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Deficiency in Rats

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John Skjaerlund

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Charles K. Whitehair

Major professor

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VITAMIN E AND SELENIUM DEFICIENCY IN RATS

By

John Skjaerlund

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ABSTRACT

VITAMIN E AND SELENIUM
DEFICIENCY IN RATS

By

John Skjaerlund

This research was undertaken to determine whether evidence of increased coagulation would be a prominent characteristic of vitamin E and selenium deficiency in young rats and to verify reports by other investigators of histopathologic lesions. Semipurified diets (containing Torula yeast) were fed to 48 rats starting at 1 month of age in a factorial experiment with 2 replicates. There were female or male rats, 0 or 50 IU vitamin E per kg diet, 0 or .1 mg added selenium per kg diet, and final age of 2, 2.5, or 3 months. Vitamin E deficiency increased fibrinogen, skeletal myopathy at 3 months, and pancreatic degeneration. Selenium deficiency increased quadriceps femoris myopathy, renal tubular mineral precipitation, and pancreatic degeneration. Combined deficiency increased pulmonary eosinophils, platelets at 3 months, hepatic necrosis, and presence of cells from seminiferous tubules within epididymal lumina. A previously unreported multifocal vacuolar degeneration of exocrine pancreatic cells was observed.

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INTRODUCTION

The understanding of the pathogenesis of lesions induced by vitamin E and selenium deficiencies is still incomplete. The suggestion that coagulopathies are involved *5(1199) has important implications. Clotting disorders complicate diseases of animals. Stroke and coronary thrombosis are major problems in human beings.

The study of selenium deficiency has particular relevance to Michigan, for the soil contains only marginal amounts of Se.*49 Vitamin E deficiency is becoming increasingly important as harvesting, storage, and feed processing methods are changed. More oxides result in significant destruction of tocopherol. There is concern that a vitamin E and selenium deficiency may be associated with poor survival and growth of calves and piglets, which are of great economic importance in Michigan.

Even after a half century of research, the opportunity to improve upon the biochemical description of the functions of vitamin E remains open. It is difficult to distinguish the causes from the effects of the lesions observed to result from vitamin E and selenium deficiencies. The focal distribution of degenerative and necrotic regions is consistent with a pathogenesis that involves thrombosis. Aberration of membranes and inadequacy of prostacyclin generation by vascular endothelium makes possible the adherence and aggregation of platelets. Thrombocytosis may predispose to this. On the other hand, diffuse

intravascular clotting can be interpreted as the consequence of the release of tissue thromboplastin from masses of acutely necrotic cells. Regardless of the actual cause of degenerative changes, there can eventually be depletion of components of the coagulation system.

This experiment is intended to test a few aspects of the following working hypothesis of coagulopathy involved in vitamin E and selenium deficiencies:

"Lack of vitamin E disrupts endothelial plasma membranes to the point of exposure of subendothelial collagen. Neighboring endothelial cells are unable to synthesize anti-aggregatory amounts of prostacyclin due to low cyclooxygenase level. Also, systemically circulating prostacyclin is unable to cause much increase in cyclic adenosine monophosphate (anti-aggregatory) in platelets, for platelet adenylate cyclase is dependent upon adequate plasma vitamin E. (Acutely necrotic masses of cells release thromboplastin, initiating pathways facilitating conversion of prothrombin to thrombin, and activate plasmin. Collagen and thrombin stimulate platelets to produce thromboxane A_2, which promotes aggregation, and to release fibrinogen from alpha granules to serve as an aggregation cofactor or 'glue'. Thrombin additionally converts fibrinogen to fibrin. Plasmin degrades both fibrinogen and fibrin, initiating a consumptive cycle of coagulation and fibrinolysis. *112)

"Deficiency of selenium permits endothelial cyclooxygenase to be degraded too rapidly; prostacyclin output diminishes. In platelets, the ratio of thromboxane A_2 to prostaglandins E_2 and $F_{2\alpha}$ enlarges. Suboptimal retention of vitamin E in plasma accounts for slight

aggravation of these mild effects.

"Combined low availabilities of vitamin E and selenium hasten the onset of a state of coagulopathy severe enough to manifest signs of disease. Protection gained by overlapping functions of the two nutrients is lost, and subtle biochemical abnormalities progress to overt pathology."

Facilities, funds, and time are quite limited for this research project. The rat is used as a model of vitamin E and selenium deficiency on account of its rapid vulnerability to this nutritional disease and its relatively low expense. The experiment utilizes a factorial design with 2 replicates, 2 sexes, 2 dietary levels of vitamin E, 2 dietary levels of selenium, and 3 final ages. Platelet counts, fibrinogen measurements, and histopathologic examination of stained tissue sections are done to test whether increased coagulation is a prominent feature early in vitamin E and selenium deficiency of young rats.

Data furnished by this experiment contribute to further understanding of vitamin E and selenium deficiency and offer evidence that there can be benefit from continued research in this area.

LITERATURE REVIEW

Introduction

There is voluminous literature of research establishing the role of vitamin E and, more recently, selenium in the nutrition of animals and human beings. Early work defines the lesions occurring in growing animals and the impairment of reproduction in older animals with deficiency of vitamin E or selenium. More recent investigations emphasize defects in circulation, particularly those resulting from altered erythrocyte and platelet structure and function. There remains a need for understanding the biochemical mechanisms of activity of vitamin E. Also, the chemical interrelationships between vitamin E and selenium in the prevention of lesions are poorly defined.

Deficiency Diseases

Growth

Young rats develop liver necrosis accompanied by kidney tubular epithelial degeneration and acute lung edema and hemorrhage when fed a diet containing 30% Torula yeast and 5% vitamin E-stripped lard. (Schwarz)*81 Addition of either .5% L-cystine or 50 ppm vitamin E alone is protective.*81 If the diet contains appreciable quantities of unsaturated fat and has purified casein as the sole protein source, rats die in 1 month due to hepatic necrosis. This is prevented by cystine, methionine, tocopherol, or selenite.*109(1331, 1371) Alopecia, cataracts, hypoplasia of dermis, and edematous spermatic tubules with fewer

than normal mature sperm result in rats fed a Torula yeast ration containing 18 ppb selenium and 60 ppm vitamin E for 2 generations. Overt muscular degeneration and focal hepatic necrosis are absent.*11(341), 87 Axonal degeneration in the brain stem is said to follow vitamin E deficiency in rats and dogs, leaving eosinophilic spherical bodies up to 150 μm in diameter.*86(996)

In ducks, combined vitamin E and selenium deficiency causes myocarditis and necrosis of the duodenum and gizzard.*111 Pancreatic atrophy is a lesion specific for selenium deficiency in chicks. A secondary impairment of lipid and vitamin E absorption follows. Chicks with muscular dystrophy due to low selenium have more tocopherol in the muscle than there is in normal muscle, though. One can infer that the dystrophic muscle has a diminished ability to utilize tocopherol.*22(113) Exudative diathesis (subcutaneous edema, mild hemorrhage) occurs only when there is deficiency of both vitamin E and selenium.*96

More than half of young pigs fed diets low in both vitamin E and selenium die at less than 3 months of age with hepatic necrosis, anemia, icterus, edema, pale muscle, and discoloration of fat.*28 Pigs fed a semi-purified diet based on casein and cod-liver oil and supplemented with selenite but lacking vitamin E develop anemia, swollen kidneys, brain perivascular edema, and degeneration of liver, skeletal muscle, and heart.*69 Myocardial vessels contain microthrombi and undergo fibrinoid medial necrosis, especially within areas of severe myocardial degeneration.*66 Increased serum creatine phosphokinase activities reflect the occurrence of subclinical muscular dystrophy in young swine deficient in vitamin E and/or selenium.*31 Characteristically in

vitamin E deficiency, hepatic degeneration is either centrilobular or mosaic with clusters of entire lobules affected without damage to adjacent lobules. Stomach ulcers may also form in swine.*73

Although the disturbance of mitochondrial structure yields reduced respiration by liver tissue, oxygen is consumed at a rate that is more rapid than normal in cardiac and skeletal muscle of vitamin E-deficient animals. Impaired lipid absorption in human beings can produce muscle weakness and creatinuria. The excessive peroxidation of unsaturated fatty acids in the endoplasmic reticulum allows release of lysosomal hydrolases in muscle.*109

Reproduction

Female rats become sterile when raised on a diet low in vitamin E. The placentae are abnormal; there is increasingly extensive blood extravasation and invariably resorption.(Evans and Bishop)*27 At day 10 of gestation there is a beginning of rarefaction of mesenchymal tissues of embryos. The livers then demonstrate decreased fetal blood cells. Uterine enlargements become grossly abnormal, and rapid uterine involution follows after day 15. By day 21, the uteri appear normal except for small decidual bodies at the mesometrial border.(Urner)*102 Similarly, rats fed bovine milk are infertile. Fetal death occurs after the first week of embryonic life and can be avoided by giving vitamin E even as late as day 5 of gestation.*109(1371) The primary metabolic defect causing death of the vitamin E-deficient rat embryo is undetermined; however, there is a severe anemia and vascular degeneration. Likewise, the vasculature degenerates in vitamin E-deficient chicken and turkey embryos.*82(359)

Vitamin E is needed for normal activity of germinal epithelium of the male rat and guinea pig but not the rabbit or mouse, according to some reports.*105(25) First the spermatozoa become immotile. Later there is degeneration of germinal epithelium.*109(1371) Others state that aspermatogenesis in the rat, along with cataract formation and decreased growth, may occur in the presence of adequate vitamin E if selenium is lacking.*12(310)

Pregnant rats suddenly changed to a vitamin E-deficient diet containing 5% cod-liver oil may experience an eclamptic disease near day 22 of gestation.(Stamler)*89 Some rats become restless and have ruffled coats, pallor, rapid respiration, and convulsions. A quiet interval precedes death. Lesions include hemorrhagic edema, hemorrhages in the kidney, hyperemia and edema in the brain, and sometimes intrauterine bleeding. Other rats abort, have signs of distress and recover, or complete pregnancy normally. Fetuses are alive and vigorous even after maternal death. Very large amounts of vitamin E prevent death of the pregnant rat (100 mg subcutaneously or .2 to .5% in the diet).*89 This observation, along with the fact that the eclamptic disease incidence is related to the amount of dietary lipid peroxide (with the total amount of lipid the same as that in a standard pelleted diet), suggests that perhaps the initiating insult is oxidant damage to membranes of the vascular system.*61(596-7)

Disseminated intravascular coagulation is described as a secondary phenomenon in which there is often consumption of clotting factors and platelets and microvascular obstruction by fibrin.*37 The process may take on either a fulminant, severe form or a chronic, low-grade form.

The generalized Shwartzman reaction is one way to trigger disseminated intravascular coagulation. Although some prefer to confine the term, generalized Shwartzman reaction, to the precise, classical, experimental situation,*37 a broader definition is sometimes applied.*42(138) The characteristics include failure of reticuloendothelial clearance of platelet thromboplastin, deposition of fibrin or altered fibrinogen within small blood vessels, and decreased fibrinolytic activity. The many thrombi do not contain platelets or leucocytes (and so differ from white cell thrombi of the local Shwartzman reaction).*84

Experimental pregnancy toxemia is described as a generalized Shwartzman reaction because of disseminated intravascular clotting. (McKay)*59-62 First there is degeneration of the placental trophoblast, and increased thrombosis is detectable in lacunae of the giant-cell trophoblast. Local deposition of fibrin in maternal blood spaces is possibly a consequence of release of a clot-promoting agent from the trophoblast. Congestion of placentas is common and may be the result of decidual and uterine vein thrombosis. Infarction, premature placental separation, and trophoblastic necrosis follow. About half of the rats have hemorrhage into the uterine cavity, which can result in vaginal bleeding. Placental damage precedes systemic involvement. Thrombosis extends to capillaries, arterioles, and venules of maternal liver, lung, spleen, adrenal gland, and kidney. The disseminated renal glomerular capillary thrombosis is characteristic of the generalized Shwartzman reaction.*59-62

Human toxemia of pregnancy by definition has at least 2 of the following 3 signs: edema, hypertension, and proteinuria.*92

Convulsions and coma also occasionally occur.*46(2) Widespread intravascular coagulation is a feature of pre-eclampsia. Fibrin is lodged in capillaries of maternal lung, brain, kidneys, and placenta. Fibrin degradation products increase, and the number of circulating platelets decreases. In severe cases one also sees "burr" cells (echinocytes) in the blood film; the same morphology of red cells is seen in vitamin E deficiency, too.*92 Thus, human deficiency manifests some similarity to the experimental toxemia of pregnant rats; however, there are other animal models in which the correspondence of lesions is more exact.*74 (386-7)

Erythrocytes

Vitamin E and selenium are essential for proper red blood cell function. Glucose provides poor protection against peroxide- or ascorbic acid-induced oxidative damage to red cells if they are from selenium-deficient animals. The reason is that selenium is a component of glutathione peroxidase.*88 Selenium, however, does not reverse the increased sensitivity of erythrocytes from vitamin E-deficient rats to the hemolyzing action of dialuric acid in vitro.*32

Vitamin E serves as a membrane antioxidant. In swine, measurements of red cell lipid peroxides is a reliable test for vitamin E deficiency. It is unaffected by selenium deficiency.*29 If there is a vitamin E deficiency, lipid peroxides oxidize membrane sulfhydryl groups, making red blood cells hyperpermeable to cations. This gives rise to osmotic swelling and hemolysis. Infants sometimes develop anemia for this reason about 2 weeks after bovine milk (which is low in vitamin E) is substituted for human milk. Susceptibility to hemolysis is normalized by

administering 10 mg α-tocopherol acetate per day orally.*52

Premature infants and infants of low birth weight are particularly vulnerable to low amounts of vitamin E in artificial formulas. They manifest irritability, edema, and hemolytic anemia. The lowest hemoglobin and highest reticulocyte count are seen after supplemental iron is given without vitamin E. This implies that therapeutic doses of iron catalyze oxidative breakdown of membrane lipids and increase red blood cell hemolysis if there is a vitamin E deficiency.*8(98), 63, 101, 105(26)

Platelets

Both primary and secondary hemostasis are influenced by vitamin E and selenium nutriture. Selenium-deficient swine have a decreased prothrombin time, decreased fibrinogen survival, and increased turnover of fibrinogen. Those lesions are suggestive of chronic, low-grade, disseminated intravascular coagulation. Swine that are deficient in either selenium or vitamin E have low platelet counts and decreased platelet turnover, which may be evidence of a platelet production defect. Thrombocytosis, however, is a feature of vitamin E deficiency in infants, monkeys, and rats.*30

Dietary α-tocopherol increases plasma and platelet vitamin E levels in the human being. Human platelets have a high content of tocopherol compared to plasma and red cells.*91(736) Vitamin E has high effectiveness in blocking arachidonic acid-induced platelet aggregation in vitro. It accomplishes this by inhibiting the enzymatic conversion of arachidonic acid into prostaglandins. Aggregation-induced platelet release of 5-hydroxytryptamine and N-acetylglucosaminidase is reduced. Thus, there

is a reduction in lipid peroxide formation (estimated by quantitative determination of malonaldehyde with thiobarbituric acid reagent *91 (733)) similar to that seen after aspirin administration, and there is inhibition of the platelet release reaction. These responses are dose-dependent but are not due to antioxidant activity because tocopheryl-quinone (the oxidative degradation product of α-tocopherol) is as effective as α-tocopherol.*90 The amount of synthesis of prostaglandin E_2 and prostaglandin $F_{2\alpha}$ in coagulating blood and the concentrations of those 2 prostaglandins in serum are inversely related to dietary and serum α-tocopherol levels. Platelets are the source of these serum prostaglandins.*44, 53

Mechanisms of Action and Interaction

Much of what is known with regard to the biochemical roles of vitamin E and selenium helps in understanding the diseases resulting from deficiency of either or both of those nutrients. Selenium is an integral and necessary part of the enzyme, glutathione peroxidase (glutathione: H_2O_2 oxidoreductase).(Rotruck et al.)*90 The antioxidant activity of vitamin E is responsible for a lot of the protection that the fat-soluble vitamin affords. Synthetic antioxidants, coenzyme Q, selenium, and some sulfur amino acids can in some cases reverse vitamin E deficiency. In addition it appears that there must be a more specific function of vitamin E than its action as an antioxidant.*107

Selenium

Each of the 4 subunits of glutathione peroxidase has an atom of selenium. The selenoenzyme catalyzes the conversion of hydroperoxides

and reduced glutathione to alcohols and oxidized glutathione. Glutathione reductase then restores oxidized glutathione to the reduced state by using NADPH + H^+ and forming $NADP^+$. Sulfur-containing amino acids can delay the onset of diseases due to selenium deficiency by serving as precursors to reduced glutathione. For example, feeding sulfur amino acids raises glutathione concentrations and delays rat liver necrosis. *12(313)

Glutathione peroxidase is present in all animal tissues studied but not plants.*103 Selenium is covalently bound to a protein of the clostridial glycine reductase system. The electron transfer process is coupled to the esterification of orthophosphate and synthesis of adenosine triphosphate. Formate dehydrogenase also contains selenium. It is likely that selenium's utility arises from the greater reactivity and lower oxidation-reduction potential of some organoselenium compounds compared to their sulfur counterparts.*88(920-1)

Highest glutathione peroxidase activity in selenium-adequate rats is found in liver, red blood cells, white blood cells, platelets, and macrophages. It is moderate in heart, lung, kidney, adrenal gland, stomach mucosa, pancreas, and adipose tissue and low in intestine, skeletal muscle, brain, testis, and lens.*103 The muscle selenoprotein is not found in animals fed a selenium-deficient diet or in animals suffering from white muscle disease.*88(919) Decreases of the normal activities of glutathione peroxidase in various tissues generally correlate with the locations of lesions caused by low selenium intake.*43 (2085) After selenium supplementation, there is a delay before the disease is halted, during which the enzyme must be synthesized.

A glutathione peroxidase which does not contain selenium is found in many tissues of animals fed selenium-deficient diets and depleted of the selenium-dependent glutathione peroxidase. The tissues in which that enzyme is found include adrenal gland, liver, kidney, fat, brain, and testis; none is found in red cells, skin, skeletal or cardiac muscle, spleen, lung, thymus, and intestine. Activity is high in guinea pigs, human beings, and sheep relative to chickens and pigs. Hamsters and rats are unable to develop appreciable levels. This is a good explanation for the lack of hepatic disease in sheep and the inability of rats to adapt to an absence of selenium.*103(322-3)

Glutathione peroxidase is distributed in the aqueous phase of plasma and cytosol,*72 where its function in reducing peroxides appears vital to cellular metabolism. Reduction of the second peroxy free radical of PGG_2 in the synthesis of endoperoxide PGH_2 from arachidonate is accomplished with glutathione peroxidase.*53(179) This facilitates prostaglandin formation. Reduced glutathione and l-epinephrine are synergistic cofactors for microsomal prostaglandin synthetase;*13(70) and catecholamines augment cyclooxygenase activity through conversion of latent forms to reactive forms.*109(640) Additionally, reduced glutathione "promotes the synthesis of stable prostaglandins from endoperoxides, thus diverting the biosynthesis pathway from the production of thromboxanes."*33(2) For example, in ram microsomes glutathione diverts metabolism of arachidonate from prostacyclin to prostaglandin E_2.*64(134)

In the erythrocyte, glutathione peroxidase is associated with the aqueous phase of cytosol and plasma. There it destroys peroxides, averting attack by peroxides on polyunsaturated fatty acids of membranes and

hemolysis of the cell. Likewise, it prevents oxidation of sulfhydryl groups of hemoglobin and the eventual formation of Heinz bodies. Other non-membrane (soluble) proteins are also protected against oxidative damage.*18(2091), 80(590), 83, 109(1001)

Selenium offers protecton against white muscle disease through the ability of glutathione peroxidase to minimize peroxidation of unsaturated fatty acids in the endoplasmic reticulum of muscle. Failure to do so leads to release of lysosomal hydrolases that can damage the muscle tissue.*109(1373)

Selenium has a sparing effect on vitamin E. The primary mechanism is that glutathione peroxidase reduces peroxides which could oxidize vitamin E. A secondary means, which seems to be of questionable importance, is the preservation of pancreatic integrity. The exocrine pancreatic secretions allow normal lipid and tocopherol absorption. It is also postulated that selenium somehow aids in the retention of vitamin E in blood plasma.*83 Selenium does not influence absorption or retention of vitamin E in the rat, but it may modify tissue distribution.*15

Vitamin E

Vitamin E is traditionally referred to as an antioxidant of lipid-associated substances. Synthetic antioxidants can sometimes substitute in treatment of vitamin E-responsive diseases. Similarly, coenzyme Q can sometimes substitute for vitamin E in neutralization, or "scavenging", of free radicals.

Chemical oxidation of α-tocopherol yields α-tocopherolquinone and α-tocopheroloxide. Apparently "α-tocopherol does not readily undergo reversible oxidation."*109(1373) After oxidation of the chromane ring

and the side chain, the metabolite is excreted as the diglucosiduronate in human bile.*109(1373)

As a dietary ingredient, α-tocopherol reduces oxidative destruction of vitamin A.*109(1372) Rats are found to have higher levels of vitamin A in the liver when they are supplemented with vitamin E, and there is a delay in onset of xerophthalmia in these animals.*100

Vitamin E protects membranes by preventing lipid hydroperoxide formation (from polyunsaturated fatty acids) and consequent autocatalytic lipid peroxidation resulting in cell damage.*43(2087) In this way the vitamin may prevent hemolysis *18(2091) and damage to vascular endothelial cells,*67 mitochondria, and muscle endoplasmic reticulum.*109

Vitamin E may diminish the rate of protein degradation by reducing the levels of lipoperoxides which oxidize sulfhydryl groups.

Vitamin E spares selenium by serving as an antioxidant. Also, at least in the rat, the activities of glutathione peroxidase and glutathione reductase are augmented.*16 Part of this cooperative effect may stem from the "degree to which selenium occurs as protein-bound selenide in the mitochondria and smooth endoplasmic reticulum."*70(10)

Summary

Many reports document the essentiality of both vitamin E and selenium for growing animals. Some species also have reproductive requirements for both substances. Deficiency has detrimental effects on red blood cells, platelets, and the coagulation system. Hemostatic mechanisms require maintenance of membrane integrity. Direction of the biosynthetic pathways of prostaglandins may depend on it as well. Some functions of

vitamin E duplicate those of selenium, and there are cases in which one substance can spare the other.

OBJECTIVES

Objectives of this research are:

1. To determine whether pulmonary thrombosis can occur in young rats fed rations deficient in vitamin E and selenium.

2. To determine whether selenium deficiency raises the platelet count in rats and whether the elevation is more severe in combined deficiency.

3. To determine whether there is an increase in fibrinogen in association with the more numerous platelets.

4. To determine whether an increase in fibrinogen is associated with hepatic necrosis.

5. To determine whether selenium increases tocopherol levels in plasma and liver of rats fed normal quantities of vitamin E and whether the amount of stainable lipid in the liver correlates with the amount of vitamin E.

6. To determine whether light-microscopic histopathology occurs in young rats fed diets singly deficient in either vitamin E or selenium from the age of weaning and to determine which nutrient is essential for maintenance of male germinal epithelium.

7. To determine whether there is any pancreatic degeneration in rats that are deprived of both vitamin E and selenium.

MATERIALS AND METHODS

Experimental Design

The experiment had a factorial design with 2 replicates at 3 levels of age and 2 levels each of sex, vitamin E, and selenium. Weanling rats were fed semipurified diets for 1, 1.5, and 2 months. Then blood and other tissues were collected from 48 of those rats at termination.

Test System

Animals

Sprague-Dawley rats fed a standard rodent diet were bred to give birth to litters in 3 clusters separated by 1 to 2 weeks. When each cluster reached 4 weeks of age, litters from that age cluster were weaned, sorted by sex, and assigned to 4 experimental dietary groups so that each would be blocked on litters.

Two survivors from each age and sex group of rats deficient in both vitamin E and selenium were chosen for necropsy. Numbering of rats within groups served as a means to preselect corresponding rats from the other dietary groups in order to control bias among groups. Thus, a balanced set of data from 48 rats was obtained.

Housing

Rats were group housed by treatment group in suspended metal cages with wire floors with the exception of the males kept until 3 months of

age, which were in plastic boxes with wire lids and sawdust bedding. They were all maintained in Building 5 of the Veterinary Research Farm.

Feeding

Water was supplied in bottles with stainless steel sipper tubes.

Feed cups were filled daily. The feed was prepared in 8-kg batches and stored in sealed plastic containers at room temperature. Aliquots were saved to verify by measurement the vitamin E and selenium levels. The basal ration was formulated with the intention of being deficient in vitamin E and selenium but otherwise meeting the requirements for growth of rats.*106(64) The feed ingredients were as listed below:

Diet Composition (per kg)

Glucose		656.4	g
Torula yeast		250	
Tocopherol-stripped corn oil		50	
$CaHPO_4.2H_2O$		18	g
$NaHCO_3$		12.5	
$CaCO_3$		6.25	
KCl		5	
$MgCO_3$		1	
$FeSO_4.H_2O$		.35	
$ZnCO_3$		.2	
$MnSO_4.H_2O$		.05	
$CoCO_3$		.05	
$CuSO_4$		.05	
KIO_3		.001	
Nicotinic acid		15	mg
Ca pantothenate		10	
Retinyl acetate	(5000 IU)	7.5	
Thiamin HCl		5	
Riboflavin		5	
Folic acid		2	
Biotin		2	
Pyridoxine HCl		1	
Menadione $NaHSO_3$		.1	
Cholecalciferol	(1000 IU)	.05	
Cyanocobalamin		.01	
*** Na_2SeO_3	(.1 mg Se)	.22	mg
*** α-tocopheryl acetate	(50 IU)	100	mg

Identification

At the time of weaning, rats were earnotched according to dietary groups. Later, they were individually marked by earnotches and clipping of hair. These numbers were used for identification while making all measurements up to examination of microscopic sections. In order to establish 'blindness' during histopathologic examination of slides, histology laboratory processing numbers were assigned in random order to the experimental subjects.

Treatments

One dietary group was fed a basal diet that was deficient in both vitamin E and selenium. The second group's diet was supplemented with 50 IU of vitamin E per kg, and the third group's diet was supplemented with .1 mg selenium per kg. The same amounts of both nutrients were added to the basal diet for the fourth group.

Rats were fed the special diets to the ages of 2, 2.5, and 3 months. This was done in order to observe changes in platelet and fibrinogen levels as deficiencies progressed to greater severity. It was found that blood sampling of the small rats by cardiac puncture often resulted in cardiac tamponade, so correlated sampling was not employed.

Due to a shipment delay, corn oil was absent for 3 weeks after weaning from the diets of the rats that were fed until 3 months of age. Similarly, animals fed to 2.5 months of age lacked corn oil the first week.

Measurements

Clinical Observations

Animals were checked daily for viability and inspected weekly for any signs of disease evident by visual observation, handling, and palpation.

Rats from all groups were weighed approximately every 2 weeks starting at the age of weaning. Individual body weights included in this experimental data set were measured the day of necropsy of each animal and one-half month previous to that.

Blood

Diethyl ether was used for anesthesia. Two ml of blood was collected by puncture and aspiration of the exposed heart and refrigerated in small culture tubes with 1 part 3.8% trisodium citrate solution per 9 parts blood.

Platelet counting was done by the manual method. Blood was mixed with lysing solution at least 10 minutes. The diluted platelets were allowed to settle for at least 12 minutes in a counting chamber kept inside a moist petri dish prior to counting under a microscope.

Fibrinogen was determined by measuring the clotting time of plasma diluted by barbital buffer when thrombin was added. Times measured by a coagulation instrument were translated into fibrinogen quantities by use of a calibration curve established with a human fibrinogen reference.

Remaining plasma was frozen in micro test tubes for storage so that measurements of vitamin E and selenium content could be made later.

Necropsy

Anesthetized animals died from exsanguination. During dissection, observations for gross lesions were made. Liver was weighed, and a portion weighing roughly 2 g was weighed and frozen for later determinations of vitamin E and selenium. These tissues were immersed in 10% formalin: abdominal skin, eye, brain, quadriceps femoris muscle, thymus, lung, heart, duodenum, pancreas, liver, spleen, kidney, adrenal gland, urinary bladder, and either ovary and uterine horn and cervix or testis and head of epididymis.

One fixed bit of liver was cryosectioned and stained with oil red O. All fixed tissues were dehydrated, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Sections of 1 type of tissue at a time from all 48 rats were examined first. Then all mounted tissues from 1 rat at a time were examined. Use of this grid technique and randomly assigned slide label numbers was an effort to provide quality control and to eliminate observer bias toward treatments during evaluation by light microscopy.

Statistical Analysis

The data set was first screened by analysis of covariance to permit discarding of a few variables observed to be artifactual or otherwise unrelated to the independent variables.

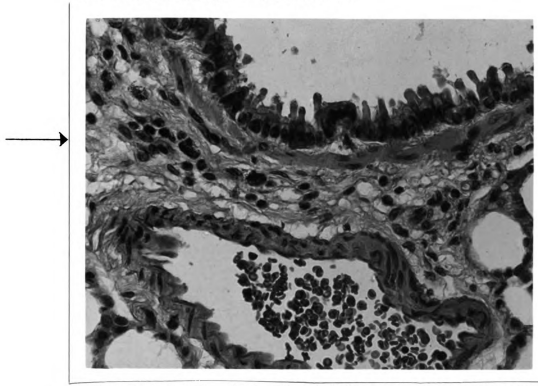
After 4-way analysis of variance, higher-order interaction terms were selectively dropped from the models to allow pooling of their sums of squares with error sums of squares.

RESULTS

See Table 8 at the end of this section for the data variables, units, and values used in statistical analysis.

1. Histopathologic examination revealed no pulmonary thrombosis in young rats lacking vitamin E and selenium. However, combined deficiency increased (doubled) the frequency of appearance of peribronchiolar and perivenular eosinophils and mast cells ($p < .05$). The prevalence in females was observed to be greater than (about one and one-half times as great as) in males ($p \simeq .01$).

See Photomicrograph 1 and Table 1.



Photomicrograph 1. Lung of rat #21: eosinophils and mast cells (arrow).

Table 1. Eosinophils: Means

Vit. E (IU/kg)	Se (mg/kg)	N	Eosinophils (arbitrary units)
0	0	12	0.79
0	0.1	12	0.33
50	0	12	0.50
50	0.1	12	0.46

Sex	N	Eosinophils (arbitrary units)
F	24	0.65
M	24	0.40

2. This experiment did not detect an elevation of the platelet count in rats that were deficient only in selenium. Combined deficiency of vitamin E and selenium caused an increase in circulating platelets (to four-thirds times the normal number) by 3 months of age ($p < .05$). See Table 2.

Table 2. Platelets: Means

Final Age (mo.)	Vitamin E (IU/kg)	Selenium (mg/kg)	N	Platelets ($10^6/mm^3$)
2	0	0	4	.55
2	0	0.1	4	.67
2	50	0	4	.66
2	50	0.1	4	.64
2.5	0	0	4	.75
2.5	0	0.1	4	.85
2.5	50	0	4	.68
2.5	50	0.1	4	.80
3	0	0	4	.98
3	0	0.1	4	.74
3	50	0	4	.60
3	50	0.1	4	.70

3. As a result of deficiency of vitamin E, fibrinogen levels increased (by one-fourth) in males at each age and in 3-month-old rats of both sexes ($p \simeq .05$). In other words, the change occurred earlier

in males, but females showed the difference, too, by 3 months.

The correlation coefficient indicated that the linear association between platelets and fibrinogen was insignificant. See Table 3.

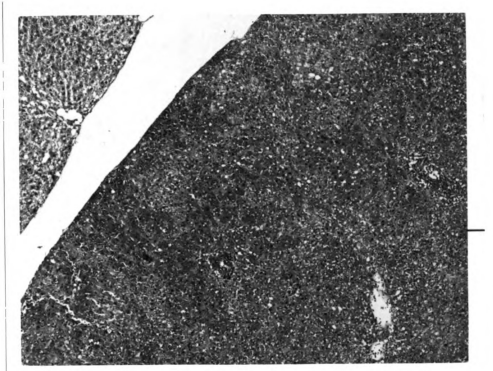
Table 3. Fibrinogen: Means

Sex	Vitamin E (IU/kg)	N	Fibrinogen (mg/dl)
F	0	12	147
F	50	12	141
M	0	12	182
M	50	12	142

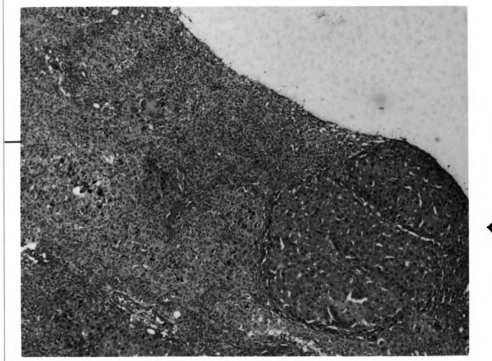
Final Age (mo.)	Vitamin E (IU/kg)	N	Fibrinogen (mg/dl)
2	0	8	160
2	50	8	152
2.5	0	8	139
2.5	50	8	131
3	0	8	195
3	50	8	140

4. Hepatic necrosis occurred in too few animals to judge whether an increase in fibrinogen followed. Two male rats fed a diet lacking both vitamin E and selenium until 2.5 months of age had severe necrosis of caudate liver lobes. One of those had necrosis of all other lobes too and additionally had cirrhosis and thymic cortical thinning. A 2-month-old, male rat in the same dietary group also had cirrhotic caudate lobes. The cirrhotic change was characterized by fibrosis, biliary proliferation, pseudolobular regeneration, calcification, granulomatous hepatitis with giant cells, and eosinophilic portal triaditis.

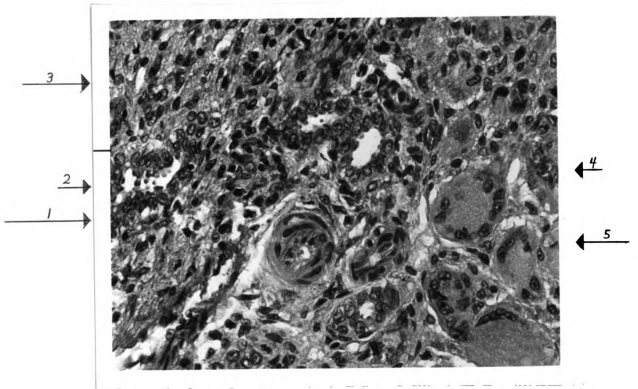
See Photomicrographs 2, 3, and 4.



Photomicrograph 2. Liver of rat #33: severe necrosis of caudate lobe (normal lobe at upper left).



Photomicrograph 3. Liver of rat #37: pseudolobular regeneration (arrow).



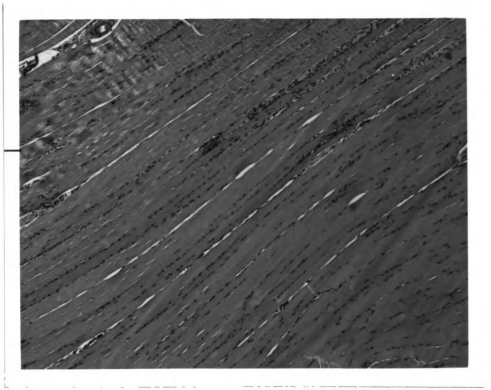
Photomicrograph 4. Liver of rat #37: eosinophilic triaditis (arrow 1), biliary proliferation (arrow 2), fibrosis (arrow 3), calcification (arrow 4), and giant cells (arrow 5).

5. There were no determinations of tocopherol and selenium levels in plasma and liver. This experiment also provided insufficient data to conclude that there was a relationship between stainable lipid in the liver and dietary level of vitamin E.

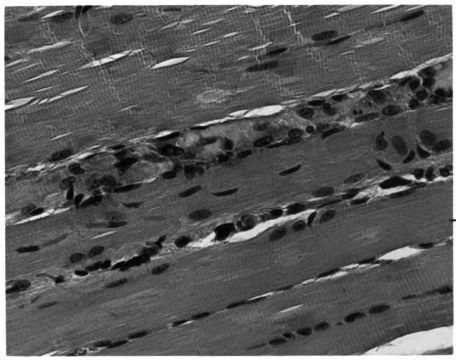
6. The incidence of flank (abdominal skin) and quadriceps femoris myopathy became significant by 3 months of age in rats fed a diet deficient in vitamin E from the age of weaning ($p < .001$). The myopathy was characterized by segmental degeneration and necrosis of scattered, individual fibers with accompanying sarcolemmal-cell proliferation and slight mononuclear infiltration. Selenium deficiency also increased the likelihood of quadriceps femoris myopathy ($p < .05$). Fifty IU of vitamin E per kg of diet in the absence of added selenium was protective to a greater extent than was .1 ppm selenium in the absence of vitamin E.

There was a positive correlation ($r \simeq .6$) between myopathy and plasma fibrinogen ($p < .0001$).

See Photomicrographs 5 and 6 and Table 4.



Photomicrograph 5. Skeletal muscle from rat #37: segmental degeneration of scattered, individual fibers.



Photomicrograph 6. Skeletal muscle from rat #37: myodegeneration and sarcolemmal-cell proliferation.

Table 4. Myopathy: Means

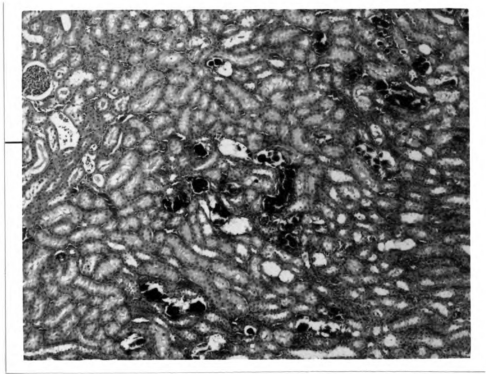
Final Age (mo.)	Vitamin E (IU/kg)	N	Flank Myopathy (arbitrary units)
2	0	8	0.6
2	50	8	0.0
2.5	0	8	0.2
2.5	50	8	0.0
3	0	8	1.9
3	50	8	0.0

Final Age (mo.)	Vitamin E (IU/kg)	N	Quadriceps Femoris Myopathy (arbitrary units)
2	0	8	0.06
2	50	8	0.0
2.5	0	8	0.3
2.5	50	8	0.12
3	0	8	1.6
3	50	8	0.0

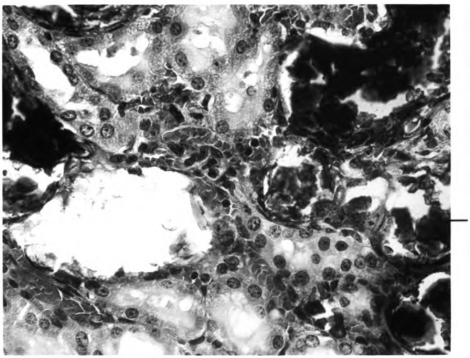
Se (mg/kg)	N	Quadriceps Femoris Myopathy (arbitrary units)
0	24	0.5
0.1	24	0.17

Selenium deficiency resulted in more severe renal tubular degeneration and mineral deposition ($p < .01$) than that which occurred with selenium adequacy. The calcification occurred mainly within necrotic tubules of the outer zone of the medulla. This study revealed that, in general, the lesions were less common in young rats and more common in female rats ($p < .05$).

See Photomicrographs 7 and 8 and Table 5.



Photomicrograph 7. Kidney from rat #21: mineral deposits in outer zone of medulla.



Photomicrograph 8. Kidney from rat #21: mineral in necrotic tubules.

Table 5. Mineral in Tubules: Means

Selenium (mg/kg)	N	Mineral in Tubules (arbitrary units)
0	24	1.7
0.1	24	0.5

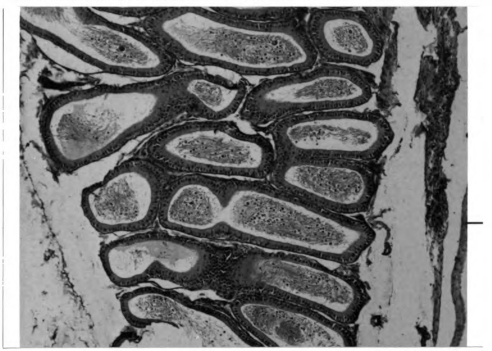
Final Age (mo.)	N	Mineral in Tubules (arbitrary units)
2	16	0.3
2.5	16	1.4
3	16	1.6

Sex	N	Mineral in Tubules (arbitrary units)
F	24	1.5
M	24	0.7

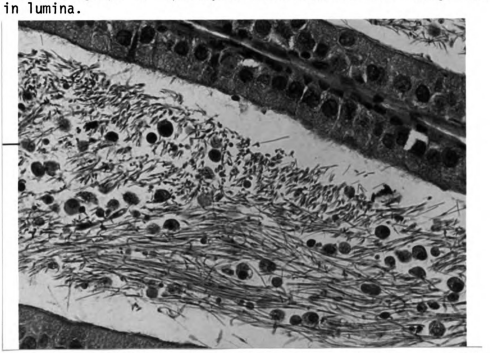
Non-sperm cells appeared in the lumina of epididymal tubules of immature rats. Most likely they were cells sloughed from seminiferous

tubules. Both vitamin E and selenium had to be absent from the diet before such cells could persist in chronologically older rats ($p < .05$).

See Photomicrographs 9 and 10 and Table 6.



Photomicrograph 9. Epididymal head from rat #37: large cells in lumina.



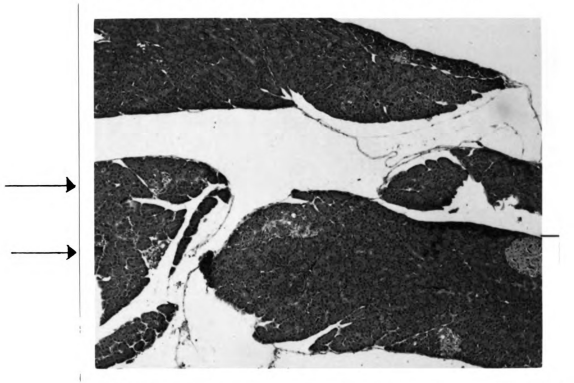
Photomicrograph 10. Epididymal head from rat #37: seminiferous tubular epithelial cells.

Table 6. Non-Sperm Cells: Means

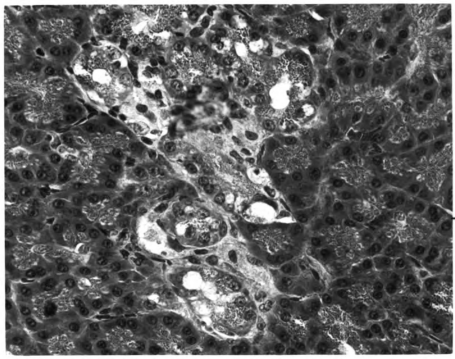
Vitamin E (IU/kg)	Selenium (mg/kg)	N	Non-Sperm Cells (arbitrary units)
0	0	6	1.6
0	0.1	6	0.6
50	0	6	0.4
50	0.1	6	0.6

7. There was multifocal, vacuolar degeneration of pancreatic exocrine cells in rats deprived of either vitamin E or selenium ($p < .05$). The question of whether a synergistic interaction existed was unanswered. (Interpretation of the results was complicated slightly by the fact that the lesion in rat #34 consisted of one large focus of exocrine cell degeneration.)

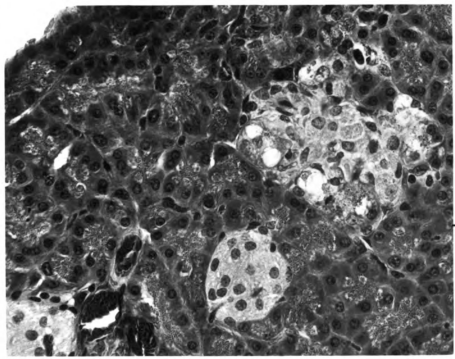
See Photomicrographs 11, 12, 13, and 14 and Table 7.



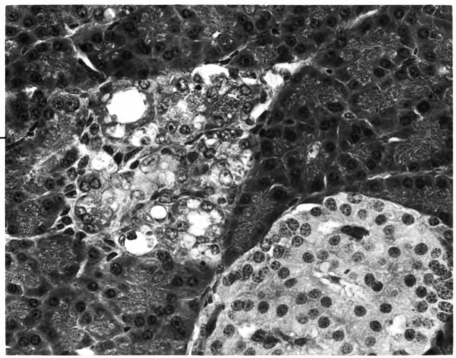
Photomicrograph 11. Pancreas from rat #41: multifocal degeneration (arrows at 2 of the foci).



Photomicrograph 12. Pancreas from rat #41: vacuoles in exocrine cells.



Photomicrograph 13. Pancreas from rat #41: degenerated acini (normal islets at lower left and lower center).



Photomicrograph 14. Pancreas from rat #41: focus of vacuolar degeneration of acini (normal islet at lower right).

Table 7. Pancreatic Degeneration: Means

Vitamin E (IU/kg)	N	Pancreatic Degeneration (arbitrary units)
0	24	0.6
50	24	0.12
Selenium (mg/kg)	**N**	**Pancreatic Degeneration (arbitrary units)**
0	24	0.6
0.1	24	0.08

Table 8. Data Set

Rat	Sex	Final Age (mo.)	Vitamin E (IU/kg)	Selenium (mg/kg)	Previous Weight (g)	Final Weight (g)	Weight Gain (g)	Liver Weight (g)
1	F	2.0	0	0.0	112	137	25	5.4
2	F	2.0	50	0.0	115	138	23	5.7
3	F	2.0	0	0.1	102	101	-1	3.5
4	F	2.0	50	0.1	112	122	10	4.9
5	F	2.0	0	0.0	118	158	40	6.7
6	F	2.0	50	0.0	112	154	42	5.9
7	F	2.0	0	0.1	86	99	13	4.5
8	F	2.0	50	0.1	123	160	37	8.0
9	F	2.5	0	0.0	168	201	33	7.5
10	F	2.5	50	0.0	151	192	41	7.5
11	F	2.5	0	0.1	118	148	30	5.0
12	F	2.5	50	0.1	182	210	28	7.4
13	F	2.5	0	0.0	133	161	28	6.8
14	F	2.5	50	0.0	178	204	26	7.4
15	F	2.5	0	0.1	151	187	36	7.0
16	F	2.5	50	0.1	147	184	37	7.2
17	F	3.0	0	0.0	149	185	36	7.4
18	F	3.0	50	0.0	159	187	28	7.9
19	F	3.0	0	0.1	132	153	21	6.9
20	F	3.0	50	0.1	104	132	28	5.6
21	F	3.0	0	0.0	186	227	41	9.1
22	F	3.0	50	0.0	134	159	25	6.1
23	F	3.0	0	0.1	160	190	30	7.4
24	F	3.0	50	0.1	165	192	27	8.2

Table 8. Continued

* = arbitrary units

Rat	Liver-to Body Weight Ratio	Eosinophils (*)	Platelets (10^6/mm^3)	Fibrinogen (mg/dl)	Hepatic Necrosis (*)	Cirrhosis (*)
1	0.039	0.5	0.66	140	0	0
2	0.041	0.5	0.66	150	0	0
3	0.035	0.5	0.62	140	0	0
4	0.040	1.0	0.60	140	0	0
5	0.042	1.0	0.57	170	0	0
6	0.038	1.0	0.66	170	0	0
7	0.045	1.0	0.69	150	0	0
8	0.050	0.5	0.52	160	0	0
9	0.037	0.5	0.96	120	0	0
10	0.039	0.5	0.70	120	0	0
11	0.034	0.5	0.70	150	0	0
12	0.035	1.0	0.82	130	0	0
13	0.042	1.0	0.80	130	0	0
14	0.036	0.5	0.68	120	0	0
15	0.037	0.0	0.98	110	0	0
16	0.039	0.5	0.69	150	0	0
17	0.040	1.0	0.76	160	0	0
18	0.042	0.5	0.61	140	0	0
19	0.045	0.5	0.58	140	0	0
20	0.042	0.5	0.65	140	0	0
21	0.040	1.0	0.89	190	0	0
22	0.038	1.0	0.64	110	0	0
23	0.039	0.5	0.84	160	0	0
24	0.043	0.0	0.77	160	0	0

Table 8. Continued

* = arbitrary units

Rat	Liver Fat (*)	Flank Myopathy (*)	Quadriceps Femoris Myopathy (*)	Mineral in Tubules (*)	Non-Sperm Cells (*)	Pancreatic Degeneration (*)
1	4.5	0.0	0.0	0.0	-	0.0
2	4.5	0.0	0.0	1.0	-	0.0
3	3.5	1.0	0.0	0.0	-	0.0
4	1.5	0.0	0.0	0.0	-	0.0
5	4.0	1.0	0.0	1.5	-	0.0
6	1.5	0.0	0.0	1.0	-	0.0
7	3.5	1.0	0.5	0.0	-	0.0
8	2.0	0.0	0.0	0.0	-	0.0
9	6.0	0.0	0.0	3.5	-	1.5
10	4.5	0.0	0.0	3.0	-	0.0
11	6.0	0.0	0.0	0.0	-	0.0
12	4.0	0.0	0.0	0.0	-	0.0
13	5.0	0.0	0.0	0.0	-	2.0
14	3.0	0.0	0.0	2.5	-	0.0
15	4.0	1.0	0.0	2.5	-	0.0
16	5.0	0.0	0.0	3.0	-	0.0
17	5.0	1.5	1.5	3.0	-	2.0
18	2.0	0.0	0.0	2.0	-	0.0
19	2.5	2.0	0.0	1.0	-	0.0
20	4.0	0.0	0.0	1.5	-	0.0
21	3.0	3.0	1.5	4.5	-	2.0
22	1.5	0.0	0.0	4.0	-	0.0
23	2.0	2.0	1.0	2.0	-	2.0
24	1.0	0.0	0.0	0.0	-	0.0

Table 8. Continued

Rat	Sex	Final Age (mo.)	Vitamin E (IU/kg)	Selenium (mg/kg)	Previous Weight (g)	Final Weight (g)	Weight Gain (g)	Liver Weight (g)
25	M	2.0	0	0.0	66	84	18	4.7
26	M	2.0	50	0.0	85	105	20	4.2
27	M	2.0	0	0.1	99	104	5	4.1
28	M	2.0	50	0.1	76	89	13	3.4
29	M	2.0	0	0.0	106	154	48	6.0
30	M	2.0	50	0.0	74	96	22	4.6
31	M	2.0	0	0.1	72	91	19	4.5
32	M	2.0	50	0.1	92	125	33	5.7
33	M	2.5	0	0.0	125	144	19	5.8
34	M	2.5	50	0.0	171	204	33	7.2
35	M	2.5	0	0.1	159	206	47	7.0
36	M	2.5	50	0.1	144	177	33	6.4
37	M	2.5	0	0.0	142	138	-4	6.6
38	M	2.5	50	0.0	137	170	33	7.3
39	M	2.5	0	0.1	118	168	50	7.2
40	M	2.5	50	0.1	198	239	41	9.6
41	M	3.0	0	0.0	125	184	59	8.9
42	M	3.0	50	0.0	154	222	68	9.4
43	M	3.0	0	0.1	102	128	26	5.4
44	M	3.0	50	0.1	124	142	18	6.0
45	M	3.0	0	0.0	170	165	-5	8.5
46	M	3.0	50	0.0	142	220	78	8.9
47	M	3.0	0	0.1	146	188	42	9.1
48	M	3.0	50	0.1	134	178	44	7.5

Table 8. Continued

* = arbitrary units

Rat	Liver-to-Body Weight Ratio	Eosinophils (*)	Platelets ($10^6/mm^3$)	Fibrinogen (mg/dl)	Hepatic Necrosis (*)	Cirrhosis (*)
25	0.056	1.0	0.44	120	0	1
26	0.040	0.5	0.78	150	0	0
27	0.039	0.0	0.80	150	0	0
28	0.038	0.0	0.78	150	0	0
29	0.039	0.5	0.52	180	0	0
30	0.048	0.0	0.52	150	0	0
31	0.049	0.0	0.58	230	0	0
32	0.046	0.0	0.68	150	0	0
33	0.040	0.5	0.72	200	1	0
34	0.035	0.5	0.58	120	0	0
35	0.034	0.0	0.95	160	0	0
36	0.036	0.0	0.87	140	0	0
37	0.048	1.0	0.53	110	1	1
38	0.043	0.0	0.74	130	0	0
39	0.043	0.0	0.77	130	0	0
40	0.040	0.5	0.84	140	0	0
41	0.048	1.0	1.00	340	0	0
42	0.042	1.0	0.58	160	0	0
43	0.042	0.0	0.63	200	0	0
44	0.042	1.0	0.59	140	0	0
45	0.052	0.5	1.25	220	0	0
46	0.040	0.0	0.59	150	0	0
47	0.048	1.0	0.89	150	0	0
48	0.042	0.5	0.80	120	0	0

Table 8. Continued

* = arbitrary units

Rat	Liver Fat (*)	Flank Myopathy	Quadriceps Femoris Myopathy (*)	Mineral in Tubules (*)	Non-Sperm Cells (*)	Pancreatic Degeneration (*)
25	10	0.0	0.0	0.0	3.0	0.0
26	2.0	0.0	0.0	0.0	1.0	0.0
27	2.0	0.0	0.0	0.0	0.5	0.0
28	2.0	0.0	0.0	0.0	2.0	0.0
29	2.5	0.0	0.0	0.0	1.0	0.0
30	7.0	0.0	0.0	0.0	1.0	0.0
31	4.0	2.0	0.0	1.0	2.5	0.0
32	2.5	0.0	0.0	0.0	1.0	0.0
33	6.0	1.0	0.0	0.0	1.0	2.5
34	6.0	0.0	1.0	0.5	0.5	3.0
35	4.0	0.0	0.0	0.0	0.0	0.0
36	3.5	0.0	0.0	0.0	0.0	0.0
37	6.0	0.0	2.5	7.0	2.5	0.0
38	5.0	0.0	0.0	0.0	0.0	0.0
39	6.0	0.0	0.0	0.0	0.5	0.0
40	2.5	0.0	0.0	0.0	0.0	0.0
41	3.0	2.0	3.0	2.0	1.0	2.5
42	5.0	0.0	0.0	0.0	0.0	0.0
43	3.5	1.0	2.5	0.0	0.0	0.0
44	2.5	0.0	0.0	0.0	0.5	0.0
45	2.5	2.0	3.0	4.5	1.0	0.0
46	1.0	0.0	0.0	1.0	0.0	0.0
47	2.0	1.5	0.0	0.0	0.0	0.0
48	3.5	0.0	0.0	0.0	0.0	0.0

DISCUSSION

Hypothetical Mechanisms

There is yet no unifying explanation for the mode of action of vitamin E. For selenium as well, some gaps in information still exist--at the biochemical level of pathogenesis--in explanations of the great variety of disease signs in different species of animals. Many studies furnish data that encourage speculation with respect to somewhat novel hypotheses of disease mechanisms involving vitamin E and selenium. Some ideas that plausibly account for actions of vitamin E or selenium are that selenium and vitamin E may favor prostacyclin synthesis, vitamin E may facilitate optimal adenylate cyclase activity, and vitamin E may induce synthesis of an enzyme such as cyclooxygenase. It is of interest to determine the pathogenesis of coagulation alterations present in deficiency diseases.

Selenium

When cyclooxygenase catalyzes the initial oxidation of arachidonate, the "extent of reaction is limited by the quantity of enzyme used. This effect appears to be a self-catalyzed destruction of the cyclooxygenase." *109(639) Perhaps glutathione peroxidase helps to slow this destruction.

Since lipid peroxides are strong and selective inhibitors of prostacyclin synthetase,*56, 65(69), 75 glutathione peroxidase may affect the balance of prostaglandin metabolic pathways as it reduces 15-hydroperoxyarachidonic acid. Glutathione peroxidase also changes

hydroperoxyeicosatetraenoic acid (the product of lipoxygenase action on arachidonic acid) to hydroxyeicosatetraenoic acid.*53(181) This probable influence of selenium in allowing blood vessels to synthesize a maximal amount of prostacyclin may have great significance, especially during pregnancy. Prostacyclin is the most potent stimulator of adenylate cyclase ever found.*34(84) It causes sequestration of platelet calcium and inhibition of platelet phospholipase A_2 and platelet cyclooxygenase. Therefore it hinders thromboxane A_2 production and release of adenosine diphosphate and serotonin from dense granules.*34(87) In this manner prostacyclin prevents and reverses aggregation of platelets. Prostacyclin also relaxes smooth muscle of blood vessel walls, which leads to dilation and lower blood pressure.*56 This counteracts the influence of thromboxane A_2. Although prostacyclin is not the major product in umbilical vessels, it is the product of more than 90% of arachidonate metabolism in the ductus arteriosus. Fetal blood vessels are known to have the greatest ability to generate prostacyclin; those of pregnant animals are intermediate.*58 Normally there is a low peripheral resistance in the fetal circulation, so inhibition of prostacyclin synthesis "may result in serious disturbances of the fetal and maternal circulation."*95(77)

Vitamin E

Equivalent thinking may apply as well to vitamin E. A recent article suggests that "it is reasonable to project the lack of antioxidants in membranes into formation of excess lipid hydroperoxides."*5(1199) Possibly that eventually leads to focal thrombosis (manifested by localized areas of ischemic necrosis) due to disturbance of the usual balance

between thromboxanes and prostacyclin.*5 Vascular endothelium of pigs degenerates during vitamin E deficiency. In the advanced stage, there is thrombosis.*67 Arachidonate-induced, acute, pulmonary thrombosis is more severe in rabbits fed rations low in vitamin E.*68

Changes in vitamin E status are often associated with alterations in prostaglandin type and/or amount. Less prostaglandin synthesis takes place in testis microsomes from vitamin E-deficient rats. Vitamin E-deficient muscle synthesizes subnormal amounts of prostaglandins. There is also increased degradation resulting from increased prostaglandin dehydrogenase activity. Rabbits require 1 month of refeeding of vitamin E to suppress the elevated prostaglandin dehydrogenase level.*14 Blood creatine phosphokinase, an index of muscle degeneration, rises when 25 mg indomethacin per day is given orally to rabbits; the cyclooxygenase inhibitor aggravates α-tocopherol deficiency.*13(67) The edema of several vitamin E-deficiency diseases perhaps is related to excessive circulating prostaglandin E_2, which causes increased capillary permeability.*109(643) Protection by vitamin E against excessive platelet aggregation and disseminated intravascular coagulation most likely happens by alteration of platelet function and prostaglandin metabolism in a way similar to that achieved indirectly by selenium.

Since prostaglandins raise or lower cyclic adenosine monophosphate values by increasing or decreasing adenylate cyclase activity or by decreasing or increasing phosphodiesterase activity,*109(642) one can postulate that a possible function of vitamin E is to maintain the proper membrane environment for adenylate cyclase. It is conceivable that catecholamines (e.g., l-epinephrine) effect a change in

cyclooxygenase via modulation of cyclic adenosine monophosphate level. Adequacy of α-tocopherol in the plasma membrane can also be thought of as a prerequisite for adrenocorticotropic hormone stimulation of cyclic adenosine monophosphate production, which in turn stimulates corticosterone secretion. Trypsin-digested adrenal cells from vitamin E-deficient rats synthesize less corticosterone in response to adrenocorticotropic hormone but not to cyclic adenosine monophosphate than do cells from vitamin E-sufficient rats. This steroid synthesis inhibition is reversed with in vitro addition or feeding of vitamin E but not other antioxidants.*48 Adrenal cortical steroids inhibit phospholipase A_2 activity.*109(638) By preventing access of arachidonate (membrane-derived) to the cyclooxygenase system, corticosteroids reduce prostaglandin biosynthesis and are anti-inflammatory.*33(2)

If vitamin E aids in stabilizing structural integrity of membranes, it may prevent leakage from platelets of granules that promote chronic, widespread coagulation.

An interesting discovery (Nair)*71 is that administration of [^{3}H]-d-α-tocopherol to tocopherol-deficient rats yields radioactive hepatocyte nuclei. Results of the experiment imply the existence of an acidic, non-histone receptor protein of chromosomal origin that binds vitamin E with high affinity.*71 As new protein synthesis is needed for a tissue to recover its ability to make prostaglandins after cyclooxygenase exhaustion,*109(639) this nuclear receptor perhaps may be utilized for induction of messenger ribonucleic acid formation. Vitamin E status affects hepatic microsomal enzyme drug hydroxylation; so do inducers and steroid hormones.*8(101) Three classes of compounds have

structures which resemble α-tocopherol: the steroid hormones, thyroid hormones, and 2,3,7,8-tetrachloro-dibenzo-p-dioxin. It seems reasonable to project that vitamin E may also travel into the cell, combine with a receptor protein, and interact with nuclear material.

Conclusions

The elevation in plasma fibrinogen concentration seems to refute the hypothesis that vitamin E deficiency accelerates consumption of fibrinogen. Another possibility is that rat vascular endothelium, in speculative contrast to that of swine for example, may be such a high producer of prostacyclin that platelet mural adherence and local fibrinogen consumption do not readily occur. Rather, the platelets may become low in cyclic adenosine monophosphate, and a trigger to platelet aggregation may cause such acute and severe coagulation that death quickly follows.

It would be helpful to know whether the increased platelet numbers are caused by increased production or increased survival. There might be a relation to prostaglandin metabolism, for .3% aspirin in the diet of vitamin E-deficient rats prevents the increased platelet counts.*54 Also, the platelet counts may be expected to change as a function of time or severity of the deficiency of vitamin E and selenium.

Either vitamin E deficiency or selenium deficiency leads to myopathy in rats by an unknown mechanism. The moderate association between fibrinogen concentration and skeletal myopathy justifies the proposal that either microthrombosis is involved in the etiology of muscle degeneration or the abnormal muscle is responsible for mediation of some

of the elevation of fibrinogen. The former seems unlikely because one would expect fibrinogen values to drop slightly as a result of thrombosis; the latter proposal is compatible with the suggestion that synthesis of subnormal amounts of prostaglandins by vitamin E- or selenium-deficient muscle initiates myodegeneration.

Hepatic necrosis, renal tubular necrosis, and pancreatic degeneration are consistent with a pathogenesis that involves failure to maintain mitochondrial membrane integrity, depletion of glutathione peroxidase, or other primary defects.

Two new (apparently unreported) observations are the increased incidence of eosinophils and mastocytes in the lung and degeneration in the pancreas of the rat. These lesions may have escaped previous detection due to their subtlety.

The finding that may be of greater importance is the correlation ($r \approx .6$) between skeletal myopathy and fibrinogen elevation. One can visualize several ways to study that relationship. An effective experiment might be to measure plasma prostaglandins, thromboxanes, prostacyclin, organ-specific enzymes, adrenal corticosteroid hormones, vitamin E, selenium, platelets, fibrinogen, and additional clotting factors in animals on diets deficient in vitamin E and/or selenium and treated with various doses (including a low dose once weekly) of a cyclooxygenase inhibitor. Use of a larger species would facilitate sequential (correlated) sampling with reversals of drug treatments and, near termination, of nutrient deficiency. Such a study could reveal the sequence of depletion and/or replenishment of coagulation factors as they respond to or cause tissue lesions and changes in prostaglandin metabolism in animals

deficient in vitamin E and/or selenium.

A few deficiencies should be considered in weighing the results of this experiment and should be corrected in future experiments. Most crucial is the failure of the control diet to produce normal growth. The population of rats from which the experimental units are selected shows no differences in growth among dietary groups before the age of 2 months. However, growth is subnormal relative to that achieved by feeding a standard, commercial diet. Use of a commercial vitamin supplement that greatly exceeds recommended amounts of most vitamins and use of casein instead of Torula yeast do not remedy the subnormal growth. The factor, age, is confounded by subclinical essential fatty acid exclusion from the experimental diets for 3 weeks and 1 week of rats fed to the ages of 3 months and 2.5 months, respectively. Two-month survival is poorest in the groups of rats kept only until the age of 2 months. Individual caging is preferable to gang housing of rodents. Correlated sampling of blood can improve the power of statistical tests. Determinations of vitamin E and selenium in plasma and liver would function as covariates for adjustment of nutritional treatment and body weight; feed determinations would establish whether the dietary treatments are indeed as stated.

SUMMARY

There is still much to be learned about the pathogenesis of vitamin E and selenium deficiency. The conclusions of the experiment reported here concur with those of other investigators and include new information. Forty-eight weanling rats fed semipurified diets supply the following results:

Vitamin E deficiency increases fibrinogen ($p \simeq .05$), skeletal myopathy at 3 months of age ($p < .001$), and pancreatic degeneration ($p < .05$).

Selenium deficiency increases quadriceps femoris myopathy ($p < .05$), renal tubular mineral precipitation ($p < .01$), and pancreatic degeneration ($p < .05$).

Combined deficiency increases pulmonary eosinophils ($p < .05$), platelets at 3 months ($p < .05$), hepatic necrosis, and presence of cells from seminiferous tubules within epididymal lumina ($p < .05$).

The association between myopathy and plasma fibrinogen level merits further investigation in connection with prostaglandin metabolism, coagulopathy, and vascular lesions.

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