/24	17	1/24	4	276
4	0·25% DPPD + 0·1 ppm Se	0/16	0	0/16	0	291
5	0·25% DPPD + 10 mg vit. E/kg	8/16	50	2/16	13	281
6	1·0% DPPD	12/24	50	9/24	38	271
7	1·0% DPPD + 0·025 ppm Se	0/24	0	0/24	0	284
8	1·0% DPPD + 0·1 ppm Se	0/16	0	0/16	0	295
9	1·0% DPPD + 10 mg vit. E/kg	4/16	25	0/16	0	278
10	0·025 ppm Se	18/24	75	13/24	54	227
11	0·1 ppm Se	0/24	0	0/24	0	310
12	10 mg vit. E/kg	12/16	75	2/16	13	242
13	100 mg vit. E/kg	0/16	0	0/16	0	262

* DPPD = N,N¹-diphenyl-*p*-phenylenediamine. Experiment terminated at 3 weeks. Diet C34 (Torula yeast). Results from 2–3 trials.
† See footnotes to Table 2.

for ethoxyquin were found necessary to obtain effects, possibly due to a difference in intestinal absorption. In contrast to ethoxyquin, DPPD rarely produced the sudden death in chicks not afflicted with exudates. As seen in Table 5, 1% of DPPD was no

* N,N¹-Diphenyl-*p*-phenylenediamine; Goodrich Chemical Co., Cleveland, Ohio.

more effective in preventing exudates, nor any more toxic, than was 0·25% (groups 2 and 6). As with ethoxyquin, 0·025 ppm of selenium significantly reduced the incidence of exudates and mortality when it was fed with DPPD (groups 3 and 7). Ten mg of vitamin E/kg diet together with 0·25% DPPD (group 5) had no effect on exudates but did reduce mortality. With 1% of DPPD there was an even more pronounced action of this low level of vitamin E (group 9). The observation that small amounts of selenium or vitamin E were more effective with 1% of DPPD than with 0·25% suggests that, even though the higher level by itself was no more efficient than the low level, more of the antioxidant was present in the tissues at 1% to permit a synergistic-like action with vitamin E and selenium.

Effect of selenium and vitamin E on the toxicity of BHT and DAH†*

Although these two antioxidants were ineffective in preventing exudates or mortality, even at the high level of 0·25%, there was a difference in that DAH produced extensive subcutaneous hemorrhages on the breast which were not seen with other antioxidants. In Table 6 it can be seen that 0·025 ppm of selenium with BHT did not alter the inci-

TABLE 6. EFFECT OF SELENIUM AND VITAMIN E ON THE TOXICITY OF BHT AND DAH*

Group	Diet supplement†	Exudates (Incidence‡)	Exudates (%)	Mortality (Incidence)	Mortality (%)	Av. wt., survivors (g)
1	None	16/16	100	15/16	94	
2	0·25% BHT	14/15	93	13/15	87	
3	0·25% BHT + 0·025 ppm Se	11/15	73	0/15	0	358
4	0·25% BHT + 20 mg vit. E/kg	2/16	13	1/16	6	409
5	0·25% DAH	6/16	100	16/16	100	
6	0·25% DAH + 0·025 ppm Se	12/15	13	0/15	0	492
7	0·25% DAH + 20 mg vit. E/kg	0/16	0	0/16	0	450
8	0·025 ppm Se	15/16	94	3/16	19	368
9	20 mg vit. E/kg	0/15	0	1/15	6	415
10	0·1 ppm Se	0/15	0	0/15	0	484
11	100 mg vit. E/kg	0/15	0	0/15	0	468

* BHT = 2,6-Di-*tert*-butyl-*p*-cresol (butylated hydroxytoluene); DAH = 2,5-*bis*(1,1-dimethylpropyl)hydroquinone (*tert* amylhydroquinone).

† Basal diet C34 (Torula yeast). Results from 2 trials.

‡ See footnotes to Table 2.

dence of exudates appreciably but completely prevented mortality. With DAH, this amount of selenium was effective against both exudates and mortality. Unfortunately, the level of vitamin E used in these experiments, 20 mg/kg of diet, prevented exudates and mortality. As with selenium, vitamin E was more effective with DAH than with BHT in reducing the incidence of exudates. The greater apparent toxicity of BHT is also reflected in the smaller body weights in the presence of selenium or vitamin E.

DISCUSSION

These experiments confirm both the observations of Scott[3] that ethoxyquin is toxic to chicks when fed in a Torula yeast diet, and those of Machlin *et al.*,[1] who found no toxicity when a soy protein diet was used. The demonstration that trace amounts of selenium, which alone are ineffective, prevent the sudden death caused by ethoxy-

* 2,6-Di-*tert*-butyl-*p*-cresol; Eastman Organic Chemicals, Rochester, N.Y.

† 2,5-Bis(1,1-dimethylpropyl)hydroquinone, Eastman Organic Chemicals, Rochester, N.Y.

quin in the Torula yeast diet suggests that the greater amount of selenium in soy protein than in the yeast is responsible for the results obtained with these two protein sources. That vitamin E, also in amounts which alone are ineffective, has an effect similar to that of selenium provides evidence that these two substances have the same or closely related modes of action.

Since the toxicities of other antioxidants in addition to ethoxyquin are also alleviated by selenium (and vitamin E), it is evident that this is a general metabolic effect. The absence of any obvious pathology at present prevents the implication of a specific tissue. An analogy might be drawn to the prevention by vitamin E of liver damage from organic compounds such as tricresyl phosphate, carbon tetrachloride, and pyridine,[7] but no discernible liver damage could be noted as a result of feeding ethoxyquin. A report[8] that the administration of selenium reduced the toxicity of carbon tetrachloride in rats is further evidence that this element has an important function in cellular metabolism.

A synergism between vitamin E and other antioxidants is easily conceivable; however, a similar relationship between selenium and antioxidants is not so readily apparent. One complicating consideration in attempting to interpret the results of these studies is the known synergistic action between vitamin E and selenium.[9] Although the chicks in these experiments were depleted of vitamin E during the course of the experiments, even after four weeks there were small, but detectable, amounts of α-tocopherol still in some tissues.[10] However, this consideration would appear to be negligible in view of the fact that the small amounts of selenium (0·025 ppm) or vitamin E (10 mg/kg) used were ineffective by themselves. It is possible that the antioxidants in some way stabilized or protected the selenium so that its efficiency was increased, but direct evidence on this point is lacking.

Since it is known that dietary selenium is rapidly incorporated into body proteins,[11] and some of these proteins have been shown to possess an antioxidant-like activity *in vitro*,[12, 13] it is conceivable that an antioxidant synergism may be occurring.

The small amount of selenium active in these experiments, which is below the level that will prevent vitamin E deficiency symptoms, emphasizes the potential importance of naturally occurring selenium in dietary constituents. In experimental work on drug toxicity, for example, this factor could have a significant influence on the results.

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