Metabolic Effects of Selenium as Related to Vitamin E[1]

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Abstract

Trace amounts of dietary selenium, which prevent certain vitamin E deficiency symptoms, have been found to affect the composition of chick tissues. Both selenium and vitamin E increased liver coenzyme A levels to normal when cystine was omitted from the diet. Liver and plasma total fatty acids were unaltered by selenium but vitamin E increased the arachidonic acid content slightly. There was no effect of either selenium or vitamin E on total lipid, cholesterol, phopholipid or coenzyme Q in liver. Low dietary levels of either selenium or vitamin E, which were insufficient in preventing deficiency symptoms, completely eliminated mortality from toxic amounts of a dietary antioxidant. The ability of dietary selenium to reduce *in vitro* lipid peroxidation in chick liver homogenates was not demonstrable with rat liver.

Introduction

IT HAS BEEN six years since the discovery that trace amounts of selenium in the diet would completely prevent certain classic vitamin E deficiency symptoms in animals (1,2). The relationship between vitamin E and selenium is one of the most improbable observations that experimental nutrition has turned up. We are faced with the interesting possibility that an element hitherto considered only as highly toxic may now be an essential trace element for some species.

Despite considerable work by numerous investigators on the metabolism of trace amounts of selenium, we do not yet have a definite understanding of how this element and vitamin E merge in the metabolic scheme. The evidence to date suggests that they produce their effects by a similar mechanism which results in the maintenance of normal cell structures. Such a statement, however, tells us nothing of the specific chemistry involved.

It should be emphasized that selenium will not prevent all aspects of vitamin E deficiency in animals. An example of this is seen in the chicken where three distinct syndromes develop in vitamin E deficiency (Table I). The lesion in the cerebellum which produces encephalomalacia is prevented by vitamin E and a variety of other antioxidants when present in the diet at relatively low levels (0.015%). Selenium has no effect on this syndrome. The condition of exudative diathesis is prevented by either vitamin E or selenium. Antioxidants are largely ineffective, but more will be said on this later. In the muscle dystrophy of chicks, only vitamin E and cystine are completely effective while selenium may reduce the severity but not the incidence. Certain dietary antioxidants will prevent the condition. This paper gives the results of experiments in which the effects of dietary selenium or vitamin E on a variety of biochemical components of tissues have been determined in an attempt to elucidate the metabolic relationship between these substances.

Experimental

In all chick studies, day-old male birds of the Arbor Acre strain were placed on purified diets deficient in both vitamin E and biologically active selenium. The protein sources were either Lake States Corp. Torula Yeast or Archer Daniel-Midland's isolated soybean protein C-1. Stripped lard from which the bulk of the tocopherols are removed by molecular distillation by Distillation Products Industries was the fat source, except where noted, and all diets contained 6% of a salt mixture, all vitamins except E, and glucose. Weanling male rats of the Holtzman strain were fed a similar deficient diet containing isolated soybean protein. Selenium was added to diets as sodium selenite and vitamin E was added as dl,α-tocopheryl acetate.

Thiobarbituric acid values were determined as described by Bieri and Anderson (3). Lipid extracts of liver were made with chloroform-methanol according to Folch et al. (4), while plasma was extracted with ethanol-diethyl ether (3:1). Total lipid in liver was determined gravimetrically and that in plasma by the procedure of Bragdon (5). Phospholipid (6) and total cholesterol (7) were also determined on the lipid extracts. Ubiquinone (8) and α-tocopherol (9) were determined on fresh tissue. Coenzyme A was assayed by the procedure of Novelli (10).

Methyl esters of fatty acids were prepared by methylation of aliquots of the total lipid extracts, after evaporation, using BF_3 as catalyst according to Metcalf and Schmitz (11). The methylated mixture, after extraction into hexane, was treated with activated alumina to remove cholesterol and other unsaponifiable material by a procedure developed by J F. Mead (personal communication). Recoveries showed that about 10% of the methyl esters were retained by the alumina but there was no preferential loss of saturated or unsaturated fatty acids.

Gas-liquid chromatography was performed with a Barber-Colman Model 15 instrument with a radium foil, argon ionization detector. Columns 6 ft x 4 mm internal diameter were packed with 15% ethylene glycol succinate on Chromosorb W, 80–100 mesh. Temperatures were: column, 180C; detector, 205C; flash heater, 240C. Inlet pressure was 20–25 psi and the relative gain was 10 or 30.

Results and Discussion

The first evidence bearing on a possible function of selenium came from the observations (12,13) that tissues from chicks fed trace amounts of selenium have an antioxidant-like property not very different from that seen in tissues of chicks given vitamin E. When tissues from vitamin E-depleted chicks are homogenized and subsequently incubated in air, the tissue polyunsaturated fatty acids undergo a non-enzymatic oxidation. The feeding of vitamin E, selenium or certain antioxidants inhibits this lipid autoxidation, particularly in liver and kidney (Table II).

TABLE I

Vitamin E Deficiency in the Chick and Its Prevention by Dietary Supplements

Syndrome	Prevented by
Encephalomalacia	α-Tocopherol; various antioxidants
Muscle dystrophy	α-Tocopherol; cystine; ethoxyquin[a]
Exudative diathesis	α-Tocopherol; selenium; ethoxyquin

[a] "Santoquin," Monsanto Chemical Co., St. Louis, Mo.

[1] Presented at the AOCS meeting in Toronto, Canada, 1962.

TABLE II

Lipid Peroxidation in Incubated Tissue Homogenates from Chicks Fed a Diet with 30% Isolated Soybean Protein

Group	Addition to diet	Average TBA values[a]					
		Liver	Kidney	Muscle	Heart	Lung	Spleen
1	None	105	130	45	80	40	150
2	0.01% dl,α-Tocopheryl acetate	20[b]	35[b]	30	15[b]	15[b]	10[b]
3	0.1% Ethoxyquin	30[b]	10[b]	35	20[b]	30[b]	90[b]
4	0.33 ppm Selenium	40[b]	70[b]	35	55[b]	40	110
5	0.3% L-Cystine	95	120	30	85	55	150

[a] A535 × 1000. Nine to sixteen chicks in each group. 5% homogenates incubated for 1 hr at 37C.
[b] Significantly lower than Group 1 ($P < 0.01$).

These results readily suggested that selenium becomes incorporated into some cellular compound which has antioxidant properties. Tappel et al. (14) have presented other evidence supporting this theory. In our continuation of these studies we have found that this antioxidant-like effect of selenium, which can be shown readily in the chick, cannot be demonstrated under similar dietary conditions in the rat (Table III). There was no effect of dietary selenium on lipid peroxidation in either liver or heart. We have also found a negative effect of selenium in guinea pig heart. A similar negative response of rat liver to selenium was reported by Corwin (15). Why selenium is not active in this respect in the rat and guinea pig in contrast to its action in the chick remains to be determined.

TABLE III

Lipid Peroxidation in Incubated Rat Liver and Heart Homogenates[a]

Addition to diet	No. of rats	Average TBA values[b]	
		Liver	Heart
None	3	337 ± 53	196 ± 18
0.5 ppm Se	3	395 ± 30	179 ± 9
0.01% α-tocopheryl acetate	3	0	0

[a] Rats fed a basal diet deficient in viatmin E and selenium with 12.5% isolated soybean protein and 4% stripped lard, for 60 days.
[b] A535 ×-1000; 1 hr incubation, 37C. Liver = 3 rats; heart = 2 rats.

It is well known that erythrocytes of vitamin E-depleted animals when removed from the body and subjected to certain oxidative treatments are more fragile than red cells from animals which are normal with respect to vitamin E. When red cells from depleted rats were incubated with dialuric acid a rapid hemolysis occurred which was paralleled by lipid peroxidation (Fig. 1). Cells from rats given dietary selenite showed an initial inhibition to hemolysis and peroxidation which disappeared after 20 min, while cells from vitamin E-supplemented animals maintained their stability.

Other experiments on erythrocytes in which their resistance to osmotic hemolysis was measured have not shown any protective action from dietary selenite. Furthermore, in the supernatant hemolysates from the vitamin E-deficient animals, there was no evidence of lipid peroxidation as measured by the thiobarbituric acid test. It would appear that osmotic hemolysis is not associated with lipid peroxidation. The apparent discrepancy between the dialuric acid and the osmotic hemolysis tests with respect to dietary selenium requires further exploration.

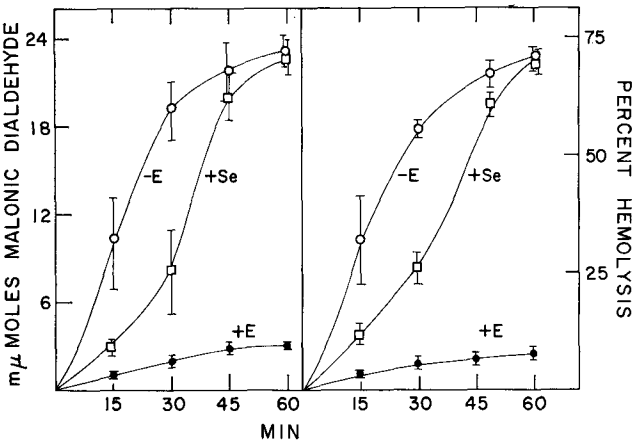

FIG. 1. Per cent hemolysis and lipid peroxidation (malonic dialdehyde production) in rat erythrocytes incubated with dialuric acid. Basal diet (−E) contained 30% Torula yeast and 4% stripped lard; + E = 200 mg α-tocopheryl acetate per kg; + Se = 0.5 ppm selenium.

In considering how dietary selenium might be affecting tissue composition, it appeared possible that alterations in the lipids, particularly the fatty acids, could occur and that these might account for the observed antioxidant-like effects. If selenium in some way decreased the tissue concentration of polyunsaturated fatty acids, then the tissue homogenates from chicks fed selenium would not undergo as much lipid peroxidation as would tissues from unsupplemented birds. To test this possibility, liver and blood plasma from chicks fed vitamin E or selenium in diets differing in the type of fat were analyzed. In three experiments, purified diets contained no added fat, 2% coconut oil, or 4% stripped lard. Analyses of liver and plasma for total lipid, phospholipid and total cholesterol (Table IV) showed that neither vitamin E nor selenium had any effect on these parameters of lipid metabolism. In only one area was a significant interaction noted, and that was in the fatty acid composition of plasma total lipid from chicks fed the diet with 4% of stripped lard (Table V). Vitamin

TABLE IV

Liver Lipid Composition of Chicks Fed Diets with or without Vitamin E and Selenium[a]

Diet characteristics	No. of weeks	Total lipid	Total cholesterol	Phospho-lipid	α-Tocoph-erol	Vitamin A	Co. Q_{10}
		mg/g	mg/g	mg/g	μg/g	μg/g	μg/g
Fat-free	10	40.7 ± 5.6	4.0 ± 0.2	24.1 ± 1.0	0	69.9 ± 3.9	45.6 ± 2.2
Fat-free + Vit.E	10	35.0 ± 2.6	4.0 ± 1.4	22.8 ± 0.9	9.4 ± 1.0	66.7 ± 9.3	61.1 ± 7.6
Fat-free + Se	10	42.7 ± 1.7	4.9 ± 0.4	25.3 ± 1.4	0	69.4 ± 10.2	49.5 ± 8.6
2% Coconut oil	6	41.4 ± 1.0	3.9 ± 0.1	26.2 ± 0.4	0	30.2 ± 2.1	54.3 ± 5.0
2% Coconut oil + Vit. E	6	47.8 ± 5.0	4.0 ± 0.1	24.3 ± 2.0	23.7 ± 5.7	33.8 ± 4.1	60.0 ± 8.3
2% Coconut oil + Se	6	38.6 ± 0.9	3.8 ± 0.8	25.4 ± 0.5	0	26.0 ± 2.6	53.9 ± 6.3
4% Stripped lard	6	38.1 ± 0.7	3.8 ± 0.2	25.4 ± 0.5	0	5.2 ± 1.2[b]	52.8 ± 4.9
4% Stripped lard + Vit. E	6	39.4 ± 1.5	3.5 ± 0.2	25.0 ± 0.7	16.6 ± 1.6	13.6 ± 2.6[b]	59.2 ± 4.5
4% Stripped lard + Se	6	39.1 ± 0.8	3.7 ± 0.2	24.6 ± 0.6	0	12.6 ± 1.0[b]	58.8 ± 5.4

[a] All diets contained 30% isolated soybean protein. Six chicks per group. Means with standard errors.
[b] Lower dietary level of vitamin A used in this experiment.

TABLE V
Effect of Vitamin E and Selenium on Plasma Fatty Acids of Chicks[a]

Fatty acid	Basal	Basal + Se	Basal + Vit. E
14	0.3 ± 0.03	0.2 ± 0.01	0.3 ± 0.01
16A[b]	0.4 ± 0.03	0.4 ± 0.04	0.3 ± 0.03
16	23.4 ± 1.0	23.5 ± 0.8	23.6 ± 0.4
16:1	3.1 ± 0.3	2.8 ± 0.3	2.5 ± 0.2
18	18.2 ± 1.2	19.3 ± 1.1	18.9 ± 0.5
18:1	32.7 ± 1.2	29.4 ± 0.5	30.1 ± 1.4
18:2	11.9 ± 0.5	12.5 ± 0.6	12.2 ± 0.4
18:3	0.6 ± 0.09	0.5 ± 0.09	0.4 ± 0.06
20:3[c]	2.2 ± 0.2	2.4 ± 0.2	1.5 ± 0.2[e]
20:3[d]	2.0 ± 0.1	1.8 ± 0.2	1.9 ± 0.1
20:4	5.2 ± 0.5	6.9 ± 0.7	8.1 ± 0.6[e]

[a] Diets contained 30% isolated soybean protein with 4% stripped lard. Four chicks per group; 28 days. Means with standard errors.
[b] Retention time corresponded to C_{18} aldehyde.
[c] 5, 8, 11-eicosatrienoic acid.
[d] Tentatively identified as 8, 11, 14-eicosatrienoic acid.
[e] Significantly different from the basal group ($P < 0.01$).

E significantly increased the arachidonic acid and decreased 5, 8, 11-eicosatrienoic acid. Similar changes due to vitamin E were noted in the liver fatty acids (Table VI); the C22:6 was also significantly elevated in this group. Although these are real dietary effects of α-tocopherol, their physiological significance, if any, is unknown. The studies show, however, that selenium

TABLE VI
Effect of Vitamin E and Selenium on Liver Fatty Acids of Chicks[a]

Fatty acid	Basal	Basal + Se	Basal + Vit. E
14	T[b]	T	T
16A[c]	0.3 ± 0.06	0.3 ± 0.02	0.3 ± 0.03
16	18.4 ± 2.0	18.8 ± 0.8	18.2 ± 1.2
16:1	1.7 ± 0.4	1.9 ± 0.2	1.3 ± 0.3
18A[c]	0.4 ± 0.07	0.2 ± 0.08	0.6 ± 0.1
18	24.6 ± 2.4	24.9 ± 0.8	26.5 ± 1.3
18:1	24.5 ± 2.2	23.3 ± 0.9	21.1 ± 2.0
18:2	11.4 ± 0.8	11.5 ± 0.8	10.6 ± 0.4
18:3	0.4 ± 0.04	0.4 ± 0.02	0.4 ± 0.05
20:3[d]	2.0 ± 0.2	1.9 ± 0.2	1.2 ± 0.1[f]
20:3[e]	2.4 ± 0.2	2.3 ± 0.3	2.1 ± 0.1
20:4	10.6 ± 1.5	11.8 ± 0.5	13.9 ± 0.6[f]
20:5	0.4 ± 0.05	0.3 ± 0.1	T
24:1	0.6 ± 0.15	0.4 ± 0.14	0.7 ± 0.05
22:5	0.6 ± 0.05	0.7 ± 0.09	0.7 ± 0.25
22:6	1.3 ± 0.1	1.4 ± 0.1	2.1 ± 0.2[f]

[a] Diets contained 30% isolated soybean protein with 4% stripped lard. Four chicks per group; 28 days. Means with standard errors.
[b] Trace = <0.1%.
[c] Retention times correspond to aldehydes.
[d] 5, 8, 11-eicosatrienoic acid.
[e] Tentatively identified as 8, 11, 14-eicosatrienoic.
[f] Significantly different from the basal group ($P < 0.01$).

does not alter the lipid composition of these tissues and indicate that the site of action of the element must be sought elsewhere.

Of special interest is the significant amount of a fatty acid tentatively identified as 8, 11, 14-eicosatrienoic acid. We have found this peak frequently in chicken liver, plasma and erythrocytes, particularly in birds fed diets containing reasonable amounts of linoleic acid, e.g., 4% corn oil or safflower oil. A similar peak has been reported by Morin et al. (16) in rat liver phospholipids, and Mead et al. (17) have postulated this fatty acid to be an intermediate in the conversion of linoleic acid to arachidonic acid.

There have been suggestions (18) that both vitamin

TABLE VIII
Prevention of the Toxicity of Ethoxyquin by Dietary Vitamin E and Selenium[a]

Additions to diet	Exudates %	Mortality %
None	100	94
0.05% Ethoxyquin	53	96
0.005% α-Tocopherol	50	0
0.025 ppm Selenium	94	38
0.05% Ethoxyquin + 0.005% α-Tocopherol	0	0
0.05% Ethoxyquin + 0.025 ppm Selenium	0	25
0.05% Ethoxyquin + 0.05 ppm Selenium	0	0
0.01% α-Tocopherol	0	0
0.05 ppm Selenium	0	0

[a] Diet contained 60% Torula yeast, 4% stripped lard and 0.3% DL-methionine. Eight chicks per group; 28 day period.

E and selenium may have a regulatory role on the concentration of coenzyme Q (ubiquinone) in tissues. We have looked into a possible interrelationship among these metabolites in the series of chick experiments mentioned above with regard to lipid composition (Table IV). It is apparent that under our experimental conditions neither vitamin E nor selenium had any effect on the coenzyme Q content of liver. Other tissues were not examined.

Olson et al. (19,20) reported that rats fed trace amounts of selenium had increased levels of coenzyme A (Co A) in their livers and that selenium greatly increased the incorporation of S^{35}-cystine into Co A. We determined the Co A levels in liver from chicks fed purified diets with 15 or 30% protein with 0.3% of added L-cystine and could find no relationship between Co A, vitamin E or selenium. When the supplementary cystine in the diets was omitted, then dietary selenium had a definite effect on liver Co A levels (Table VII). In the basal groups (experiment B), there was a decrease to about one-half that in the groups receiving cystine (experiment A). Both vitamin E and selenium prevented the decrease in Co A concentration, with the effect of selenium being more pronounced in the 15% protein diet. The metabolic significance of these differences has not been explored. These results, however, confirm that an interrelationship exists between the sulfur amino acids, vitamin E and selenium.

We have recently made an observation of another physiological effect of selenium which has not been noted heretofore. As reported by Machlin (21), we have found that the antioxidant ethoxyquin would replace vitamin E and prevent all three deficiency syndromes (Table I) in the chick. In contrast, the group at Cornell University reported that ethoxyquin would not prevent the symptom of exudative diathesis except at a level of 0.05% which they stated to be toxic (22). Since neither in Machlin's or our own work had any toxicity of ethoxyquin been noted at this, or even higher levels, there appeared to be a discrepancy in these results. This was resolved when we found that ethoxyquin was indeed toxic when

TABLE VII
Effect of Vitamin E and Selenium on the Coenzyme A Content of Chick Liver[a]

	Units of coenzyme A per mg N					
	15% Protein			30% Protein		
	Basal	+ Se	+ Vit. E	Basal	+ Se	+ Vit. E
Experiment A (+0.3% L-cystine)	4.6 ± 0.6	5.0 ± 0.4	4.5 ± 0.1	4.4 ± 0.2	4.7 ± 0.3	3.8 ± 0.5
Experiment B (No L-cystine)	2.7 ± 0.3[b]	4.7 ± 0.5[b]	3.8 ± 0.3[b]	2.9 ± 0.5[c]	3.7 ± 0.2	4.4 ± 0.3

[a] Basal diets contained isolated soybean protein with 6% stripped lard. No added methionine. Four chicks per group except where indicated. Twenty-eight day feeding period. Values are means with standard errors.
[b] Six chicks per group. Compared to basal group, selenium = $P < 0.01$; vitamin E = $P < 0.05$.
[c] Significantly less ($P < .05$) than the basal group with cystine. The selenium and vitamin E groups are not significantly different from this value.

fed in a vitamin E and selenium-free diet containing torula yeast, but was innocuous when fed in a soybean protein type diet. Subsequent work revealed that the adverse effects of ethoxyquin in the torula yeast diet could be completely overcome by adding selenium, as selenite, in amounts which by themselves had very little effect in preventing exudates or mortality (Table VIII). Low dietary concentrations of α-tocopherol which were not completely active in preventing deficiency symptoms also eliminated the toxicity. It is probable that there is sufficient selenium in the soybean protein diet to prevent the toxicity of ethoxyquin. This protective action of selenium appears to be similar to that exerted by vitamin E under a variety of toxic conditions (23).

These various studies provide further information of metabolic effects of dietary selenium as related to vitamin E. The similarity of action of these two substances, as for example their relationship to coenzyme A and their prevention of certain toxicities, suggests a common biochemical mechanism. The hypothesis that vitamin E and selenium act *exclusively* as tissue antioxidants and thereby stabilize critical polyunsaturated fatty acids in the cell is weakened by the negative results with rat and guinea pig tissues; a general ground-theory should apply to a variety of species. On the other hand, there is insufficient evidence to support a hypothesis that α-tocopherol and selenium act specifically in one or more pathways of intermediary metabolism. Clearly, more information must be obtained to enable us to unravel the intriguing interrelationship between these substances.

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The Biological Consequences of Feeding Polyunsaturated Fatty Acids to Antioxidant-Deficient Animals

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Abstract

The addition of polyunsaturated fatty acids (PUFA) to diets deficient in vitamin E and other effective antioxidants results in a variety of symptoms in animals. For example, the feeding of such diets to rats results in muscular dystrophy, testis degeneration, dental depigmentation, brown discoloration of the fat and and uterus and creatinuria. Similar diets fed to rabbits and ruminants results in muscular dystrophy. In chickens the symptoms observed are encephalomalacia, lowered egg production, and poor hatchability. The addition of PUFA to diets is known to result in the destruction of vitamin E in the diet or in the tissues of animals as a result of free radicals produced during the autooxidation of the PUFA. However, in several studies, this possible explanation for the development of vitamin E deficiency symptoms has been made untenable. In such studies the more likely explanation for development of symptoms is the *in vivo* peroxidation of PUFA in the tissues of animals following incorporation of large amounts of PUFA in lipid structures and depletion from the tissues of vitamin E and other biologically effective antioxidants.

Introduction

THE TOPIC of antioxidant-unsaturated fatty acid relationships is not new and, in fact, began shortly after the initial discovery of our most important *in vivo* antioxidant vitamin E. In 1923, Evans recognized that vitamin E was necessary for the reproduction of the rat and, just a few years later, Agdur (1) and Evans (2) found that vitamin E deficiency symptoms were exaggerated by the addition of cod liver oil to the diet. Why the recent interest in this topic? One of the reasons is the popularization of the concept that unsaturated fatty acids will decrease blood cholesterol in humans, with the inference that this lowered cholesterol would reduce atherosclerosis. The increased consumption of PUFA has caused some concern, that excessively high intakes of unsaturated fatty acids may precipitate vitamin E deficiencies in humans (3). Another reason for renewed interest is the accumulation of evidence in the last few years that many synthetic antioxidants can prevent the deleterious effects of unsaturated fatty acids in animals. Thirdly, we now have available elegant procedures for the analysis of fatty acids in tissues, and also more refined nutritional techniques. This has led to experiments which help clarify and quantitate the initial observations on the relationships between polyunsaturated fatty acids (PUFA) and vitamin E.

Recent works which have helped establish some of the following points are briefly reviewed below:

1) PUFA are necessary for the development of most vitamin E deficiency symptoms. Where they are not absolutely necessary, PUFA almost invariably will exaggerate the requirement (3,4,5).

2) PUFA-induced symptoms can be prevented by a number of synthetic antioxidants of quite varied chemical structures (5,6,7,8,9).