Studies on Vitamin E and Selenium Deficiency in Young Pigs
I. Hematological and Biochemical Changes

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ABSTRACT

Pigs which were deficient in vitamin E and/or selenium had the following parameters weekly determined from six to 13 weeks of age: Packed cell volume, hemoglobin concentration, red cell and white cell counts, red cell indices, reticulocyte count, serum iron, serum total iron binding capacity, myeloid: erythroid ratio, serum glutamic-oxaloacetic transaminase and creatine phosphokinase activities and body weight. Except for the myeloid:erythroid ratio and serum creatine phosphokinase activity, these parameters were not found to be significantly affected by either vitamin E deficiency, selenium deficiency or deficiency of both. The myeloid:erythroid ratio was increased ($p<0.01$) in association with selenium deficiency, which tends to indicate decreased erythropoiesis but was not reflected in the peripheral red cell picture. Evidence of dyserythropoiesis was not found to be a significant feature in serial bone marrow aspiration biopsies of vitamin E and/or selenium deficient pigs. Even if the serum glutamic-oxaloacetic transaminase activities were not found to be significantly affected by either vitamin E deficiency, selenium deficiency or deficiency in both as compared to replete animals, a few animals, especially in the group deficient in both vitamin E and selenium, presented quite marked transient increases of serum glutamic-oxaloacetic transaminase activity which was interpreted to reflect the occurrence of acute episodes of hepatosis dietetica. Serum creatine phosphokinase activities were found to be increased in association with vitamin E deficiency ($p<0.01$), selenium deficiency (<0.05) and the interaction was also significant ($p<0.01$). It was concluded that the serum creatine phosphokinase activity increases reflect the occurrence of subclinical muscular dystrophy and that vitamin E and selenium deficiencies have marked additive effects in the induction of skeletal muscular dystrophy.

RÉSUMÉ

Cette expérience visait à déterminer hebdomadairement, de la sixième à la 13e semaine d'âge, chez des porcelets atteints de déficiences simultanées ou non en vitamine E et en sélénium, les paramètres suivants: l'hématocritie, la teneur du sang en hémoglobine, la numération des hématies et des leucocytes, les indices érythrocytaires, la numération des réticulocytes, la teneur du sérum en fer, la capacité de saturation totale de la transferrine, le rapport granulo-érythroblastique, l'activité de la transaminase glutamique-oxaloacétique et de la créatine phosphokinase sériques, ainsi que le poids corporel.

Sauf pour le rapport granulo-érythroblastique et l'activité de la créatine phosphokinase sérique, les paramètres sanguins ne subirent pas de changements appréciables à cause de déficiences simultanées ou non en vitamine E et en sélénium.

Le rapport granulo-érythroblastique augmenta ($p<0.01$) lors d'une déficience en vitamine E, laissant supposer une diminution de l'érythropoïèse qui ne se manifesta toutefois pas dans le sang périphérique. L'examen d'aspirations répétées de moelle osseuse, chez les porcelets atteints de déficiences simultanées ou non en vitamine E et en sélénium, ne révéla pas de dysérythropoïèse appréciable.

Même si l'activité de la transaminase glutamique-oxaloacétique sérique n'accusa pas d'altération appréciable, à la suite de déficiences simultanées ou non en vitamine E et en sélénium, par comparaison avec les sujets témoins, quelques porcelets appartenant au groupe

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Based on a thesis submitted by M. Fontaine in partial fulfilment of the requirements for the Ph.D. degree at the University of Guelph.

Submitted October 21, 1975.

souffrant de déficiences simultanées en vitamine E et en sélénium présentèrent des augmentations transitoires marquées de l'activité de cette transaminase; on interpréta ce phénomène comme l'indice de crises aiguës d'hépatose diététique.

L'activité de la créatine phosphokinase sérique subit une augmentation, lors de déficiences en vitamine E ($p<0.01$) et en sélénium ($p<0.05$); de plus, une déficience simultanée de ces deux substances provoqua une interaction appréciable ($p<0.01$).

Les auteurs ont conclu qu'une augmentation de l'activité de la créatine phosphokinase sérique reflète la présence d'une dystrophie musculaire subclinique et qu'une déficience simultanée en vitamine E et en sélénium accentue le développement de la dystrophie musculaire.

INTRODUCTION

Over five decades have passed since the discovery of vitamin E or alpha tocopherol by Evans and Bishop (11). Since then vitamin E deficiency has been associated with a wide variety of syndromes in several animal species and in man but the precise biological function of tocopherol has not yet been determined. This pleomorphism in the manifestations of the deficiency of a single nutritional agent has prevented the formulation of a satisfactory generalized hypothesis for its mode of action at the cellular level.

As investigators became involved in the field of vitamin E deficiency the element selenium was soon found to have a sparing effect on the occurrence of many tocopherol deficiency associated syndromes. The discovery of this interaction between two quite dissimilar compounds increased the complexity of explaining their mechanism(s) of action.

In swine nutrition the importance of vitamin E became apparent with the work of Obel (40). A few years later selenium was shown to have a preventive effect on the occurrence of the so-called vitamin E deficiency syndrome in pigs (9). Thereafter for the past 20 years, much effort has been devoted to experimentally reproducing the vitamin E and selenium deficiency syndrome in pigs and the pathological lesions have been well characterized (19, 35, 38, 49).

Several investigators (1, 19, 34, 36, 37, 40) have reported an anemic condition to occur as part of the vitamin E deficiency syndrome in swine as it is observed in vitamin E deficient premature human infants (30, 32, 44, 45, 47) and in primates under experimental vitamin E deficiency (8, 14, 15, 46). The character of the anemia having not been further investigated in swine, prompted this study to measure and characterize the anemia reported to occur in association with vitamin E deficiency in pigs. Since selenium was recently found to be a component of the enzyme glutathione peroxidase in erythrocytes of rats (48), chicks (39), cattle (16) and sheep (41), selenium deficiency was also considered as a possible cause of hemolytic anemia in swine.

The primary organ involved in the clinical diagnosis of vitamin E and selenium deficiency in swine is difficult to determine because complex pathological changes involving the skeletal muscles, the liver and the heart, usually occur simultaneously (4, 19, 25, 33, 38, 50, 56). The situation is further complicated by the fact that vitamin E and selenium deficient pigs will often die suddenly without premonitory signs. Various laboratory procedures have been devised to supplement the clinical examination for the diagnosis of vitamin E and selenium deficiency (19, 24). The determination of vitamin E and selenium in blood and in tissues is a direct and specific test. Unfortunately, however, this approach is not suitable for routine diagnosis. The peroxidative hemolysis of erythrocytes has been used as an index of the vitamin E status, but the results which have been reported are conflicting (17, 19, 21). Another group of diagnostic methods includes the determination of biochemical changes which take place in connection with the lesions in the various organs such as creatinuria, myoglobinuria, increased plasma potassium and increased serum bilirubin. Hyldgaard-Jensen (24) made a survey of this latter group of diagnostic procedures and concluded that they were of very little diagnostic value for vitamin E deficiency.

In swine, a growing body of knowledge concerning the enzymes activities in various tissues has greatly enhanced their usefulness as diagnostic tools. Wretlind *et al* (59) investigated tissue activity distribution of glutamic-oxaloacetic transaminase (GOT) in pigs and recorded the following

values (times 10^4 Reitman-Frankel units):

Heart	5.63
Liver	4.10
Skeletal muscle	1.47

Those results clearly indicate that GOT is not organ specific in swine and that any of the vitamin E and selenium deficiency associated lesions, alone or in combination, may give rise to an increased GOT activity. Serum GOT activity has, however, been extensively used as an index of tissue damage in association with vitamin E and/or selenium deficiency (12, 19, 20, 28, 31, 43, 54, 55, 56, 58).

In mammals, the use of serum creatine phosphokinase (CPK) has been considered to be the most sensitive and specific enzyme test of myopathic conditions, this enzyme being relatively specific to striated muscles (3, 6, 10, 18, 24, 53).

Serum GOT and CPK activities were therefore determined concurrently in vitamin E and/or selenium deficient pigs to evaluate the usefulness of those parameters as indices of tissue damage.

MATERIALS AND METHODS

Experimental Animals

Two groups of 24 crossbred (purebred Yorkshire boar and Landrace sows) white piglets from seven different litters, were bought from the same farm and the sows placed on the vitamin E and selenium deficient diet (Table I) as soon as the piglets started to eat (i.e. about three weeks of age). The male piglets were castrated at three weeks of age. The piglets were thereafter weaned onto the experimental diet, which was finely ground, at five weeks of age. The diet was selected on the basis of the high incidence of vitamin E and selenium deficiency associated lesions recorded by Sharp *et al* (51) using a similar high moisture corn-soybean meal diet for its low vitamin E and selenium concentrations. After weaning, the animals were moved to the Ontario Veterinary College where they were tagged and assigned to a two x two factorial arrangement. When possible an equal number of piglets of each sex was assigned to each treatment and the body weight was also considered during allotment of piglets. The factors were two levels of supplemental vitamin E[1] (zero, 250 I.U.) and two levels of supplemental selenium[2] (zero, 1 mg). All the animals were given the same diet and the vitamin E and selenium were given parenterally each week from six weeks of age, according to the two by two factorial arrangement. To get replications for each of the four treatments (+ E + Se, + E −Se, −E +Se, −E −Se), the pigs were kept four per pen. During the period of study, the animals had free access to water, were fed *ad libitum* and kept on a wood shaving bedding on concrete.

TABLE I. Composition of the Experimental Diet[a] Used for the Induction of Vitamin E and Selenium Deficiency in Pigs

Ingredients	% (weight basis)
Propionic acid treated corn (75.3% dry matter)	79.08
Soybean meal (49% crude protein)	18.11
Trace mineral premix[b]	0.91
Vitamin premix[c]	0.45
Cobalt iodized salt	0.45
Calcium phosphate	0.91
Limestone	0.91

[a]Dry matter: 79.68%
Crude protein: 19.97% (dry matter basis)
[b]Trace minerals added in ppm of complete diet; manganese 60, iron 70, copper 10, zinc 100
[c]Vitamins added per kg of complete diet; riboflavin 4.4 mg, d-calcium pantothenate 8.8 mg, niacin 19.8 mg, choline chloride 110.2 mg, B_{12} 19.8 µg, A 3,307 I.U., menadione sodium bisulfite 2.2 mg. D_2 551.2 I.U.

Experimental Procedure

The various determinations were done weekly from six weeks of age to 13 weeks of age. At six weeks of age all the animals were in the same condition (i.e. deficient in both vitamin E and selenium), therefore the determinations done at that time represent the base level. The parenteral supplementations were started after the first sampling at six weeks of age.

The following parameters were studied weekly in both groups of 24 pigs which gave 12 replications for each treatment: packed cell volume, hemoglobin concentration, red cell and white cell counts, red cell indices (mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscu-

[1]Vitamin E (dl, alpha-tocopherol acetate) Injectable, Agricultural Research Department, Hoffman-La Roche Inc., Nutley, New Jersey.

[2]Selenium (sodium selenite) Injectable prepared by Stevenson, Turner and Boyce for experimental use.

lar hemoglobin concentration), serum glutamic-oxaloacetic transaminase (GOT) and creatine phosphokinase (CPK) activities and body weight. In addition, the following weekly determinations were performed for the first group of 24 pigs: serum iron, serum total iron binding capacity, myeloid:erythroid ratio and reticulocyte count.

To verify the efficacy of the experimental diet and the parenteral supplementations upon the vitamin E and selenium status of the animals, serum vitamin E and selenium concentrations were determined for all the pigs at three times during the period of study (i.e. six, nine and 13 weeks of age). These determinations were performed on serum samples collected one week after the last parenteral supplementations. Furthermore, the experimental diet was assayed for its vitamin E and selenium content on three random samples taken from the bags of feed during the period of study.

TECHNIQUES

a) *Blood Collection* — All the blood samples were collected by the orbital sinus bleeding technique as described by Huhn *et al* (23), the animal being held in a dorsal recumbent position in a V-shaped trough. A 16 ga, 1½ inch needle was used instead of a glass bleeding pipette.

b) *Routine Hematology* — A 2 ml blood sample drawn in EDTA was used for the routine hematology determinations. Packed cell volumes (PCV) were determined using a Clay Adams[3] microhematocrit centrifuge[4] and microhematocrit reader. Hemoglobin determinations were done with a Coulter Hemoglobinometer[5] and erythrocyte and leukocyte counts were determined using a Coulter Counter model Z_{BI}[5]. The red cell indices were calculated using the standard formulas (5).

Reticulocytes were stained with new methylene blue (5) and blood smears prepared in a routine manner, were counterstained with Wright's stain in an automatic slide stainer.[6] Reticulocytes were counted according to the procedure of Brecher and Schneiderman (2) using a Miller ocular micrometer disc.

c) *Serum Iron and Total Iron Binding Capacity* — The serum iron and total iron binding capacity determinations were performed using Hyland Ferro-Check II Test[7] according to the manufacturer's specifications.

d) *Bone Marrow Sampling and Examination* — Bone marrow aspiration biopsies were taken from sternebrae using sterile 18 ga, one inch needles[8] and new sterile 12 ml disposable plastic syringes.[9] A small amount of material from each marrow aspirate was discharged onto a glass slide. The slide was tilted slightly and excess blood was drawn off the granules onto a gauze sponge. A spreader slide was used to pick off a few marrow granules which were smeared onto a clean glass slide in such a manner as to leave a thin trail of cells behind each granule (7). Air drying of the smears was hastened by waving. The smears were stained with Wright's in the automatic slide stainer.

Myeloid:erythroid (M:E) ratios were evaluated on the cells found in the trail behind the smeared marrow granules. Two hand counters[10] were used in recording the cell types. Cells were classified into either the myeloid or erythroid series until 100 cells of the erythroid series had been counted, leaving out the lymphocytes, monocytes, plasma cells and all reticulum cells (including bare nuclei). Multinucleated erythroid precursors, when encountered, were recorded and their number expressed as percentage multinuclearity in the erythroid series.

e) *Vitamin E and Selenium Determinations* — The serum selenium concentrations as well as the estimations of the selenium present in the diet were determined according to the procedure used by Sharp (49) which is essentially the fluorometric procedure of Hoffman *et al* (22) utilizing 2,3-diaminonaphthalene[11] which forms a flu-

[3]Clay Adams Co., Canlab Supplies, Toronto, Ontario.

[4]R.C.F. of 15,000 x gravity.

[5]Coulter Electronics Inc., Toronto, Ontario.

[6]Ames Hema-Tek Slide Stainer, Fisher Scientific Co., Toronto, Ontario.

[7]Hyland Ferro-Check II Test, Canlab Supplies, Toronto, Ontario.

[8]Becton Dickinson, Osgood Biopsy Needle, Canlab Supplies, Toronto, Ontario.

[9]Monoject Sherwood, Canlab Supplies, Toronto, Ontario.

[10]Hand Counter, Canlab Supplies, Toronto, Ontario.

[11]Aldrich Chemical Co. (Daniels Chemical Co., Montréal, Québec).

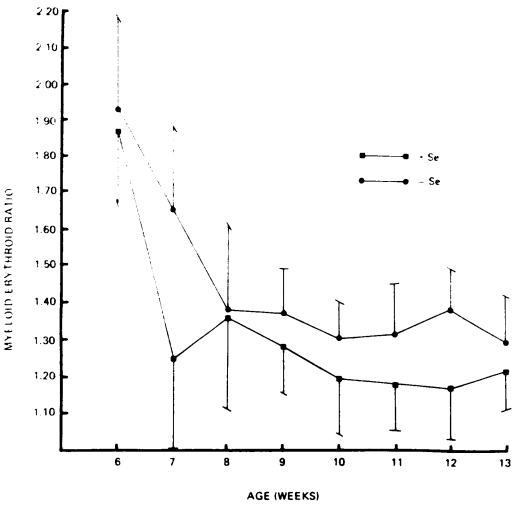

Fig. 1. Effect of selenium supplementation on myeloid: erythroid ratio (each point represents the mean value from 12 animals and brackets indicate 95% confidence intervals).

orescent piazselenol. A 1 ml serum sample and 1 gm diet sample were respectively used in that procedure.

The serum vitamin E determinations were conducted according to the method of Fabianek *et al* (13) where tocopherol is oxidized by ferric chloride and the zinc complex formed by ferrous ions with 4,7-diphenyl-1,10-phenanthroline[12] (bathalphenanthroline) is determined spectrophotometrically. The method was modified in two respects: 2.5 ml serum was used instead of 0.4 ml, with corresponding adjustments of volume of reagents, and hexane was substituted to xylene for tocopherol extraction. The technique described by Sharp (49) was used for diet vitamin E determinations.

f) *GOT and CPK Determinations* — For the enzyme determinations, the serums were separated within two hours after blood collection and stored at –20°C until the determinations were done. Serum GOT activities were measured according to the reaction rate method of Karmen (26) and Karmen *et al* (27), adapted to the Reaction Rate Analyser[13] and using commercially available GOT reagents.[14] Similarly, serum CPK activities were determined according to the reaction rate method of Oliver (42) adapted to the Reaction Rate Analyser and commercially available CPK reagents. For both GOT and CPK determinations, abnormal human serum[15] was used as quality control to get activity in the same range as recorded with swine serums, especially for CPK.

g) *Statistical Analysis* — The statistical analysis on the results of weekly determinations was done by analysis of variance, according to the following plan:

Sources of Variation	Degrees of Freedom
Replications	5 (11)
Treatments	3
E	1
Se	1
E x Se	1
Error	15 (33)
Total	23 (47)

RESULTS

The results for the parameters weekly determined from six weeks of age (base level) to 13 weeks of age in vitamin E and/or selenium deficient pigs are summarized in Tables II to IV where the weekly means for each of the four different treatments (+E+Se, +E–Se, –E+Se, –E–Se) and the F-values derived from the analysis of variance, are given. As indicated in Table III and illustrated in Fig. 1, the myeloid: erythroid ratio was found to be increased ($p<0.01$) in association with selenium deficiency. Serum CPK activities were increased in association with vitamin E deficiency ($p<0.01$), selenum deficiency ($p<0.05$) and the interaction was also significant ($p<0.01$). The weekly means for serum CPK activity were plotted for each level of vitamin E and selenium (Fig. 2). Packed cell volume, hemoglobin concentration, red cell count, red cell indices, white cell count, reticulocyte count, serum iron, serum total iron binding capacity, serum GOT activity and body weight were not found to be affected by either vitamin E and/or selenium deficiency. Furthermore,

[12]Frederick Smith Chemical Co., Columbus, Ohio.

[13]LKB 8600 Reaction Rate Analyser, Fisher Scientific Co., Toronto, Ontario.

[14]Boehringer Mannheim GMBH, Montréal, Québec.

[15]Validate-A, Warner-Chilcott Co., Toronto, Ontario.

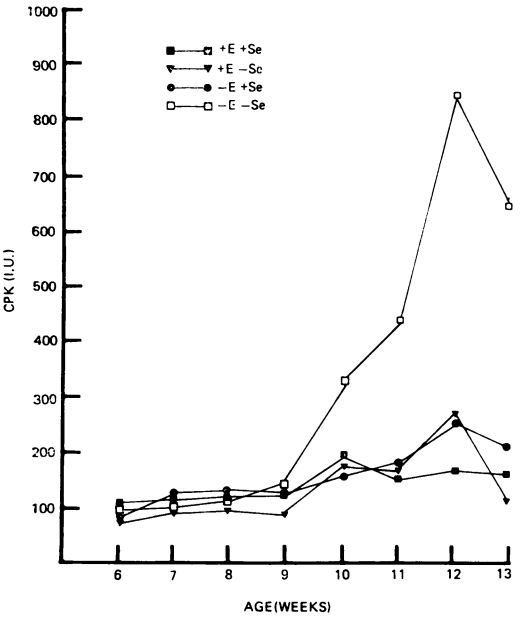

Fig. 2. Effect of vitamin E and/or selenium supplementation on serum CPK activity (each point represents the mean from 12 animals).

the presence of multinucleated erythroid precursors was not found to be a significant feature in bone marrow smears of vitamin E and/or selenium deficient animals, an occasional multinucleated erythroid precursor being observed in bone marrow smears of deficient animals as well as in replete animals.

The mean (± one standard deviation) vitamin E and selenium levels of the experimental diet, determined at three different times during the period of study, were respectively 1.20 ± 0.07 and 0.023 ± 0.002 $\mu g/g$ of feed. Results of the serum vitamin E and selenium determinations are illustrated in Figs. 3 and 4.

DISCUSSION

The packed cell volume, hemoglobin concentration, red and white cell counts, red cell indices, reticulocyte count, serum iron,

TABLE II. The Effect of Vitamin E and /or Selenium Deficiency in Swine on Various Parameters Determined Weekly from Six to 13 Weeks of Age

Parameter	Treatment	Age (weeks) 6	7	8	9	10	11	12	13	F-Values	
Packed Cell Volume (%)	+ E + Se	33.5[a]	31.8	32.4	33.5	33.1	34.2	35.6	36.5	Vitamin E	0.01[b]
	+ E − Se	32.3	32.8	31.3	32.7	32.3	33.8	34.7	35.0	Selenium	1.25[b]
	− E + Se	33.3	32.4	33.0	32.9	33.8	33.8	34.8	35.0		
	− E − Se	32.4	31.8	31.9	32.8	33.3	34.2	34.9	35.3	E x Se	0.30[b]
Hemoglobin (g/100 ml)	+ E + Se	11.1	10.5	10.9	10.6	10.9	11.4	11.7	12.3	Vitamin E	0.03[b]
	+ E − Se	10.6	10.8	10.6	10.3	10.6	11.3	11.5	11.6	Selenium	2.52[b]
	− E + Se	10.9	11.0	11.3	10.5	11.1	11.4	11.6	12.0		
	− E − Se	10.7	10.4	10.7	10.2	10.7	11.2	11.6	11.8	E x Se	0.01[b]
Red Cell Count (times $10^6/mm^3$)	+ E + Se	6.06	5.59	5.83	5.78	5.69	5.83	6.16	6.46	Vitamin E	0.07[b]
	+ E − Se	5.48	5.91	5.42	5.67	5.60	5.84	6.07	6.04	Selenium	0.92[b]
	− E + Se	5.79	5.65	5.76	5.69	5.75	5.91	6.11	6.18		
	− E − Se	5.55	5.62	5.74	5.73	5.77	6.06	6.28	6.20	E x Se	1.22[b]
MCV[c] (μ^3)	+ E + Se	56.2	57.0	55.9	58.4	58.1	58.9	57.9	56.5	Vitamin E	0.03[b]
	+ E − Se	58.6	55.1	57.9	57.6	57.7	57.2	57.2	58.1	Selenium	0.12[b]
	− E + Se	57.5	58.5	57.4	57.8	58.9	56.8	56.9	56.8		
	− E − Se	58.5	56.8	55.8	57.4	57.9	56.7	55.8	57.0	E x Se	0.22[b]
MCH[d] ($\mu\mu g$)	+ E + Se	18.6	18.9	18.8	18.3	19.1	19.7	19.1	19.0	Vitamin E	0.00[b]
	+ E − Se	19.3	18.5	19.6	18.2	18.9	19.3	18.9	19.3	Selenium	0.76[b]
	− E + Se	18.9	19.8	19.6	18.5	19.3	19.4	19.0	19.4		
	− E − Se	19.3	18.6	18.7	17.9	18.7	18.6	18.5	19.0	E x Se	1.21[b]
MCHC[e] (%)	+ E + Se	33.1	33.3	33.5	31.5	32.9	33.5	33.0	33.6	Vitamin E	0.05[b]
	+ E − Se	32.9	33.5	33.8	31.5	32.8	33.8	33.1	33.3	Selenium	2.96[b]
	− E + Se	33.0	33.9	33.9	32.0	32.8	34.4	33.5	34.2		
	− E − Se	32.9	32.8	33.5	31.2	32.3	32.9	33.1	33.4	E x Se	4.02[b]

[a]Mean (n = 12)
[b]Not significant ($F_{0.05}$; 1, 33 degrees of freedom = 4.14)
[c]Mean corpuscular volume
[d]Mean corpuscular hemoglobin
[e]Mean corpuscular hemoglobin concentration

serum total iron binding capacity, serum GOT activity and body weight were not found to be significantly different in vitamin E and/or selenium deficient animals as compared to replete animals. These results in regard to packed cell volume, hemoglobin concentration, red cell count and reticulocyte count, are in disagreement with the observations of Obel (40), Grant (19), Nafstad (34, 36) and Baustad and Nafstad

TABLE III. The Effect of Vitamin E and/or Selenium Deficiency in Swine on Various Parameters Determined Weekly from Six to 13 Weeks of Age

Parameter	Treatment	Age (weeks) 6	7	8	9	10	11	12	13	F-Values	
Serum Iron (μg/100 ml)	+ E + Se	197.7[a]	174.3	190.7	191.0	225.0	219.8	223.7	287.5	Vitamin E	0.42[b]
	+ E − Se	226.2	199.2	142.2	213.0	215.0	199.2	272.0	335.5	Selenium	2.84[b]
	− E + Se	197.5	206.5	148.3	172.8	224.2	168.3	235.0	288.2		
	− E − Se	258.2	213.0	217.3	209.0	262.2	234.0	286.5	392.5	E x Se	1.19[b]
TIBC[d] (μg/100 ml)	+ E + Se	516.2	476.7	485.3	484.0	505.3	558.8	539.0	524.5	Vitamin E	0 42[b]
	+ E − Se	486.5	467.5	486.3	486.7	549.0	572.5	523.7	527.2	Selenium	0.24[b]
	− E + Se	503.0	506.7	502.5	475.2	547.2	563.5	565.5	568.0		
	− E − Se	512.0	503.7	491.7	399.0	560.8	574.7	542.8	523.8	E x Se	0.32[b]
Reticulocytes (%)	+ E + Se	2.64	3.20	3.95	2.96	2.11	2.72	1.50	1.70	Vitamin E	0.39[b]
	+ E − Se	2.24	3.24	3.97	3.03	2.73	2.81	1.49	1.94	Selenium	0.15[b]
	− E + Se	2.64	3.02	3.59	2.95	2.47	2.82	2.07	2.17		
	− E − Se	2.35	2.80	3.82	3.66	2.49	2.63	2.14	2.10	E x Se	0.03[b]
Myeloid: Erythroid Ratio	+ E + Se	1.88	1.52	1.34	1.28	1.25	1.28	1,25	1.26	Vitamin E	2.66[b]
	+ E − Se	1.85	1.62	1.35	1.51	1.24	1.29	1.36	1.39	Selenium	10.07[c]
	− E + Se	1.86	1.00	1.38	1.29	1,13	1.09	1.09	1.18		
	− E − Se	2.00	1.68	1.40	1.23	1.35	1.32	1.40	1.19	E x Se	2.38[b]

[a]Mean (n = 6)
[b]Not significant ($F_{0.05}$; 1, 15 degrees of freedom = 4.54)
[c]($p < 0.01$)
[d]Total iron binding capacity

TABLE IV. The Effect of Vitamin E and/or Selenium Deficiency in Swine on Various Parameters Determined Weekly from Six to 13 Weeks of Age

Parameter	Treatment	Age (weeks) 6	7	8	9	10	11	12	13	F-Values	
White Cell Count (times $10^3/mm^3$)	+ E + Se	17.9[a]	17.0	16.7	14.4	14.6	19.8	14.7	14.7	Vitamin E	0.00[b]
	+ E − Se	16.2	17.9	17.9	16.1	14.7	18.9	15.4	14.4	Selenium	0.62[b]
	− E + Se	16.8	16.4	15.6	16.7	15.9	17.6	14.6	13.5		
	− E − Se	17.5	19.8	17.8	15.5	15.7	17.6	15.6	15.0	E x Se	0.23[b]
Body Weight (lbs)	+ E + Se	20.2	23.6	30.1	37.3	44.2	52.1	60.7	71.8	Vitamin E	0.17[b]
	+ E − Se	20.9	24.6	30.4	37.8	44.5	53.4	61.9	73.2	Selenium	0.67[b]
	− E + Se	21.6	25.5	30.9	38.3	45.5	53.8	63.4	75.2		
	− E − Se	19.0	22.5	27.3	34.7	41.2	49.2	57.3	66.3	E x Se	1.43[b]
GOT[e] (I.U.)	+ E + Se	15.7	14.9	19.7	19.8	19.4	25.3	18.4	40.9	Vitamin E	1.98[b]
	+ E − Se	15.2	15.8	19.8	18.7	18.3	22.1	18.5	25.0	Selenium	2.32[b]
	− E + Se	15.2	16.7	17.8	16.0	18.5	22.0	19.7	19.7		
	− E − Se	16.0	15.8	17.1	95.4	38.3	43.3	83.5	54.8	E x Se	3.40[b]
CPK[f] (I.U.)	+ E + Se	110.2	136.5	160.7	155.3	290.8	201.3	222.3	213.8	Vitamin E	9.84[d]
	+ E − Se	81.8	133.1	140.9	123.8	240.1	233.0	333.3	155.4	Selenium	6.55[c]
	− E + Se	87.7	148.7	163.8	158.1	211.0	227.2	313.4	216.8		
	− E − Se	98.3	139.3	150.0	175.8	344.9	442.5	846.6	673.8	E x Se	7.86[d]

[a]Mean (n = 12)
[b]Not significant ($F_{0.05}$; 1, 33 degrees of freedom = 4.14)
[c]($p < 0.05$)
[d]($p < 0.01$)
[e]Glutamic-oxaloacetic transaminase
[f]Creatine phosphokinase

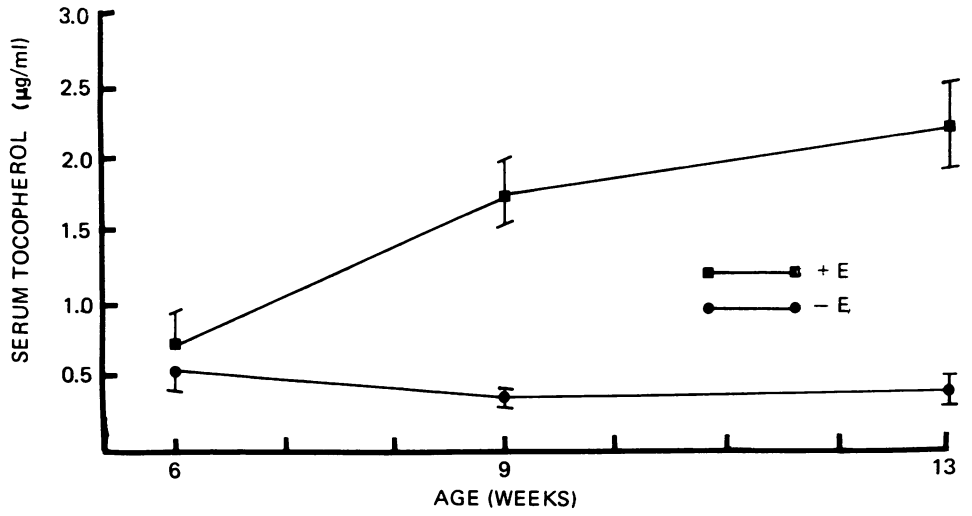

Fig. 3. Effect of vitamin E supplementation on serum vitamin E concentration (each point represents the mean value from 24 animals and brackets indicate 95% confidence intervals).

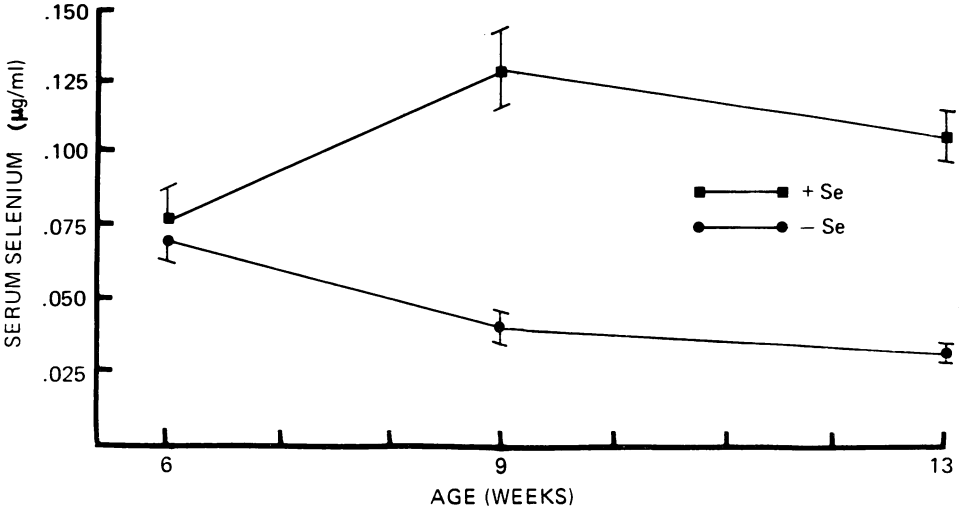

Fig. 4. Effect of selenium supplementation on serum concentration (each point represents the mean value from 24 animals and brackets indicate 95% confidence intervals).

(1) who reported an anemic condition to be associated with vitamin E deficiency in swine. The observations of Stowe (52), Michel *et al* (33) and Vrancken (57) are, however, in accordance with the present results. Interestingly, the myeloid:erythroid ratio was found by analysis of variance to be increased ($p<0.01$) in selenium deficient animals which tends to indicate decreased erythropoiesis although, the 95% confidence intervals overlap when the weekly means of each group are plotted (Fig. 1). The biological significance of that increase in the myeloid:erythroid ratio may be important but the clinical significance is probably questionable, the overall mean for the seven week period of study, being 1.45 for selenium deficient animals as compared to 1.31 for replete animals. The increase of the myeloid:erythroid ratio in selenium deficient animals while biologically interesting is unlikely a limiting factor and so, not reflected in the peripheral red cell picture. It would probably be very interesting to investigate the ability of marrow of selenium deficient pigs

to respond to an artificially induced anemic condition as compared to replete pigs. The presence of multinucleated erythroid precursors as described by Nafstad (34, 36), Nafstad and Nafstad (37) and Baustad and Nafstad (1) in association with vitamin E deficiency was not found to be a significant feature in bone marrow smears of vitamin E and/or selenium deficient animals, an occasional multinucleated erythroid precursor being observed in bone marrow smears of deficient animals as well as in replete animals. Michel *et al* (33) conducted bone marrow studies in pigs fed a vitamin E and selenium deficient diet and found that nuclear abnormalities of erythroid precursors in bone marrow smears were relatively common with vitamin E deficiency. Multinuclearity of the erythroid precursors also occurred, however, in the marrow of pigs fed the standard growing ration supplemented with vitamin E. Evidence of dyserythropoiesis is, however, quite frequently observed in post mortem bone marrow smears from pigs dying spontaneously with lesions of vitamin E and selenium deficiency syndrome. Whether the occurrence of multinucleated erythroid precursors is a typical feature of vitamin E deficient swine or is a terminal event or the result of post mortem changes which are vitamin E dependent, remains to be elucidated.

The serum GOT activities were not found to be significantly increased in association with either vitamin E deficiency, selenium deficiency, or deficiency in both. Such findings are not in agreement with previous reports (12, 19, 28, 43, 53, 55, 56). It is, however, evident that even if a significant increase of serum GOT activity was not detected by analysis of variance, a few animals especially among those deficient in both vitamin E and selenium, presented quite marked transient increases of GOT activity at certain times during the period of study. Furthermore, since these individual increases of GOT activity were not usually associated with a simultaneous increase of CPK activity, they probably reflect the occurrence of acute episodes of liver damage (hepatosis dietetica).

Serum CPK activities were increased in vitamin E deficient animals ($p < 0.01$), selenium deficient animals ($p < 0.05$) and the interaction was also significant ($p < 0.01$). As CPK is quite specific to skeletal muscles and myocardium, and since serum GOT activities were not concurrently increased, it can be assumed that the increase of serum CPK activities reflects the occurrences of subclinical muscular dystrophy mainly in skeletal muscles. Furthermore, the highly significant interaction between vitamin E and selenium deficiency and the increase of serum CPK activities indicated that vitamin E and selenium deficiencies have marked additive effects in the production of skeletal muscular dystrophy. Interestingly, the serum CPK activities of the vitamin E and selenium deficient pigs did not increase appreciably until the animals reached nine weeks of age (Fig. 2), which indicate that the induction of a significant degree of muscular dystrophy is quite lengthy.

Serum GOT and CPK activities are therefore useful indices of tissue damage in association with vitamin E and selenium deficiency in swine and when determined concurrently, are of value in the differentiation of the tissues primely damaged.

As evidenced in Figs. 3 and 4, the serum plasma tocopherol concentrations as well as the serum selenium concentratitons were low in all animals at the beginning of the study (base level). During the seven week period of study, the serum vitamin E levels of the unsupplemented pigs presented a further slight decrease from the base level as compared to the animals supplemented with dl, alpha-tocopherol acetate (250 I.U./week) which presented a gradual increase up to a mean ($\pm$ one standard deviation) of 2.22 ± 0.69 μg/ml at 13 weeks of age as compared to 0.40 ± 0.24 μg/ml for the deficient animals. The serum selenium levels showed a gradual slight decrease from the base level for the unsupplemented animals as compared to the pigs supplemented with sodium selenite (1 mg/week) which presented quite a marked increase at nine weeks of age, then a slight decrease from that peak, at 13 weeks of age. That slight drop of the serum selenium concentrations of the supplemented animals between nine and 13 weeks of age probably means that the dosage of the weekly sodium selenite injections should have been increased according to the increase in body weight of the animals. The mean ($\pm$ one standard deviation) serum selenium level at 13 weeks of age was 0.107 ± 0.020 μg/ml for the supplemented animals and 0.032 ± 0.010 μg/ml for the unsupplemented group.

The serum tocopherol levels of the unsupplemented animals compare well with the values obtained by Hill (21) and Naf-

stad (36). Lindberg (29) reported normal plasma tocopherol level in growing swine between 20 and 35 kg to be 1.45 ± 0.36 (standard deviation) μg/ml, which indicates that the dosage of the weekly vitamin E injections used in the present study was adequate.

ACKNOWLEDGMENTS

The technical assistance of Mr. Ted Fountain, Mr. E. Grift, Mrs G. Binnington and Mrs Jean Claxton is gratefully acknowledged. Financial support was provided by the National Research Council of Canada and the Shute Institute of London, Ontario.

M. Fontaine was a Fellow of the Medical Council of Canada. Injectable vitamin E was kindly provided by Hoffman-La Roche Inc. Nutley, New Jersey and injectable selenium was kindly prepared by Stevenson, Turner and Boyce. Professor G. Ashton guided the statistical analysis of the data. Financial support was provided by the Ontario Ministry of Agriculture and Food.

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