PATHOLOGY OF TOCOPHEROL-DEFICIENT MINK AND SWINE

Thesis for the Degree of Ph. D.

MICHIGAN STATE UNIVERSITY

Howard Denison Stowe

1962

This is to certify that the

thesis entitled

Pathology of Tocopherol-Deficient Mink and Swine

presented by

Howard Denison Stowe

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Veterinary Pathology

C. A. Whitehair
Major professor

Date July 10, 1962

O-169

ABSTRACT

PATHOLOGY OF TOCOPHEROL-DEFICIENT MINK AND SWINE

by Howard Denison Stowe

Three experiments, two mink and one swine, were conducted to study the pathology of tocopherol deficiency. Semipurified type rations were employed and the development of the tocopherol deficiency was evaluated by means of growth rates, symptomatology, biweekly hematological and serological studies, gross pathology and histopathology. Also investigated were the effects of tocopherol deficiency upon Salmonella pullorum antibody production, the tocopherol-depleting activity of cod liver oil (mink) and ethyl linoleate (swine), the tocopherol-sparing activity of selenium and ethoxyquin and the species requirement for tocopherol.

Sudden deaths among the tocopherol-deficient mink and swine were considered significant manifestations of tocopherol deficiency. The sudden deaths of mink usually followed an exposure to a stress factor. No growth alterations or consistent physical symptoms were observed in surviving tocopherol-deficient animals.

The most significant hematological alteration that coincided with tocopherol depletion was an increased erythrocyte fragility that was indicated in both species by a 48-hour layered hemolysis in refrigerated saline. The increased fragility was also detected in swine by the dialuric acid

hemolysis test. An absolute neutrophilia was associated with tocopherol-deficient mink that had steatitis. Packed cell volumes and hemoglobin concentrations increased with the age of mink and were unaffected by tocopherol deficiency in either mink or swine.

Prolonged tocopherol deficiency of mink (Experiment II) resulted in decreased serum albumin and increased alpha and beta globulin fractions while the serum protein fractions were not altered by tocopherol deficiency in swine. Elevated serum glutamic-oxalacetic and glutamic-pyruvic transaminase values and serum tocopherol values between 50 and 70 micrograms/100 ml. were associated with tocopherol deficiency lesions.

Grossly, internal intercostal and adductor myopathy was common in tocopherol-deficient mink while subcutaneous edema, hemorrhagic myositis and a perilobular to generalized fatty infiltration of the liver were noted in the tocopherol-deficient swine.

Histologically, the skeletal myopathy of tocopherol-deficient mink consisted of swollen, differentially stained fibers, vacuolar degeneration, sarcolemmal and myoblastic proliferation and calcification of the non-phagocytized myofibrillae. Also associated with the deficiency in mink were calcified necrotic myocarditis, centrolobular hepatic hemorrhage, coagulation necrosis with calcification of the convoluted tubules and calcified necrotic foci in the adrenal cortex.

Microscopic lesions characteristic of tocopherol deficiency in swine were centrolobular hepatic hemorrhage,

endomysial edema, hemorrhagic myositis, a slight myoblastic proliferation, hyalinized adductor fibers containing rowed internal nuclei, and Purkinje fiber degeneration. Sarcolemmal proliferation and calcification of the myofibrillae were not observed in swine while the internal nuclear rowing of skeletal muscle was not characteristic of tocopherol deficiency in mink.

Tocopherol deficiency did not impair or enhance the antibody response of mink to Salmonella pullorum antigen; however, tocopherol-deficient swine demonstrated a greater ability to produce Salmonella pullorum antibody than did tocopherol-supplemented swine.

Isocaloric supplementation of 8% cod liver oil to tocopherol-deficient ration fed to mink caused the deposition of a yellow, acid-fast pigment in the interstices of the adipose tissue. An isocaloric supplement of 5% ethyl linoleate to the tocopherol-deficient ration fed to swine, on the other hand, did not cause the acid-fast-pigmented steatitis. Neither supplement hastened tocopherol depletion as measured by biweekly serum tocopherol values.

Selenium supplementation at the rates of 0.1 and 1 p.p.m. prevented fatal tocopherol deficiency lesions in mink. Neither selenium nor ethoxyquin was effective in preventing the 48-hour hemolysis phenomenon in mink or swine. Alpha-tocopherol supplementation of the basal mink ration at the rate of 25 p.p.m. and the swine ration at 100 p.p.m. was adequate to prevent all lesions associated with a tocopherol deficiency.

PATHOLOGY OF TOCOPHEROL-DEFICIENT MINK AND SWINE

By

Howard Denison Stowe

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Veterinary Pathology

1962

ACKNOWLEDGMENTS

The author is grateful to the following for helping to make this research and thesis possible:

Dr. C. K. Whitehair, my major professor, whose encouragement for research and whose counsel in the preparation of the manuscript were invaluable.

Dr. C. C. Morrill, department chairman, for granting department facilities for this research and for his guidance in writing this thesis.

Dr. R. F. Langham for his assistance with the histopathology.

Dr. M. L. Calhoun for her helpful suggestions in the preparation of the manuscript.

Drs. R. W. Luecke and D. E. Ullrey for constructive criticisms of the manuscript, help with analytical procedures and the use of laboratories under their supervision.

Mr. Robert Maronpot for his assistance with analytical determinations and photography.

Mrs. Nancy Anderson, Mrs. Ann Goatley, Mrs. Delorise Palombo, Mrs. Jackie Bradley and Mr. William Truitt for their technical assistance.

National Institutes of Health and the Mink Farmer's Research Foundation of Milwaukee for their financial support.

Distillation Products Industries, Rochester, New York, and Lake States Yeast and Chemical Division of St. Regis

Paper Company, Rhinelander, Wisconsin, respectively, for providing the molecularly distilled lard and torula yeast for these experiments.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The nutritional importance of tocopherol has been extensively investigated in many species since Evans and Bishop (1922) identified the fat-soluble substance. Recently, semi-purified tocopherol-deficient and unsaturated-fatty-acid-low rations were fed to mink to determine if a relationship existed between tocopherol deficiency and urinary incontinence. Although no such relationship was found, several cases of tocopherol-deficiency-myopathy were produced without any evidence of "yellow fat" which Gorham et al. (1951) considered characteristic of tocopherol deficiency in mink fed high levels of fish products. Thus, the mink was considered to be a suitable animal in which to study the pathology of tocopherol deficiency uncomplicated by steatitis.

Mink Experiment I was undertaken to re-examine the uncomplicated tocopherol deficiency, to study the effects of selenium supplementation and to obtain information relative to the tocopherol requirement of mink under the conditions of the experiment.

Mink Experiment II was designed to demonstrate the differences between the uncomplicated tocopherol deficiency and one complicated by the effects of an elevated intake of unsaturated fatty acids. The study of a chronological relationship between the onset of increased fragility of tocopherol-deficient erythrocytes (observed in Experiment I)

and the onset of other clinical pathology of tocopherol deficiency was also of interest.

The importance of tocopherol in swine nutrition was emphasized when Adamstone *et al.* (1949) noted a hemorrhagic condition in 10 mm. pig embryos identical histologically to that observed in tocopherol-deficient chick embryos. Most investigators of tocopherol deficiency in swine have used unsaturated fatty acids as tocopherol-depleting agents. The resulting tocopherol deficiencies were complicated with a steatitis related to unsaturated fatty-acid toxicity. The uncomplicated tocopherol deficiency produced in mink suggested the feasibility of studying the same deficiency in the baby pig.

REVIEW OF LITERATURE

Tocopherol in Nutrition

General

The fat-soluble dietary factor necessary for reproduction in rats was discovered by Evans and Bishop (1922) and called vitamin E. For this biological alcoholic antioxidant, George Calhoun coined the term 'tocopherol' from tokos - childbirth; phero - to carry; and ol - alcohol. Of the seven known tocopherol isomers, alpha, beta, gamma, delta, epsilon, zeta and eta, Dam (1957) reported that alpha is physiologically the most important, accumulates to the greatest degree in animal tissues and, in vitro, elicits the least antioxidant effect.

While the rat and chick have been the most frequently used laboratory animals for tocopherol experiments, the following species have also been utilized: monkey (Marvin et al., 1960), calves (Maplesden and Loosli, 1960), cattle (Andersson, 1960), sheep (Muth et al., 1959), foals (Dodd et al., 1960), dogs (Brinkhous and Warner, 1941), cats (Cordy and Stillinger, 1953), rabbits (Borgman, 1959), hamsters (West and Mason, 1958) and guinea pigs (Bender et al., 1959).

The major manifestations of tocopherol deficiency include exudative diathesis, encephalomalacia, adipose tissue discoloration, [illegible] depigmentation, reproductive failure,

liver necrosis, lung hemorrhage, erythrocyte fragility, and muscular degeneration. Some less frequently observed effects include adrenal necrosis, renal tubule degeneration, arterioscleroses, Purkinje fiber degeneration, reticular and elastic fiber discontinuity, duodenal and gastric ulcers, increased cellularity of bone marrow, sarcolemmal proliferation and altered clotting time.

Metabolic Role

That the tocopherols inhibit autoxidation of fats in contact with molecular oxygen is evidenced by two facts: certain signs of tocopherol deficiency occur only when easily oxidized unsaturated fatty acids are present in the diet; and many antioxidants and redox substances structurally unrelated to tocopherol afford partial to complete protection against certain manifestations of tocopherol deficiency. Bouman and Slater (1957) indicated that the tocopherols are also associated enzymatically with tissue respiration, specifically with the cytochrome c reductase activity in heart mitochondria where large quantities of cellular tocopherols are found. Contrary to the reports of Bouman and Slater, Pollard and Bieri (1959) found the DPNH cytochrome c reductase activity was not significantly affected by tocopherol deficiencies. Simple homogenization of the enzyme extracts was found to affect their inactivation, which could be overcome by the addition of lipid substances, by lyophilizing or by centrifuging. Also, Pollard and Bieri found that the iso-octane phase used by Bouman and Slater

had a narcotizing effect upon the enzymes. Removal of the bound isooctane from the respiratory chain preparations resulted in their reactivation.

Zalkin and Tappel (1960) found tocopherol inhibited mitochondrial lipid peroxidation and concluded that tocopherol functions solely to stabilize cellular unsaturated lipids against oxidative deterioration, thus maintaining their functional integrity at the subcellular level. Schwarz (1961) reported that the only detectable defect in tocopherol deficient rat mitochondria is in their succinate utilization in the presence of diphosphopyridine nucleotide and was evidenced by respiratory failure. An inhibitory factor, possibly iron, was found to hasten the respiratory failure. Schwarz concluded that iron inactivates various sulfhydryl dehydrogenase substrate systems and that the physiologic function of tocopherol is related to thiol or dithiol groups of enzyme systems because sulfur amino acids decrease the tocopherol requirement to approximately one-tenth that of normal. The metabolically active form of tocopherol may act at active sulfhydryl group sites, force the equilibrium toward the S-S form and eliminate the point of attack for inhibitory heavy metals. Corwin (1960) has also indicated alpha tocopherol functions in maintaining sulfhydryl groups. Considerably more work is necessary to elucidate positively the metabolic roles for the various tocopherols.

Tocopherol-Sparing Compounds

Selenium

Schwarz and Foltz (1957) demonstrated that the so-called Factor 3, which prevented hepatic necrosis in rats fed tocopherol deficient rations, was actually selenium. Since 1957, sodium selenite has been reported therapeutically effective against exudative diathesis of chicks by Dam and Sondergaard (1957), stiff lamb disease by Muth et al. (1959), ill thrift in lambs by Drake et al. (1960), white muscle disease of calves by Sharman et al. (1959) and Jolly (1960) and muscular dystrophy, necrotic hepatitis, hemorrhagic lymphadenitis and microangiopathy in swine by Grant (1961).

Yang et al. (1959) and Lam et al. (1961) suggested that some metabolic relationship exists between selenium and co-enzyme A. Dietary selenium at .05 p.p.m. was found by Yang and associates to stimulate co-enzyme A biosynthesis from S^{35} labeled cystine and to elevate co-enzyme A levels in the livers of rats on necrogenic diets. Rosenfield (1961) found that seleno-cystine and seleno-methionine are formed by bacteria in the ovine rumen from inorganic selenium. Rosenfield (1960) also showed that selenite interfered with protein synthesis by inhibiting the transmethylation of homocystine or nomocysteine by betaine or choline. Corwin and Schwarz (1959) did not find selenium effective in preventing the decline of succinate peroxidation which is observed in livers of tocopherol-deficient rats. Schwarz (1960) reported that, in cystine, a trace contamination of

1 atom of selenium in 350,000 atoms of the sulfur ion is adequate to protect against hepatic necrosis in the rat.

Selenium, as well as alpha-tocopherol, has been shown by Edwin et al. (1961) and Green et al. (1961) to increase the ubiquinone levels in rat heart, liver and ovaries. The ubiquinones are considered by Diplock et al. (1960) and Redfearn and Punphrey (1960) to function in oxidative phosphorylation or electron transport and the essential biological role of selenium may be associated with its ability to increase ubiquinone levels in vivo.

Bieri (1961) indicated that selenium increases the antioxygenic potential of cells by, in some way, altering the composition of the cell proteins. Bieri, however, observed no correlation between the dietary concentration of selenium and its antioxidant effect. Selenium supplementation lowered tissue thiobarbituric acid values, indicating decreased peroxidation.

Redox Substances

Dam and coworkers (1951) reported the following to afford considerable protection against symptoms of exudative diathesis in tocopherol-deficient chicks: methylene blue @ .126% of diet, thionine @ .089%, thiophenylamine @ .675%, disulfiram @ .025%, and ascorbic acid @ .5%. If any protection were afforded, it was reflected by elevated tocopherol values in adipose tissue. Against liver necrosis in rats, Dam and Granados (1951) found methylene blue to afford complete protection and magnesium supplementation afforded

slight protective action with the necrogenic diets.

Antioxidants

The tocopherol sparing effects of NN'-diphenyl-p-phenylenediamine (DPPD) have been extensively studied by many including Sharman and Moore (1958), Draper (1959) and Crider et al. (1961). In tocopherol deficient rats DPPD was found to prevent the brown uterus, testicular degeneration and the abnormal tendency of the erythrocytes to hemolyze. Oser and Oser (1956), found DPPD, however, caused prolonged gestations which led to dystocias in rats. Machlin and Gordon (1960) found that .1% ethoxyquin added to a tocopherol-deficient-torula-yeast ration and fed to chicks prevented both exudative diathesis and encephalomalacia.

Others

Moore and Sharman (1958) found isoniazid, an agent effective against tubercle bacilli, prevented the rapid post mortem autolysis in kidneys and the mottling of the incisor teeth of tocopherol deficient rats. Mason and Rao (1960) showed that alpha-tocopherolhydroquinone, thought only to contain antidystrophic activity, does in fact possess antisterility activity approximating 1/20th that of alpha-tocopherol. Corwin (1960) found S-H glutathione and 2,3-dimercaptopropanol (BAL) prevented the decline in oxidation rates of alpha-ketoglutarate or succinate in liver homogenates from tocopherol deficient rats. Green et al. (1960) found that intraportal injections of the cobalt ion have high

activity in preventing respiratory decline in liver slices from tocopherol-deficient rats.

Tocopherol Antagonists

Unsaturated Fatty Acids

Although Agduhr (1926) first reported the harmful effects of cod liver oil fed to children, Goettsch and Pappenheimer (1931) were among the first to find that the unsaturated fatty acids contained in cod liver oil aggravated a tocopherol deficiency. Madsen (1936) experimented with fats and oils in synthetic rations and concluded that fats alone were not responsible for the muscular dystrophy produced. Morgulis and Spencer (1936) reported two factors were probably involved in nutritional muscular dystrophy, one in wheat germ oil and the other in green lettuce and dry alfalfa. Leaf fats of pasture herbage have been found by Hilditch (1956) to be highly unsaturated yet Keith and Schneider (1957) found no correlation between the dystrophogenicity of roughages and their tocopherol content.

Although Brown (1953) has shown cod liver oil to contain appreciable tocopherol, the polyenoic fatty acids, especially in cod liver oil, have been used extensively as tocopherol depleting agents in nutritional experiments since about 1945. Filer and associates (1947) found C_{18} acids with 3 double bonds most effective in producing histological changes in the tocopherol-deficient rat. Kokatnur *et al.* (1960) found the most effective polyenoic acid for the

acceleration of encephalomalacia in chicks is 12 oxo cis-9-octadecenoic acid, especially when used with corn oil. Nishida et al. (1960) produced encephalomalacia in chicks in from one to five hours by intravenously injecting 10 mg of linoleic acid previously emulsified in 1 ml. of serum. Parenterally administered alpha-tocopherol prevented this encephalomalacia which seemed to be initiated by the in vivo accumulation of lipohydroperoxides.

Horwitt and coworkers (1961) indicated that the requirement for tocopherol is a function of the amounts of polyunsaturated fatty acids in the diet and body tissues and that past dietary habits affect tocopherol needs. Diet changes were found to alter the mitochondrial lipids of all tissues including the brain. In studies undertaken to investigate the relationship between tocopherol and various types of dietary fat for the rat, Alfinslater et al. (1961) found that tocopherol was required by the rat in the absence as well as in the presence of dietary fat.

Other Tocopherol Antagonists

Sulfathiazole and sulfaguanidine therapy, according to Daft et al. (1943), resulted in lesions similar to tocopherol deficiency. Pindborg (1949) confirmed this and speculated that bacteria of the alimentary tract synthesized some tocopherol and when the gut is sterilized by sulfa therapy, tocopherol deficiency may result. Tedeschi and De Cicco (1953 and 1954) found o-cresol succinate and guaiacol acetate, injected into rats, produced fetal resorption, germinal

epithelial degeneration and muscular dystrophy. All these were preventable with simultaneous administration of alpha-tocopherol.

Hove (1953) found pyridine administered to tocopherol-deficient rats caused liver damage and death which could be prevented by alpha-tocopherol, methylene blue and yeast nucleic acids. Pyridine was found to depress the antioxidant activity of alpha-tocopherol. Hove (1955) included carbon tetrachloride and sodium sulfate as tocopherol stress factors and found tri-o-cresyl phosphates to interfere with tocopherol absorption from the gut. Sharman and Richards (1960) noted chlorine dioxide-bleached commercial flours, when fed to rats, were able to cause positive dialuric acid hemolysis values indicative of a tocopherol deficiency.

Clinical Indications of Tocopherol Depletion

Tissue Tocopherol

Direct tissue tocopherol analyses provide the most direct indicators of tocopherol depletion. However, the number of different procedures available is indicative of their variable reliability. Determinations for total tocopherol in use presently are basically of two types. One measures the reducing capacity of ethanolic extracts of tocopherol in the presence of ferric chloride and alpha-alpha dipyridyl (Quaife et al., 1949). The other method by Duggan (1959) is based upon the ability of tocopherol extracts to fluoresce.

For specific tocopherols, two basic methods are also available. The method of Bro-Rasmussen and Hjarde (1957) depends upon the capacity of chromatographic columns of activated secondary magnesium phosphate to adsorb the tocopherols. These are then selectively eluted depending upon the percentage of ethyl ether in the petroleum ether elutriant. The second and reportedly more reliable method (Edwin et al., 1960) incorporates the purification of the tocopherol extracts on a fluoricil column followed by two dimensional paper curtain chromatography to separate the individual tocopherols.

Creatine-Creatinine Excretion

The presence of creatine and its anhydride, creatinine, in the urine has been used as an indicator of tocopherol deficiency. Bicknell and Prescot (1948) considered creatinuria, at least in the rat, to be the first clinical sign of tocopherol deficiency. Although Melville and Hummel (1951) indicated that a creatinuria preceded histological changes in a tocopherol deficiency, they were unable to correlate degrees of creatinuria positively with the severity of the deficiency. Butturini (1949) judged hypercreatinuria to be a nonspecific clinical symptom found in all vitamin deficiencies. Hove and Harris (1947) with rabbits and Bauer and Berg (1943) with mice demonstrated altered urinary creatinine levels during a tocopherol deficiency; however, Bacigalupo (1952) found tocopherol deficiency in lambs had no effect upon the creatinine excretion.

Blood Enzymes

Blood enzyme determinations have become diagnostic aids in nutritional myopathies since alterations of some blood enzyme levels were observed in the human following myocardial infarction and viral hepatitis. Possibly instigated by the work of White and Hess (1957) on blood enzymes and muscular dystrophy in the human, Kuttler and Marble (1958) and Blincoe and Dye (1958) found elevated serum glutamic oxalacetic transaminase (SGOT) values in natural and artificially induced cases of white muscle disease in calves. Swingle *et al.* (1959) have found similar results in lambs with nutritional muscular dystrophy. Blincoe and Marble (1960) found a significant positive linear correlation between SGOT and serum lactic dehydrogenase levels in lambs having nutritional myopathy induced by feeding cod liver oil. In severe dystrophy, serum alkaline phosphatase decreased to one-half its normal value. Normal values for SGOT and serum glutamic pyruvic transaminase (SGPT) in domestic animals have been compiled by Cornelius *et al.* (1959). Only in the dog liver did these workers find an appreciable SGPT activity and likewise only in the dog was there an elevation of SGPT following a hepatic necrosis induced by carbon tetrachloride.

Erythrocyte Fragility

An increased susceptibility for erythrocytes to hemolyze is indicative of tocopherol depletion. Dialuric acid was found by Christensen and Dam (1951) in Denmark to produce *in vitro* hemolysis of erythrocytes from tocopherol-deficient

rats. This hemolysis was inhibited by alpha-tocopherol and partially by methylene blue. These workers postulated that alpha-tocopherol inhibited the hemolysis by preventing a free radical, involved in the autoxidation of dialuric acid, from reacting with a substance within the erythrocyte.

Rose and György (1952) showed intravenously administered dialuric acid to cause intravascular hemolysis. These workers also found that 1.5 micrograms of alpha-tocopherol added to a substrate of erythrocytes gave complete protection against the hemolytic action of dialuric acid; that in the order given, beta, gamma and delta tocopherols showed a decreasing protective action against dialuric acid and that hydrogen peroxide caused hemolysis to both tocopherol-deficient and normal erythrocytes. Friedman et al. (1958) established a bioassay for vitamin E by the dialuric acid hemolysis test and indicated that the activity of dialuric acid is readily destroyed upon contact even with such inert substances as polyethylene.

Gitler et al. (1958) found selenium, methionine, cystine, methylene blue and butylated hydroxy toluene (BHT) non-effective, under the conditions employed, in altering the susceptibility of tocopherol-deficient erythrocytes to hemolyze with dialuric acid. Bunyan et al. (1960) indicated that the presence of methyl groups, ortho to the hydroxy group in tocopherol, enhanced the in vitro potency of the tocopherol in preventing dialuric acid induced hemolysis. Ions found active in this regard were Co, Mn, Sn, CrO_4, and

Cr_2O_7. The former two appeared to act synergisticly with alpha-tocopherol.

Alfinslater *et al.* (1961) correlated plasma tocopherol, erythrocyte tocopherol and erythrocyte fatty-acid composition with the susceptibility of tocopherol-deficient erythrocytes to dialuric acid hemolysis. Leat (1960) indicated that dietary fat plays a more important part in the hemolytic susceptibility than does dietary tocopherol in swine rations.

Erythrocyte Survival

Marvin *et al.* (1960) and Horwitt *et al.* (1960), using Cr^{51} tagged autologous erythrocytes, demonstrated an accelerated turnover of erythrocytes in the tocopherol-deficient monkey and man respectively. In monkeys, survival times of 35-49 days were associated with tocopherol deficiency while normal erythrocytes had a survival period of 100 days.

Glucose Tolerance

Another indirect tocopherol depletion indicator was reported by Mertz and Schwarz (1955) to be an impaired intravenous glucose tolerance. Although not significantly affected by 50 mg. of alpha-tocopherol acetate, 2.15 mg. of Schwarz's Factor 3 fraction increased glucose clearance rate to that of the control animals. In this regard, Horn *et al.* (1955) concluded that tocopherol protects liver reserves of glycogen.

Tocopherol Deficiency Myopathy

Biochemical Lesions

One of the first biochemical lesions associated with tissue from tocopherol deficient animals, an increased requirement for oxygen, was described by Victor (1934). Houchin and Mattill (1942) confirmed Victor's work and reported that striated muscle from tocopherol-deficient animals required from 125-250% more oxygen than normal muscle and that following oral administration of alpha-tocopherol, normal oxygen consumption was restored in 22 hours. Roderuck (1949) also confirmed this increased oxygen requirement in cavia. Further proof of an increased oxygen requirement was shown somewhat differently by Teleford *et al.* (1954) who subjected tocopherol-deficient, normal and tocopherol-supplemented rabbits to a series of three decompression exposures. During the course of these decompressions, all the tocopherol-deficient animals, one supplemented animal and no normal animals died.

Bonetti *et al.* (1952) noted that the content of contractile elements (myosin and actomyosin) in tocopherol-deficient rabbit skeletal muscle decreases gradually during the deficiency. Keeler and Young (1961) electrophoretically characterized the protein extracts from normal and dystrophic ovine muscles. Actomyosin, myosin and a rapid moving myoalbumin were found elevated in extracts from affected longissimus dorsi and quadriceps femoris muscles while the myogen fraction was decreased. In blood serum from the affected animals, the alpha globulin fraction increased while beta and

gamma globulins decreased. The myoglobin concentrations of both pale and red skeletal muscle of tocopherol-deficient cavia have been found by Schottelius et al. (1959) to decrease following 15 days on tocopherol low rations.

Dinning and Day (1957), at Arkansas, have shown the tocopherol-deficient monkey to have an increased skeletal muscle DNA and a reduced skeletal muscle creatine. Lawrie (1960) found a rapid post mortem decline in the pH of dystrophic longissimus dorsi muscle of swine to an ultimate of 5.11 compared to 5.5 for the normal longissimus dorsi.

Pathogenesis

The best documented and most convincing study relative to the pathogenesis of tocopherol deficiency myopathy has been made by West and Mason (1959) using hamsters. Animals were sacrificed at ten day intervals during an 80-day deficiency period and a subsequent 20-day recovery period. Sections were taken from the adductor magnus, sacrospinalis, subscapularis, masseter and tongue muscles. In order to observe much longer segments of unsectioned muscle fibers, thin stained spreads of the skeletal muscle from the cheek pouch were prepared.

The microscopic changes were classified as reversible and irreversible. The former were characterized by alignment of muscle nuclei in chain-like rows within the fibers. The phenomenon, called internal rowing, could remain static for indefinite periods. The irreversible injury included changes in this order: focal degeneration of myofibrils

which were converted into highly cellular masses composed of surviving muscle nuclei, investing sarcoplasm, macrophages and leukocytes; formation of contraction clots, similar to those produced instantaneously by trauma and coagulation necrosis of segments of fibers. Macrophages then enter to remove the necrotic material.

Muscle elements or myoblasts form a syncytium from which reconstitution of the fiber segment occurs. Regeneration occurs, to a lesser extent, through terminal budding or plasmodial outgrowth from viable portions of affected fibers. Later phases of regeneration are more or less indistinguishable from the sublethal reaction described as internal rowing.

Following tocopherol therapy, degenerative changes are promptly prevented, all evidence of previous necrosis is abolished in 5-10 days, and there remain only normal, regenerating and rowed fibers. The latter gradually transform into normal fibers and the tissue restoration, at least structurally, appears complete.

West and Mason (1958) preferred to consider the prominent feature coagulative necrosis rather than Zenker's or hyaline degeneration because of the chances for misinterpretation in overstained iron and phosphotungstic acid-hematoxylin sections. Adams et al. (1953), it is interesting to note, did not include tocopherol deficiency in their list of factors producing waxy, hyaline or Zenker's degeneration in the human, nor did they consider tocopherol deficiency

myopathy a true muscular dystrophy because, in the latter, no regenerative activity is present.

Recognition must be given the fact that all striated muscle does not react alike during tocopherol deficiency. In this regard, for instance, West and Mason mentioned nothing about the calcium infiltration of muscle fibers in myopathy due to tocopherol deficiency, reported especially in calves by MacDonald et al. (1952).

According to Semenova (1958) the neuropathology associated with a nutritional myopathy, at least in the rat, is confined to the motor end plates and neuromuscular spindles. Following the early degenerative changes in the muscle, changes occurred which consisted of separation of terminal ramifications together with some thickening and an increase in nuclei at the base of motor end plates. As the myopathy progressed, the number of nerve endings was reduced and degenerative changes occurred in the neuromuscular spindles. Upon tocopherol supplementation, the muscle fibers regenerated and then the nerve endings were restored.

Tocopherol Deficiency in Mink

Relation to Steatitis

The existing information relative to the tocopherols in mink nutrition, however meager, has evolved almost entirely from studies of steatitis in mink. Natural cases of yellow fat in mink in the United States were first reported by McDermid and Ott (1947). Hartsough and Gorham (1949)

considered this disease to be histologically similar to that experimentally produced by Dam (1944) in the rat.

According to Hartsough and Gorham, the disease in mink is clinically manifested by a leukocytosis of 25-35,000, an erythrocytopenia of approximately 3 million and a hemoglobinuria. The affected mink, usually kits, appear unusually wide through the abdomen, have an unnatural gait, have been ravenous eaters and may die suddenly following a short anorexic period. Morbidity ranges up to 50% and mortality to 75%. The gross pathology consists of lumpy palpable abdominal fat, marked subcutaneous edema, and brownish yellow coloration of the subcutaneous and visceral fat which may have a rancid odor. Less common findings include splenomegaly, mesenteric lymphadenitis, mesenteric and omental hyperemia and petechial hemorrhages in the affected adipose tissue.

Quortrup et al. (1948) described the microscopic pathology of early field cases as a disseminated non-suppurative inflammation of the subcutaneous and visceral fat which is infiltrated primarily with polymorphs. Fat necrosis is minimal and the interlobular septae of the adipose tissue are edematous. More advanced steatitis, according to Quortrup, shows fibroblastic proliferation and many macrophages containing fat in the inflammatory area. An atrophic epidermis, often only one cell thick, and dermal edema may also accompany the steatitis.

Gorham et al. (1951) found that 20 mg. tocopherol/mink/day added to a basal ration consisting of 85% fresh frozen

fish scrap, 13% commercial mink cereal and 2% brewers yeast were sufficient to prevent any acid-fast pigmentation of adipose depots. Lalor et al. (1951) reported a daily intake of 5 mg. of alpha-tocopherol was required to prevent steatitis on rations containing relatively high amounts of trienoic acids as are present in horse fat to the extent of 16%.

Dalgaard-Mikkelsen et al. (1958) demonstrated that 50 mg. tocopherol/kg. of feed, 3% hardened pig fat and methylene blue at the rate of 50-100 mg/kg. of feed protect against yellow fat even when the diet contains 10% fish oil. They also showed that if the fish oil were limited to 3-4% of the diet, no antioxidants are required to prevent steatitis.

Leekly and Cabell (1959) found that 112 gm. DPPD/ton of ration composed largely of frozen stored fish canner waste prevented steatitis. However, reproductive performance was affected by DPPD. Butylated hydroxytoluene was tested with similar diets and found to prevent steatitis without any reproductive impairment.

Relation to Myopathy

Zenker's degeneration or "white heart" disease was reported by Benson (1959) to be most common in older mink and tocopherol supplementation of mink rations provided effective prevention of the cardiac degeneration.

Tocopherol Deficiency in Swine

Reproductive Failure

Early investigations relative to the tocopherols in swine

nutrition were designed to demonstrate the effectiveness of tocopherol in preventing reproductive failures in farm swine. In this regard, Bay and Vogt-Miller (1934) found wheat germ oil effective in combating sterility in sows. Considerably later, Carpenter (1949) found no benefit from supplementing the sow's ration with wheat germ oil shortly before farrowing; however, supplementation prior to conception and during gestation did improve reproductive performance and the livability of the pigs. Garton and Naftalin (1953) produced an exudative diathesis of swine with tocopherol-low rations containing lard and cod liver oil as the fat sources.

Unstable Pork

With regard to the role of tocopherols in stabilizing animal tissues, Burr (1945) and Watts *et al.* (1948) found supplemental tocopherol decreased the susceptibility of pork fat to rancidity. Fish-product-rich swine rations became associated with unstable pork fat which Breirem (1952) attributed to oxidative destruction of the available tocopherol. Dammers *et al.* (1958) found 40 mg. tocopherol/day adequate for maximum keeping quality of pork fat. Zaehringer *et al.* (1959) found thiobarbituric acid values of frozen pork varied inversely with the number of weeks on a tocopherol supplemented ration. The maximum rate of alpha-tocopherol supplementation was 530 IU/day for six weeks prior to slaughter.

Steatitis

As the antioxidant role of the tocopherols became realized, cod liver oil became the most common tocopherol depleting agent in experimental tocopherol deficiencies and porcine steatitis became associated with these deficiencies. Robinson and Coey (1951) fed a tocopherol-low ration supplemented weekly with five ounces of cod liver oil and produced a brown fat which was prevented by 50 mg. tocopherol/day. These workers also found the iodine values of carcass fat were elevated in proportion to the cod liver oil intake.

Gorham et al. (1951) and Davis and Gorham (1954) produced yellow fat in swine with a diet containing 85% fish scrap and found that 500 mg. tocopherol/day prevented the deposition of the acid fast pigment. Burr (1945) had postulated that the tocopherols act through an oxidase system to effect oxygen uptake of tissue and in turn determine the stability of meat products.

Garton and Duncan (1954) raised pigs from weaning on tocopherol-low rations containing up to 50% cod liver oil and lard. The resulting fats were dark brown and contained the absorbed lard and oil practically unchanged. Gedigk and Fischer (1959) postulated the origin of the lipid pigments of the depot fats during tocopherol deficiency. They stated that, in the absence of tocopherol, the unsaturated fats accumulate gradually in the area of protein containing metabolically active cytoplasmic foci and are then oxidized and polymerized. The lipid pigments were found in macrophages

following their absorption of fat containing tissue particles.

Necrotic Hepatitis

A naturally occurring disease, necrotic hepatitis of swine, which Hjarre (1951) reported to affect ten percent of all the pigs seen annually at the Stockholm Veterinary Medical Institute, is clinically similar to that reported by Dam and Granados (1951) in vitamin E deficient rats and to that reported by Naftalin and Howie (1949) to result from the stresses of a cold and damp environment upon swine. Obel (1953) has extensively studied and experimentally produced the disease to which she has given the name "hepatosis diaetetica" (h.d.). It occurs in pigs under six weeks of age and is associated with anemia, discolored adipose tissue, subcutaneous edema, moderate ascites, gastric ulcers, renal and splenic congestion, mottled liver and waxy degeneration of skeletal and cardiac musculature. Histologically, the liver changes include centrolobular necrosis, hemorrhage, dystrophic calcification of the hepatic cells, anoxic vacuoles at the periphery of the lobules, purulent cholangiolitis and thrombi in the bile canaliculi. Reparative processes appear early and consist of neutrophilic and lymphocytic infiltration, bile duct proliferation and apparent hepatic cell regeneration. Histologically associated with hepatosis diaetetica is a steatitis, fibrinoid degeneration of medium sized arteries, nephrosis of Henle's loop and edematous lymphadenopathy.

Obel produced h.d. with a basal diet of 73% carbohydrates, 18% dried brewer's yeast, 6% cod liver oil and 3% minerals. Supplementation of the basal ration with tocopherol failed to elicit a protective effect on the liver but if lard replaced the cod liver oil and alpha-tocopherol were added, h.d. was prevented. With neither torula yeast or baker's yeast could h.d. be produced. Supplementation of the basal diet with .5% methionine or cystine prevented the h.d. Unfortunately, the significance of selenium was not recognized at the time of Obel's experiments. She considered hepatosis diaetetica to be caused by a combination of toxic products, reflex anoxia and diminished detoxifying property of the liver due to limited sulfur-containing amino acids and tocopherol.

Hove and Seibold (1955) described a fatal liver necrosis which developed in growing swine fed a diet deficient in vitamin E and containing 6% protein (soybean meal) and 2% cod liver oil. Deaths were attributed to acute hemorrhagic liver necrosis and the liver fat had less concentrations of linoleic and pentaenoic acids than did the liver fat from tocopherol supplemented animals. In New Zealand, Dodd and Newling (1960) reported a natural outbreak of hepatosis diaetetica in swine receiving a diet consisting of cheese whey, barley, meat meal and a fish liver oil supplement of 8 ounces/500 gallons of whey.

Myopathy

Forbes and Draper (1957) experimentally produced skeletal

and cardiac muscle degeneration in baby pigs receiving semi-purified type rations containing 20% methionine-supplemented casein, 45% cerelose, 29% vitamin E free lard, minerals and vitamins. Lannek et al. (1961) in Sweden demonstrated that a stress factor such as cod liver oil is required to experimentally provoke muscular dystrophy in swine and in field cases of the dystrophy in swine; they considered the stress factor to be a toxic substance in the natural grains.

General Characteristics

Pelligrini (1958) characterized the manifestations of vitamin E deficiency in growing pigs receiving a semipurified type, 32-45% torula yeast ration from 10 days of age and studied the effectiveness of alpha-tocopherol acetate, sodium selenite and L cystine in preventing the deficiency symptoms. A fatal liver necrosis accompanied by degeneration of the semitendinosus, semimembranosus and latissimus dorsi muscles, developed in growing pigs after 50-70 days on the basal diet. L-cystine supplementation prevented the liver necrosis, but not the muscle degeneration. Fifty mg. alpha-tocopherol acetate and .045 p.p.m. sodium selenite prevented deaths and the pathology of liver and muscles. Rates of gain and feed efficiency were satisfactory in all groups prior to death. Occurring with lesser frequency in the fatal cases were splenic infarcts, portal triad endarteritis, bile duct proliferation, ascites, hydrothorax, lymphadenitis and hyperemia of the intestines. In a similar study in swine, Eggert et al. (1957) reported sudden deaths, liver necrosis, steatitis,

hemorrhagic lymphadenitis, and hemorrhagic gastroenteritis, all of which were prevented by 40 p.p.m. alpha-tocopherol acetate or 1 p.p.m. sodium selenite.

Plasma Enzymes

With reference to plasma enzymes and tocopherol deficient swine, Orstadius et al. (1959) demonstrated elevated SGOT and SGPT and ornithine-carbamyl transferase values in cases of spontaneous liver dystrophy of swine while spontaneous muscular dystrophy provoked only an elevation of the transaminases. Augustinsson et al. (1960) showed arylesterase, a phenotypically characteristic enzyme of swine plasma, to be unaffected by an induced hepatic dystrophy preventable by sodium selenite.

Erythrocyte Fragility

Forbes and Draper (1957) and Leat (1961) reported that tocopherol deficiency in swine was not accompanied by an increased susceptibility of the erythrocytes to hemolyze with dialuric acid. Leat (1961), however, reported that a spontaneous hemolysis in .9% saline occurred earlier in the erythrocytes from the animals given 2% olive oil than from animals on a low-fat diet. Only once though was this hemolysis affected by tocopherol supplementation.

The material presented in this review in no way represents a complete coverage of the available literature relative to the tocopherols in nutrition. Rather, it is intended

to highlight some current concepts relating especially to the metabolic roles of tocopherol and selenium and to review the literature most pertinent to the experiments reported herein.

MINK EXPERIMENTS

Materials and Methods
Experiment I

Forty six-weeks-old dark male mink kits were obtained from the Michigan State University Fur Project on June 11, 1959, and placed on the semipurified tocopherol-deficient ration described in Table 1.

Table 1.--Composition of the tocopherol-deficient diet.

Ingredient	Per Cent
Vitamin free casein	16.0
Isolated soy assay protein[a]	8.0
Torula yeast[b]	16.0
Sucrose	25.5
Molecularly distilled lard[c]	24.0
Solka-Floc (alpha-cellulose)[d]	6.0
Phillips and Hart Salt Mix (IV)[e]	4.0
Amino acid and vitamin supplement	0.5

[a]From Archer-Daniels-Midland Company, 2795 Sharon Rd., Cincinnati, Ohio.
[b]Furnished by Lake States Yeast and Chemical Division of St. Regis Paper Company, Rhinelander, Wisconsin.
[c]Furnished by Distillation Products Industries, Rochester, New York.
[d]From Brown Company, 150 Causeway St., Boston 14, Mass.
[e]Phillips and Hart (1935).

The amino acid and vitamin supplement for this ration was prepared to furnish the constituents at rates shown in Table 2, page 30. The ration was prepared dry in a Hobart mixer and stored in polyethylene bags under refrigeration at all times. Quantities sufficient for each day's feeding were removed from the refrigerator immediately prior to feeding, mixed with water to the consistency of mashed potatoes and fed on

Table 2.--Amino acid and vitamin supplementation rate.

Component	Rate*	Component	Rate*
Arginine HCl	250.0	Inositol	50.0
DL methionine	100.0	p-amino benzoic acid	100.0
Riboflavin	1.0	Folic acid	0.2
Pyridoxine	0.5	Cyanocobalamin	0.16
Calcium pantothenate	3.6	Biotin	0.05
Nicotinic acid	5.0	Vitamin A acetate	0.52
Choline chloride	400.0	Vitamin D_2	0.01
Thiamine HCl	0.5	Menadione	0.5

*-g./100 gm. dry diet

metal feed doors once per day with additional feedings as indicated. The feed doors were scraped regularly to prevent the mink from having access to any rancid feed.

All animals remained on the above ration for four weeks at which time eight animals were assigned to lot A and their basal ration was supplemented with alpha-tocopherol at the rate of 150 p.p.m. on a dry basis.

Four weeks later, when sudden deaths were frequent among the tocopherol-depleted animals, lots B, C and D were established with eight animals per lot. The basal rations for lots B and C were supplemented with alpha-tocopherol at the levels of 50 and 25 p.p.m. respectively. The basal ration for lot D was supplemented with selenium, as sodium selenite, at the level of 1 p.p.m.

Eight other kits of the same age but which had been adapted to a semipurified type ration on another experiment were acquired late in July. These kits were placed on the tocopherol depletion diet along with the remaining unallotted tocopherol-deficient kits from the original forty animals.

These unallotted animals were used as replacement animals.

All animals were observed daily and at the time of weighing each week, all were examined for indications of urinary incontinence. Biweekly blood samples of 10-12 ml. were taken via cardiac puncture from one-half of the animals in each lot, thereby permitting biweekly analyses but subjecting each animal to the bleeding stress only once monthly. The differential counts, total white counts, packed cell volumes and hemoglobin values were obtained from the whole blood samples according to the procedures described by Coffin (1953). Near the termination of the experiment, a few preliminary red blood cell fragility determinations were made in saline according to the procedure of Dacie *et al.* (1938).

The following determinations were made on the blood serum fraction: total serum tocopherol according to a method of Quaife *et al.* (1949) modified to a macro-technique, serum glutamic-oxalacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT) according to the methods of Reitman and Frankel (1957) as outlined in Sigma Chemical Company's 1959 Technical Bulletin No. 505, and paper electrophoresis analyses of serum proteins on a Spinco Model R paper electrophoresis system at room temperature.

Biweekly 24-hour urine samples were obtained and analyzed for creatine, creatinine and specific gravity according to the procedures of Coffin (1953). Surface tension determinations were also made on the urine samples using a du Nouy tensiometer.

Animals that died or were sacrificed were examined via standard necropsy procedures. Sections from the following organs were preserved in acetate-buffered ten per cent formalin for histological examination: internal intercostal, adductor magnus and cardiac muscles, trachea, lung, parotid salivary gland, small intestine, pancreas, liver, kidney, adrenal gland, ureter, urinary bladder, urethra and any other tissue considered important at the time of necropsy. Testicular tissue was preserved in Bouin's fluid. Appropriate sections from each of the tissues were stained with hematoxylin and eosin for general characteristics and with Von Kossa's stain for demonstrating calcium deposition within the tissue. The histological procedures followed were according to the Armed Forces Institute of Pathology Manual of Histologic and Special Staining Technics.

Experiment II

Thirty nine-weeks-old brown male mink kits were purchased from the J. S. Dyer Mink Ranch, Delta River Rd., Lansing, Michigan, on June 27, 1961, and were placed on the semipurified tocopherol-deficient ration shown in Table 3, page 33. The ration was prepared as described for Experiment I. Three weeks later, on July 18, 1961, when the mink had become fully accustomed to the ration, the mink were assigned to the experimental lots as indicated in Table 4, also on page 33.

The biweekly hematological and serological analyses completed in this experiment were the same as those completed

Table 3.--Composition of the tocopherol-deficient diet.

Ingredient	Per Cent
Vitamin free casein	8.0
Isolated soy assay protein	16.0
Torula yeast*	20.0
Sucrose	26.5
Molecularly distilled lard*	20.0
Solka-Floc*	5.0
Phillips and Hart Salt Mix (IV)*	4.0
Amino acid and vitamin supplement*	0.5

*As previously described in Experiment I.

Table 4.--Assignment to experimental lots.

Lot	Number of Mink Per Lot	Ration
A	10	Basal tocopherol deficient
B	8	Basal plus eight per cent cod liver oil to replace an equal amount of lard.
C	8	Basal plus .1 p.p.m. selenium as sodium selenite.
D	4	Basal plus 25 p.p.m. alpha-tocopherol.

in Experiment I. Red blood cell fragility determinations were made during the entire course of this experiment according to the method described by Dacie (1953) but modified to include a 48-hour refrigeration period. This was found necessary in Experiment I to demonstrate the increased fragility as a layering hemolysis (LH) of the erythrocytes from the tocopherol-deficient mink.

The onset of LH was compared chronologically with the onset of alterations in other hematological and serological determination values. Dialuric acid hemolysis determinations

by the method of Friedman *et al.* (1958) were also conducted routinely on the erythrocytes from the tocopherol-deficient and supplemented animals during the experiment.

Near the termination of this experiment, the ability of the various lots of mink to produce antibody in response to injected antigen was studied. Four mink from each group were given intraperitoneal injections of *Salmonella pullorum* antigen daily for six days. The amount of antigen injected was determined by the formula

$$\frac{\text{Lbs. body wt. x per cent blood volume}}{10 \times \text{dilution of the antigen}} = \text{Amt. of antigen.}$$

The blood volume was estimated at seven per cent and the antigen was diluted 1/80 with sterile saline. After the last injection of antigen, all animals in the study were bled to obtain post-antigen-injection serum samples. The agglutination titers of these samples, together with those of the pre-antigen-injection serum obtained on the last routine bleeding before this study was started were determined via the method of Stafseth *et al.* (1959).

Also, at the termination of the experiment, the ability of an orally administered commercial antioxidant, ethoxyquin (*Santoquin*)*, to eliminate the LH of erythrocytes from tocopherol-deficient mink was studied. The results of this study were compared to those obtained from a study of the rapidity with which oral alpha-tocopherol was able to eliminate LH in previously tocopherol-deficient mink. The animals for this experiment, unlike the first experiment which

*Monsanto Chemical Company, St. Louis, Missouri.

was conducted at the Michigan State Fur Project area, had to be maintained at the Dyer Mink Ranch. Facilities were not available there for collecting urine samples; therefore, the urinalyses conducted during Experiment I were not repeated.

Both formalin and Zenker's fixatives were used as general fixatives for tissues from this experiment and sections of liver were fixed in Carnoy's fixative to be later stained with Best's carmine for glycogen. The same tissues taken at necropsy in Experiment I were also taken in Experiment II. Both the formalin and Zenker's fixed tissues were stained with hematoxylin and eosin for general characteristics. Appropriate sections were stained with Best's carmine, Von Kossa, periodic acid-Schiff, Sudan IV and Ziehl-Neelsen stains.

Results

Experiment I

Growth

The data on weight gains for the tocopherol-deficient, tocopherol-supplemented and tocopherol-deficient-selenium-supplemented mink for the course of the experiment are given in Table 5. The surviving tocopherol-deficient mink at 102 days of age had grown as rapidly as the tocopherol-supplemented animals fed 150 p.p.m. alpha-tocopherol. Even following tocopherol supplementation in lots B and C and selenium supplementation of lot D, no significant growth differences were observed by 147 days of age.

Table 5.--Average weights in grams of tocopherol-deficient mink of different ages and following their tocopherol and selenium supplementation.

Lot	Treatment	Days of Age		
		47	102	147
A	150 p.p.m. tocopherol	275 (0)	984 (34)	1193 (79)
B	50 p.p.m. tocopherol	285 (0)	1015 (0)	1136 (45)
C	25 p.p.m. tocopherol	294 (0)	968 (0)	1258 (45)
D	1 p.p.m. selenium	285 (0)	1010 (0)	1170 (0) 45*

Values in ( ) are number of days of tocopherol supplementation.

*value indicates number of days of selenium supplementation at 1 p.p.m.

Mortality

Twenty animals died during the experiment and the circumstances pertaining to their deaths are presented in Table 6.

Of the animals that died following a stress factor, three had grossly visible myopathy. Of the fifteen tocopherol-deficient animals to succumb during the experiment, five died within a three-day period after approximately 70 days on the tocopherol-deficient regimen. No animals died due to a tocopherol deficiency after their tocopherol-deficient rations were supplemented with as little as 25 p.p.m. of alpha-tocopherol or with 1 p.p.m. of selenium as sodium selenite.

Table 6.--Probable causes and number of deaths among tocopherol-deficient and supplemented mink receiving semipurified type rations.

Probable cause of death	Number of deaths for each status	
	Deficient	Supplemented
Stress of bleeding procedure	3	-
Stress of metabolism cage	4	-
Extensive myopathy	2	-
Urinary calculi	1	-
Cardiac tamponade	2	2
Undetermined	3	3

Hematology

Total white blood cell counts, packed cell volumes and hemoglobin determinations were not significantly different among the experimental groups during the experiment. Values for each determination, however, increased gradually during the course of the experiment as had been observed in a previous mink experiment utilizing similar rations. Maximum values were reached when the mink were between 116 and 137 days of age. The data are recorded in Tables 7, 8, and 9, pages 38, 39, and 40.

Table 7.--Mean leukocyte counts/c.m.m. for tocopherol deficient mink and following their supplementation with alpha-tocopherol at 150 p.p.m. (A), 50 p.p.m. (B), 25 p.p.m. (C), and selenium at 1 p.p.m. (D).

Lot	Days of Age										
	60	74	86	88	102	110	116	137	153	174	Av.
A	3700 ±410	4650 ±670	*	3750 ±730	6700 ±1580		5062 ±1800	6700 ±240	4387 ±30	6775 ±1000	5215
B	3433 ±670	4075 ±990		4112 ±870	6162 ±360	*	6375 ±1040	5217 ±880	6737 ±1410	4450 -	5070
C	3725 ±480	3700 ±780		3300 ±630	5025 ±740	*	6466 ±370	6867 ±640	6900 ±1050	5066 ±170	5131
D	4887 ±350	4275 ±790		3387 ±680	6412 ±2180	*	8525 ±990	7625 ±450	6525 ±570	6162 ±990	5974

*Start of respective supplementation for each lot.
±Standard error.

Table 8.--Mean packed cell volumes (per cent) for tocopherol-deficient mink and following their supplementation with alpha-tocopherol at 150 p.p.m. (A), 50 p.p.m. (B), 25 p.p.m. (C) and selenium at 1 p.p.m. (D).

Lot	Days of Age										
	60	74	86	88	102	110	116	137	153	174	Av.
A	37.1 ±1.0	40.1 ±0.8	*	40.9 ±1.7	50.6 ±0.7		55.7 ±0.2	51.2 ±0.2	53.5 ±0.6	55.8 ±1.4	48.1
B	36.5 ±1.7	40.4 ±1.2		42.7 ±3.7	47.5 ±1.9	*	52.6 ±1.0	57.2 ±1.9	54.1 ±1.7	56.5 -	48.4
C	36.7 ±1.4	41.8 ±1.2		40.2 ±2.3	47.7 ±1.6	*	53.9 ±2.3	50.9 ±0.8	55.1 ±0.7	51.6 ±1.2	47.2
D	38.7 ±0.8	40.8 ±1.3		44.0 ±0.9	48.3 ±0.9	*	51.4 ±1.5	52.0 ±0.2	53.5 ±0.9	52.5 ±1.0	47.6

*Start of respective supplementation for each lot.
‡Standard error.

Table 9.--Mean hemoglobin values (gm/100 ml. blood) for tocopherol deficient mink and following their supplementation with alpha-tocopherol at 150 p.p.m. (A), 50 p.p.m. (B), 25 p.p.m. (C), and selenium at 1 p.p.m. (D).

Lot	Days of Age										
	60	74	80	88	102	110	116	137	153	174	Av.
A	10.9 ±0.4	12.5 ±0.8	*	13.5 ±0.7	18.1 ±0.4		19.3 ±0.3	17.7 ±0.4	18.4 ±0.3	18.1 ±0.3	16.0
B	10.5 ±0.4	12.3 ±0.3		14.7 ±0.8	17.2 ±0.8	*	18.3 ±0.3	19.1 ±0.7	18.3 ±0.8	19.3 -	16.3
C	11.0 ±0.4	11.9 ±1.1		13.3 ±2.2	16.2 ±1.6	*	19.2 ±1.6	17.3 ±0.6	18.6 ±0.5	17.3 ±0.8	15.8
D	11.6 ±0.8	12.2 ±1.3		14.4 ±0.9	17.6 ±0.9	*	18.2 ±1.5	17.1 ±0.2	18.6 ±0.8	17.5 ±1.1	15.9

*Start of respective supplementation for each lot.
±Standard error.

Data relative to the differential counts during the experiment are summarized in Table 10, page 42. The pre- and post-tocopherol and selenium supplementation values show no characteristics indicative of a differential response to these supplements. The data for all lots do indicate a shift in the lymphocyte/neutrophil ratios from 49:47 at 60 days of age to 34:63 at 153 days of age.

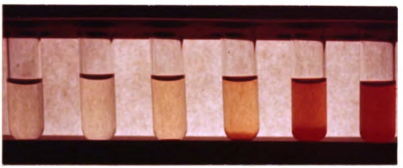
In the preliminary study of erythrocyte fragility, after the recommended 24-hour refrigeration period, no differences were observed between the sets of tubes with erythrocytes from the tocopherol-supplemented animals and the sets of tubes with erythrocytes from the tocopherol-deficient animals. On occasion, however, sets of these fragility tubes were observed following a 48-hour refrigeration period. Little change had taken place in the set of control tubes (see Figure 1, page 43) containing the tocopherol-supplemented erythrocytes. Intact erythrocytes were still visible at the bottoms of the tubes of nearly isotonic saline while hemolysis occurred gradually in the more hypotonic saline.

In tubes of equally hypotonic saline containing erythrocytes from the tocopherol-deficient mink, lysis had taken place and the lysed fraction remained concentrated (layered) at the bottoms of the tubes as illustrated in Figure 2 (see page 43). It was noted that the lowest level of alpha-tocopherol supplementation (25 p.p.m.) was adequate to protect the erythrocytes from this type of hemolysis and that 1 p.p.m. of selenium, as sodium selenite, did not protect the erythrocytes from this type of hemolysis.

Table 10.--Summary of differential leukocyte counts from tocopherol-deficient mink and following their supplementation with alpha-tocopherol at 150 p.p.m. (A), 50 p.p.m. (B), 25 p.p.m. (C), and selenium at 1 p.p.m. (D)

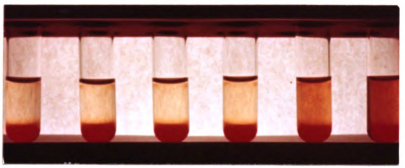
Lot	Leukocyte	Days of Age										
		60	74	86	88	102	110	116	137	153	174	Av.
	Neutrophils											
	Segmented	37.0	50.0	*	58.0	51.0		48.0	48.0	60.0	42.0	49.0
	Nonsegmented	4.0	5.0		2.0	5.0		6.0	2.0	2.0	4.0	4.0
A	Lymphocytes	56.0	40.0		35.0	39.0		44.0	47.0	36.0	52.0	44.0
	Monocytes	3.0	3.0		1.0	.5		.75	0	.25	.25	1.0
	Eosinophils	.25	2.0		4.0	3.0		.75	2.0	1.0	2.0	2.0
	Basophils	.25	.5		0	0		2.0	.25	.25	.25	.3
	Neutrophils											
	Segmented	48.0	49.0		56.0	55.0	*	58.0	59.0	60.0	42.0	53.0
	Nonsegmented	3.0	4.0		2.0	5.0		7.0	3.0	3.0	3.0	4.0
B	Lymphocytes	44.0	42.0		36.0	38.0		33.0	36.0	35.0	53.0	40.0
	Monocytes	4.0	3.0		1.0	.5		0	0	.5	0	1.0
	Eosinophils	1.0	2.0		5.0	2.0		1.0	0	.75	2.0	2.0
	Basophils	0	.25		0	0		.5	.5	0	0	.2
	Neutrophils											
	Segmented	45.0	45.0		65.0	52.0	*	52.0	52.0	53.0	61.0	53.0
	Nonsegmented	2.0	5.0		1.0	7.0		9.0	2.0	6.0	5.0	5.0
C	Lymphocytes	51.0	44.0		31.0	38.0		37.0	41.0	38.0	34.0	39.0
	Monocytes	2.0	4.0		.75	.25		1.0	0	.33	0	1.0
	Eosinophils	.25	2.0		1.0	2.0		1.0	3.0	3.0	1.0	2.0
	Basophils	.25	.25		0	0		.33	2.0	0	0	.3
	Neutrophils											
	Segmented	43.0	51.0		59.0	65.0	*	49.0	59.0	67.0	54.0	56.0
	Nonsegmented	5.0	4.0		2.0	6.0		8.0	7.0	3.0	5.0	5.0
D	Lymphocytes	48.0	42.0		36.0	27.0		41.0	32.0	29.0	39.0	37.0
	Monocytes	4.0	2.0		1.0	.25		.25	.25	.25	.25	1.0
	Eosinophils	.5	.5		1.0	1.0		1.0	1.0	.5	1.0	1.0
	Basophils	0	.75		0	0		.25	.25	.25	0	.2

*Start of supplementation for the respective lots.



0.70 0.65 0.60 0.55 0.50 0.45
Per Cent Saline

Figure 1.--Tocopherol-supplemented mink erythrocyte fragility test following 48-hour refrigeration period.



0.70 0.65 0.60 0.55 0.50 0.45
Per Cent Saline

Figure 2.--Tocopherol-deficient mink erythrocyte fragility test following 48-hour refrigeration period. Note lysed fraction concentrated (layered) at bottom of tubes.

Serology

The pre- and post-tocopherol and selenium supplementation data from the electrophoretic studies are presented in Table 11, page 45, and reveal no consistent pattern alterations associated with either supplement. However, as in the hematology data, trends associated with the age of mink were noted. Combining the data from all the lots during the course of the experiment, the per cent albumin increased from 48.6 to 60.1, the per cent alpha globulin decreased from 20.3 to 11.4, the per cent beta globulin decreased from 22.8 to 14.4 and the per cent gamma globulin increased from 8.2 to 14.3.

The alterations in the average serum values for glutamic-oxalacetic and glutamic-pyruvic transaminase before and following tocopherol or selenium supplementation are given in Table 12, page 47. Considered of significance in the SGOT data are the elevated values for lots B, C and D on day 116. Sera for these determinations were obtained only six days after the start of supplementation for the respective lots. Insufficient time had elapsed to correct the probable myopathy and thereby lower the SGOT values as was accomplished by the succeeding bleeding.

The total serum tocopherol values for the experimental animals are presented in Table 13, page 48. An immediate response followed the tocopherol supplementation of lot A while a more moderate response followed the supplementation of lots B and C. Values of less than 50 micrograms/100 ml. of serum were observed during the time the sudden deaths among the tocopherol-deficient animals were most frequent.

Table 11.--Average per cents for albumin and globulin fractions of serum from tocopherol-deficient mink and following their supplementation with alpha-tocopherol at 150 p.p.m. (A), 50 p.p.m. (B), 25 p.p.m. (C) and selenium at 1 p.p.m. (D).

Serum Fraction	Lot	Days of Age										
		60	74	86	88	102	110	116	137	153	174	Av.
Albumin	A	46.7	51.2	*	54.3	57.1		62.6	60.3	61.5	60.8	56.8
		± 3.8	3.0		4.4	1.4		2.7	2.2	3.5	4.0	
	B	40.9	53.0		51.2	51.8	*	62.9	51.3	60.8	59.9	54.0
		± 1.0	2.0		4.4	4.0		1.8	5.4	4.4	-	
	C	54.9	52.7		55.2	51.0	*	61.4	58.5	61.3	61.5	57.0
		± 1.0	1.4		1.0	5.3		2.1	2.2	1.3	2.0	
	D	52.1	55.5		52.6	50.0	*	55.0	58.5	54.0	58.0	54.5
		± 1.2	2.0		3.6	2.6		4.6	2.0	6.2	3.1	
Alpha Globulin	A	20.6	20.6	*	16.0	15.8		12.5	14.0	12.6	11.5	15.4
		± 1.3	2.3		2.3	1.0		.7	.6	1.1	1.4	
	B	18.8	19.5		14.4	19.7	*	14.1	19.2	15.9	11.7	16.7
		± 1.0	0.8		1.3	1.4		0.4	3.0	1.7	-	
	C	21.0	18.1		11.1	18.2	*	12.4	14.2	11.1	11.4	14.7
		± 1.1	0.9		0.8	2.0		1.0	0.2	0.9	0.3	
	D	21.0	18.8		13.3	18.0	*	13.1	15.2	10.8	11.2	15.2
		± 0.9	0.6		1.8	0.7		0.8	0.8	1.5	0.5	
Beta Globulin	A	25.5	19.7	*	18.6	17.4		13.2	14.2	12.5	12.6	16.8
		± 2.1	0.9		0.5	0.2		0.8	0.5	1.2	1.4	
	B	30.0	17.8		27.5	16.7	*	14.1	12.6	14.5	13.2	18.3
		± 2.1	1.8		6.0	2.3		0.8	1.1	1.5	-	
	C	16.4	20.3		18.3	21.0	*	15.7	14.6	14.8	13.6	16.7
		± 0.7	1.0		1.6	2.4		2.4	0.5	2.0	0.5	
	D	19.2	18.4		24.8	21.1	*	20.8	13.8	25.2	18.4	20.1
		± 1.3	1.0		4.4	3.1		5.9	0.5	2.2	3.0	

Table 11.--(Continued)

Serum Fraction	Lot	Days of Age										
		60	74	86	88	102	110	116	137	153	174	Av.
Gamma Globulin	A	7.0	8.5	*	11.1	9.9		11.7	11.0	12.5	14.6	10.8
	±	0.7	1.1		1.1	0.6		1.5	1.8	1.1	2.5	
	B	10.2	9.5		6.8	11.8	*	8.9	12.7	8.7	15.2	10.5
	±	1.1	1.2		1.0	0.6		1.2	1.2	1.3	-	
	C	7.8	8.9		11.7	10.7	*	10.4	12.7	12.2	13.4	11.0
	±	0.3	0.7		0.8	1.6		1.0	0.3	0.5	0.9	
	D	7.8	7.2		9.7	10.8	*	11.1	13.1	9.6	14.1	10.4
	±	0.9	1.1		2.0	0.5		1.4	0.8	1.5	1.1	

*Start of respective supplementation for each lot.
±Standard error.

Table 12.--Average serum glutamic-oxalacetic and glutamic-pyruvic transaminase values (Sigma Frankel Units) for tocopherol-deficient mink and following their supplementation with alpha-tocopherol at 150 p.p.m. (A), 50 p.p.m. (B), 25 p.p.m. (C) and selenium at 1 p.p.m. (D).

Serum Transaminase	Lot	Days of Age									Av.
		60	74	86	88	102	110	116	137	153	
Glutamic-Oxalacetic	A	122	144	*	116	150		156	103	131	131
	±	17	19		7	5		15	14	17	
	B	149	218		195	134	*	181	149	97	160
	±	15	7		31	11		13	33	9	
	C	130	193		113	144	*	210	99	122	144
	±	11	48		17	17		36	21	23	
	D	117	105		119	133	*	314	108	194	156
	±	23	14		22	16		90	10	26	
Glutamic-Pyruvic	A	43	93	*	84	78		79	67	90	76
	±	13	2		13	7		8	9	22	
	B	45	81		128	94	*	101	110	62	89
	±	14	10		19	10		7	19	5	
	C	37	94		100	91	*	112	64	90	84
	±	9	18		16	7		27	10	30	
	D	51	69		55	96	*	109	77	82	77
	±	9	11		14	12		16	5	3	

*Start of supplementation of respective lots.
±Standard error.

Table 13.--Mean serum tocopherol values (micrograms/100 ml. serum) for tocopherol-deficient mink and following their supplementation with alpha-tocopherol at 150 p.p.m. (A), 50 p.p.m. (B), 25 p.p.m. (C) and selenium at 1 p.p.m. (D).

Lot	Days of Age									
	60	74	86	88	102	110	116	137	153	174
A	40	57	*	617	703		532	368	289	444
	± 7	8		162	90		74	36	76	78
B	51	41		31	25	*	86	47	145	132
	± 15	1		7	7		22	23	22	-
C	69	51		43	49	*	58	47	96	77
	± 15	15		7	17		21	19	16	6
D	48	58		51	38	*	18	17	19	16
	± -	6		23	3		-	8	5	2

*Start of supplementation for the respective lots.
±Standard error.

Urinalyses

Data from the five 24-hour urine collections made during the course of the experiment are presented in Tables 14 and 15, pages 49 and 50. The absence of consistent trends for a given determination, immediately following tocopherol or selenium supplementation, is evidence that significant differences among these data are not present.

During the experiment, twenty different animals were observed to have the form of urinary incontinence known to the mink rancher as "wet belly." The incidence of transient and chronically incontinent mink for the respective lots is given in Table 16, page 51. It was in conjunction with these observations that the surface tension measurements reported in Table 15 were made. Little evidence was found for an association between tocopherol deficiency and urinary

Table 14.--Average urinary creatine and creatinine excretion values for tocopherol deficient mink and following their supplementation with alpha-tocopherol at 150 p.p.m. (A), 50 p.p.m. (B), 25 p.p.m. (C) and selenium at 1 p.p.m. (D).

Determination	Lot	Days of Age							Av.
		67	81	86	95	109	110	143	
Creatine(1)	A	0.69	0.61	*	0.42	0.41		0.26	0.48
		±0.02	0.08		0.02	0.04		0.08	
	B	0.78	0.54		0.35	0.63	*	0.37	0.53
		±0.14	0.03		0.02	0.28		0.04	
	C	0.96	0.84		0.50	0.45	*	0.23	0.60
		±0.11	0.22		0.06	0.08		0.12	
	D	0.96	0.31		0.62	0.48	*	0.57	0.59
		± .10	.03		.16	.12		.01	
Creatinine(1)	A	1.40	1.54	*	1.47	1.35		1.41	1.43
		±0.16	0.15		0.14	0.16		0.28	
	B	1.61	1.63		1.55	1.35	*	1.51	1.53
		±0.06	0.26		0.17	0.12		0.26	
	C	1.59	2.29		1.75	1.61	*	2.65	1.98
		±0.12	0.29		0.13	0.25		1.1	
	D	1.86	1.44		2.12	1.53	*	1.76	1.74
		±0.12	0.12		0.14	0.18		0.10	

*Start of supplementation of respective lots.
(1)Mg./100 gm. body weight/24 hours.
±Standard error.

Table 15.--Average specific gravity and surface tension values for urine from tocopherol-deficient mink and following their supplementation with alpha-tocopherol at 150 p.p.m. (A), 50 p.p.m. (B), 25 p.p.m. (C) and selenium at 1 p.p.m. (D).

Determination	Lot	Days of Age							Av.
		67	81	86	95	109	110	143	
Specific Gravity (gm./ml.)	A	1.061	1.040	*	1.029	1.035		1.044	1.042
		± .003	.006		.004	.005		.006	
	B	1.052	1.036		1.028	1.035	*	1.047	1.040
		± .005	.002		.004	.006		.009	
	C	1.056	1.042		1.032	1.044	*	1.038	1.042
		± .002	.008		.009	.007		.011	
	D	1.058	1.042		1.049	1.046	*	1.051	1.049
		± .003	.004		.007	.003		.005	
Surface Tension (dynes/cm.)	A	51.6	45.2	*	39.8	45.2		46.8	45.7
		± 0.6	2.8		1.1	0.7		1.2	
	B	51.2	42.9		39.2	46.5	*	43.9	44.7
		± 1.0	1.4		1.0	1.3		1.8	
	C	48.2	43.4		47.3	45.3	*	49.9	46.8
		± 0.6	0.6		0.6	1.6		2.7	
	D	49.9	47.7		47.0	46.8	*	49.8	48.2
		± 1.0	1.4		3.1	0.7		0.9	

*Start of supplementation of respective lots.
±Standard error.

Table 16.--Incidence of transient and chronic urinary incontinence in mink fed purified type rations supplemented with alpha-tocopherol at 150 p.p.m. (A), 50 p.p.m. (B), 25 p.p.m. (C) and selenium at 1 p.p.m. (D).

Lot	Duration of Respective Supplement	Number of Incontinent Mink	
		Transient	Chronic
A	79 days	5	1
B	45 "	4	1
C	45 "	3	1
D	45 "	5	2

incontinence in mink. Although the experimental average for the urine surface tension in lot D was 2.5 dynes greater than the average for lot A, no consistent alterations in surface tension followed the tocopherol supplementation of lots B or C or the selenium supplementation of lot D.

Symptomatology

Three tocopherol-deficient mink that died with gross evidence of myopathy showed ante-mortem symptoms. One had lost weight during the last weigh period, one had been anorexic for 24 hours prior to death and the third had a partial posterior paralysis. Tocopherol-deficient mink without gross evidence of myopathy at necropsy manifested no unusual physical symptoms.

Gross Pathology

Skeletal myopathy and what appeared to be a perilobular fatty infiltration of the liver were observed with equal regularity in the tocopherol-deficient mink at necropsy. The myopathy involved the internal intercostal muscles (Figure 3, page 55), the insertion end of the adductor

muscles (Figure 4, page 56) and the diaphragm. Although the myopathy was usually bilateral, some unilateral lesions were observed in the intercostal muscles.

Gross cardiac myopathy was observed in two tocopherol-deficient mink. One case was observed on the endocardial surface of the right ventricle (Figure 10, page 61) and the second case was visible on the epicardial surface of the right ventricle near the apex.

Histopathology

Skeletal muscle.--Three phases of skeletal myopathy were observed in the tocopherol-deficient mink. The first phase was characterized by swollen, differentially stained muscle fibers, vacuolar degeneration (Figure 5, page 57) and/or an increased number of irregularly spaced sarcolemmal nuclei (Figure 6, page 57). In the second phase, illustrated in Figure 7, page 58, the previous features were present along with an extensive nuclear element composed primarily of sarcolemmal nuclei and myoblasts. Lymphocytes and myophages were often present. The third or reparative phase was characterized by focal calcium deposits amid normal muscle fibers (Figure 8, page 59). Occasionally the myophagic reaction and calcification of individual myofibrils shown in Figure 9 (page 60) were observed.

While gross evidence of myopathy was more frequent in the internal intercostal muscles, microscopic lesions were observed with greater frequency in the adductor muscles. The increase in sarcolemmal nuclei was more characteristic

of myopathy of the adductors than of the internal intercostals or diaphragm. Internal nuclear rowing was not a prominent feature of skeletal myopathy in mink.

Cardiac muscle.--Early cardiac myopathy was distinguished by a relative increase in the number of internal nuclei which showed a tendency to row. Advanced cardiac myopathy (Figures 11 and 12, pages 61 and 62) was characterized by calcified necrotic foci immediately adjacent to normal cardiac muscle. No intermediate stages of cardiac myopathy were observed.

Liver.--The perilobular infiltration of fat was confirmed upon histopathological examination. In addition, there was centrolobular congestion and the veins of the portal triads were unusually large. Tocopherol-deficient-selenium-supplemented mink sacrificed at the end of the experiment had a lymphocytic infiltration around the portal triads.

Kidney.--Coagulation necrosis of the proximal and distal convoluted tubules was observed in mink with extensive myopathy. Tocopherol-deficient mink with early myopathy had hemorrhagic peritubular foci in the renal cortex and medulla. Hemosiderin was evident in the cytoplasm of the proximal and distal convoluted tubule cells. A rather homogenous sero-sanguinous fluid was present in the intertubular spaces of the kidneys of sacrificed tocopherol-deficient mink and around Bowman's capsules of the sacrificed tocopherol-deficient-selenium-supplemented mink. Bowman's capsules were often distended with a proteinaceous material which appeared to

have produced some glomerular atrophy. There was evidence of hyperplasia of the juxta-glomerular apparatus cells of one mink that had an early myopathy.

Adrenal gland.--A basophilic reaction of the acinar cells of the medulla, a sero-sanguinous exudate at the cortico-medullary junction and focal necrosis with calcification of the cortex (Figure 13, page 63) were observed and believed to have resulted from tocopherol deficiency.

Adipose tissue.--Mink that survived a prolonged tocopherol deficiency had a form of steatitis illustrated in Figure 14 (page 64). Tiny non-acid-fast droplets were present in the interstitial spaces of the adipose tissue.

Lungs.--A pneumonitis (Figure 15, page 64) was present in all the experimental animals but was more extensive in the tocopherol-deficient mink. This pneumonitis was accompanied by atelectasis, emphysema and congestion of the alveolar capillaries.

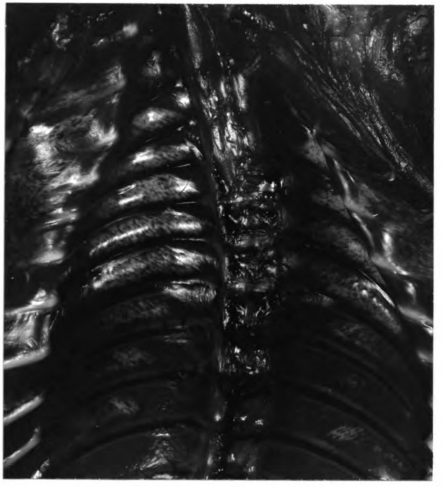


Figure 3.--Bilateral internal intercostal myopathy in a tocopherol-deficient mink.

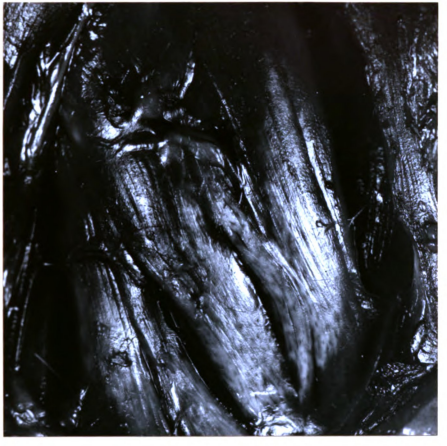


Figure 4.--Necrosis near the insertion end of an adductor from a tocopherol-deficient mink.

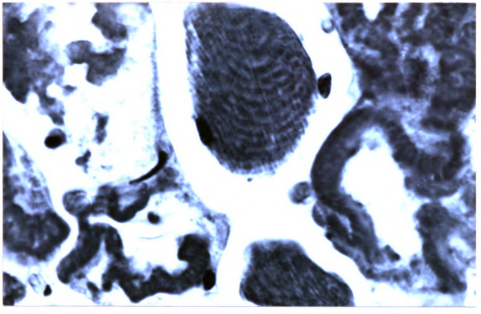


Figure 5.--Vacuolar degeneration of internal intercostal muscle fibers from a tocopherol-deficient mink. (x660)

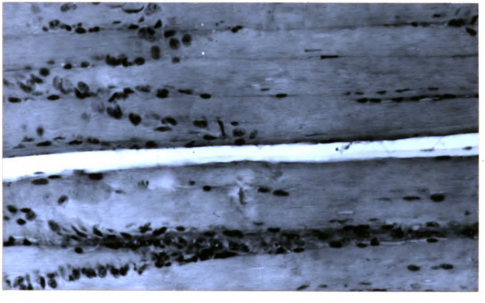


Figure 6.--Sarcolemmal proliferation in an adductor from a tocopherol-deficient mink. (x300)

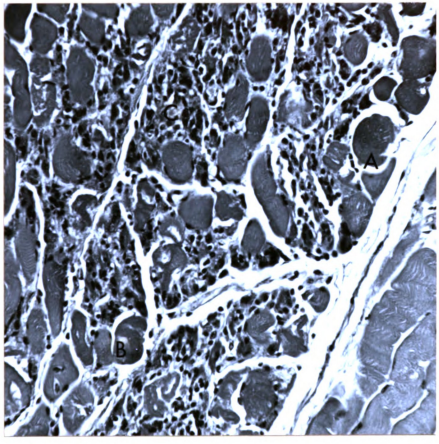


Figure 7.--Adductor from a tocopherol-deficient mink; A. Swollen, differentially stained fibers; B. Vacuolar degeneration; and C. Proliferating sarcolemmal cells and myoblasts. (x350)

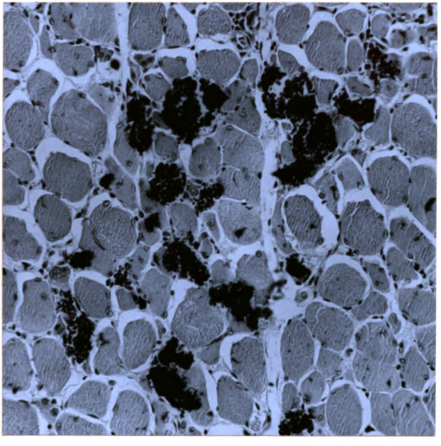


Figure 8.--Calcified foci (black) in an adductor from a tocopherol-deficient mink. (Von Kossa x350)

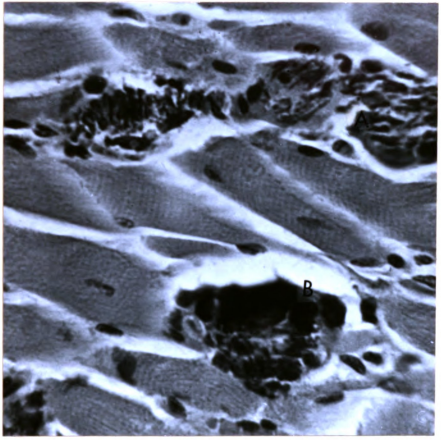


Figure 9.--Internal intercostal muscle from a tocopherol-deficient mink; A. Early myofibrillar calcification; and B. Myophagia of calcifying muscle fiber. (x540)

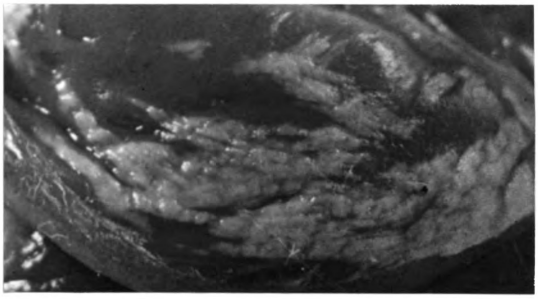


Figure 10.--Calcified necrotic right-ventricular myocarditis of a tocopherol-deficient mink. Endocardial surface is exposed.

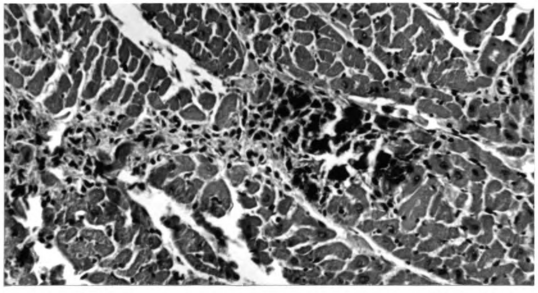


Figure 11.--Focal calcified necrotic myocarditis of a tocopherol-deficient mink. (x320)

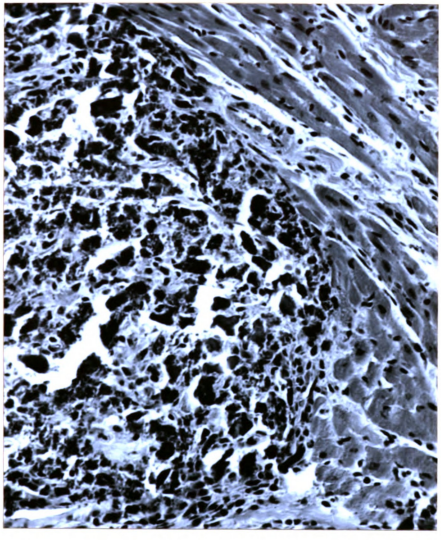


Figure 12.--Extensive calcified necrotic myocarditis of a tocopherol-deficient mink. (x320)

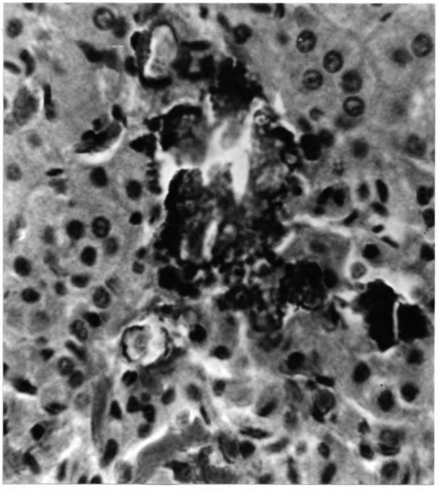


Figure 13.--Calcified necrotic focus in the adrenal cortex of a tocopherol-deficient mink. (x750)

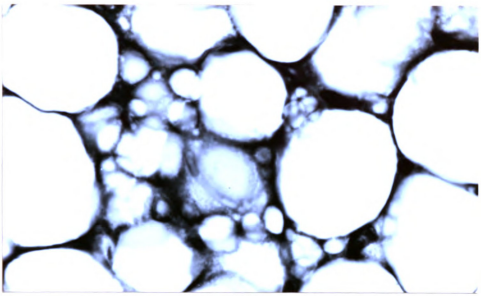


Figure 14.--Steatitis of a mink made chronically tocopherol-deficient without unsaturated fatty acid supplementation. Spheres in the interstices are not acid-fast. (Ziehl-Neelsen x860)

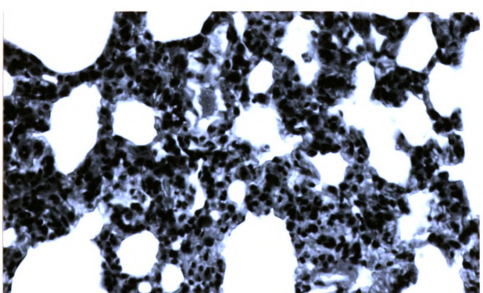


Figure 15.--Pneumonitis characteristic of both tocopherol-deficient and tocopherol-supplemented mink. (x300)

Experiment II

Growth

The data on weight gains of the mink on their respective rations are presented in Table 17.

Table 17.--Mean weights in grams for mink fed purified type tocopherol-deficient ration (A) supplemented with 8% cod liver oil (B), 0.1 p.p.m. selenium (C) and 25 p.p.m. alpha-tocopherol (D).

Days of Age	Lots			
	A	B	C	D
60	484 (10)	467 (8)	423 (8)	503 (4)
67	669 "	692 "	686 "	699 "
74	733 "	744 "	733 "	771 "
81	832 "	869 "	847 "	853 "
89	937 "	981 "	956 "	978 "
96	1003 "	1043 "	932 "	988 "
103	1005 "	1094 (7)	1014 "	1094 "
110	1086 (9)	1174 "	1049 "	1119 "
129	1107 (8)	1106 (6)	1123 "	1063 "
144	1279 (7)	1255 "	1321 "	1228 "
165	1275 (5)	1216 "	1281 "	1256 "

Values in ( ) indicate number of animals per lot.

Among the surviving animals at 165 days of age, no growth differences were observed. A growth lag attributed to the severely hot summer weather occurred in all lots when the mink were between 90 and 110 days of age. (The owner of the ranch indicated feed consumption and growth for all his mink were decreased during this same period.) Slight weight losses in lots A, B and C occurred during the last weigh period. The heaviest surviving mink in lots A, B, C and D weighed 1550, 1440, 1755 and 1435 grams respectively.

Mortality

During the course of the experiment, six animals died

and the circumstances pertaining to their deaths are given in Table 18.

Table 18.--Probable causes and number of deaths among mink fed tocopherol-deficient ration (A) supplemented with 8% cod liver oil (B), 0.1 p.p.m. selenium (C) and 25 p.p.m. alpha-tocopherol (D).

Probable Causes	Number of Deaths in Each Lot			
	A	B	C	D
Stress of bleeding procedure	2 (57)(109)		--	--
Stress of antigen-antibody reaction	2 (96)(98)		--	--
Steatitis		1 (38)	--	--
Cardiac tamponade		1 (77)	--	--

Values in ( ) indicate number of days on the respective rations.

No selenium or alpha-tocopherol supplemented mink died during the experiment.

Hematology

The mean leukocyte counts for all lots are presented in Table 19, page 67. Based upon the total leukocyte counts considered normal from Experiment I, the counts for all lots at 74 days of age were apparently elevated. Lot A, the uncomplicated tocopherol-deficient lot, except at 74 days of age, had higher total leukocyte counts than lots C and D for the duration of the experiment. Lot B, that was fed 8% cod liver oil, had a significant leukocytosis between 102 and 132 days. The packed cell volume and hemoglobin data are summarized in Tables 20 and 21 respectively (see pages 68 and 69). The packed cell volumes for lot B were substantially lower from 102 to 164 days of age than lots

Table 19.--Mean leukocyte counts/cmm for mink fed tocopherol-deficient ration (A) supplemented with 8% cod liver oil (B), 0.1 p.p.m. selenium (C) and 25 p.p.m. alpha-tocopherol (D).

Lot	Days of Age							Av.
	74	88	102	118	132	164	171	
A	8533	7933	9175	6237	8388	5217	8770	7750
	± 520	435	871	685	964	1855	1232	
B	8950	6875	14790	15470	10450	5021	7383	9848
	± 1552	1349	640	3091	360	1122	591	
C	9950	4375	6281	4913	7250	3117	5808	5956
	± 317	510	600	640	425	142	1170	
D	10200	4850	5500	5700	4400	4650	5950	5893
	± -	20	1249	948	1649	1007	1012	

±Standard error.

A, C and D. The hemoglobin values for lot B remained approximately 1 gram lower than the control lot (D) for the duration of the experiment. There was a tendency for packed cell volume and hemoglobin values to increase with the age of mink. These values reached maximum at 164 days of age.

The differential count data presented in Table 22, page 70, indicate that the relative leukocytosis in lot B resulted from an absolute neutrophilia and was accompanied by a decrease in the percentage of lymphocytes. The differential counts for lots A, C and D were not considered altered by the respective treatments. As in Experiment I, the lymphocyte/neutrophil ratio reversed during the experiment with a ratio of 60:37 at 74 days of age changing to 39:55 at 164 days of age.

Table 20.--Mean packed cell volumes (per cent) for mink fed tocopherol-deficient ration (A) supplemented with 8% cod liver oil (B), 0.1 p.p.m. selenium (C) and 25 p.p.m. alpha-tocopherol (D).

Lot	Days of Age							Av.
	74	88	102	118	132	164	171	
A	40.4	46.3	50.1	50.7	53.1	56.0	54.0	50.1
	± 0.7	1.4	0.6	1.1	0.7	3.0	0.3	
B	40.7	44.0	47.3	40.8	46.6	45.3	51.0	45.7
	± 1.4	2.0	1.0	5.5	4.3	2.4	1.2	
C	40.1	42.7	51.3	49.7	52.0	53.2	50.8	48.2
	± 0.3	1.1	1.5	1.5	1.2	1.0	2.4	
D	40.6	45.0	52.4	50.9	54.2	53.1	51.7	48.0
	± -	1.4	2.0	2.5	0.7	0.6	1.0	

‡Standard error.

Table 21.--Mean hemoglobin values (gm./100 ml. blood) for mink fed tocopherol-deficient ration (A) supplemented with 8% cod liver oil (B), 0.1 p.p.m. selenium (C) and 25 p.p.m. alpha-tocopherol (D).

Lot	Days of Age							Av.
	74	88	102	118	132	164	171	
A	13.9	14.9	16.9	16.5	17.9	19.2	17.8	16.7
	± 0.2	0.2	0.3	0.2	0.1	0.8	-	
B	13.8	13.9	15.9	16.2	16.9	17.4	16.8	15.8
	± 0.4	0.7	0.1	0.5	0.6	2.6	0.6	
C	13.6	13.6	17.5	16.9	18.0	18.3	17.6	16.5
	± 0.2	0.2	0.6	0.6	0.3	0.4	0.7	
D	13.5	14.7	17.4	16.7	18.6	18.5	18.1	16.8
	± -	0.3	0.4	0.6	0.1	0.8	0.3	

±Standard error.

Table 22.--Summary of the differential leukocyte counts for mink fed tocopherol-deficient ration (A) supplemented with 8% cod liver oil (B), 0.1 p.p.m. selenium (C) and 25 p.p.m. alpha-tocopherol (D).

Lot	Leukocytes	Days of Age							Av.
		74	88	102	118	132	164	171	
	Neutrophils								
	Segmented	31	51	33	40	44	54	34	51
	Nonsegmented	1	0	0	1	2	3	1	1
A	Lymphocytes	64	47	64	55	49	32	57	53
	Monocytes	2	2	1	0	0	2	1	1
	Eosinophils	2	1	2	4	5	5	6	3
	Basophils	0	0	0	0	0	1	1	0
	Neutrophils								
	Segmented	35	46	51	63	50	52	54	52
	Nonsegmented	0	0	0	4	5	2	2	2
B	Lymphocytes	60	56	45	28	41	39	41	44
	Monocytes	1	0	1	1	0	1	1	1
	Eosinophils	3	0	2	3	3	5	1	2
	Basophils	0	0	0	0	0	1	0	0
	Neutrophils								
	Segmented	42	48	33	41	50	53	54	47
	Nonsegmented	0	1	0	3	4	2	1	2
C	Lymphocytes	53	47	60	49	38	42	43	47
	Monocytes	1	1	2	1	0	1	1	1
	Eosinophils	5	3	5	6	5	1	1	3
	Basophils	0	0	0	0	1	1	0	0
	Neutrophils								
	Segmented	39	46	33	40	40	50	51	43
	Nonsegmented	0	0	0	5	3	3	2	2
D	Lymphocytes	64	53	61	52	55	43	38	52
	Monocytes	2	0	2	1	0	2	1	1
	Eosinophils	0	0	5	2	2	2	2	2
	Basophils	0	0	0	1	1	1	1	1

The 48-hour erythrocyte fragility test for LH was conducted on each blood sample taken. All samples were negative on the first bleeding. By the time the mink in lots A, B and C were 88 days of age, the 48-hour LH occurred as illustrated in Figure 2. All the remaining fragility tests for LH of erythrocytes from these animals were positive for the duration of the experiment. The longer animals in lots A, B and C remained on the experiment, there was an increasing tendency for LH to occur in less than 48 hours.

Layering hemolysis never occurred in the fragility tubes containing erythrocytes from lot D, the control animals; however, erythrocytes from these animals did become more fragile during the experiment. At 74 days of age, hemolysis began in 0.47 per cent saline and was complete in 0.35 per cent saline. At 171 days of age, hemolysis began in 0.67 per cent saline and was complete in 0.37 per cent saline.

Near the end of the experiment, several animals from lots A and C were changed to the tocopherol-supplemented ration and bled every 24 hours for several days thereafter. Within 48 hours after these animals consumed their first tocopherol-supplemented ration, their erythrocytes were protected from the layering hemolysis.

In a similar trial, Santoquin,* a commercial antioxidant, added to the basal tocopherol-deficient ration at a level of

---

*Monsanto Chemical Company, St. Louis, Missouri.

100 p.p.m., was found ineffective in preventing LH even after the mink had had access to the antioxidant-supplemented rations for seven days. The dialuric acid hemolysis procedure, with all the precautions listed by Friedman et al. (1958) proved an unreliable indicator of tocopherol depletion in mink. From the optical density readings from tube 3 of each determination, however, erythrocyte fragility indexes for each group were obtained and are shown in Table 23.

Table 23.--Average indexes of fragility for erythrocytes from mink fed tocopherol-deficient ration (A) supplemented with 8% cod liver oil (B), 0.1 p.p.m. selenium (C) and 25 p.p.m. alpha-tocopherol (D).

Lot	Days of Age				
	74	88	102	118	132
A	86	98*	156	266	335
	± 12	16	33	95	143
B	76	78*	162	364	573
	± 27	6	19	110	72
C	86	81*	125	185	296
	± 1	7	15	98	72
D	65	49	63	56	67
	± 4	1	10	4	2

*Layering hemolysis was present at this bleeding.
±Standard error.

Although the fragility indexes for lots A, B and C on day 88 were little different from those for day 74, LH had already occurred by day 88. From 102 days the fragility index for lot B fed 8% cod liver oil was greater than the index for all other lots. Supplementation of the tocopherol-deficient ration with 0.1 p.p.m. selenium (lot C) was ineffective in preventing the increased fragility index.

Serology

Information obtained from the electrophoretic study of the serum proteins is presented in Table 24 on page 74. Tocopherol deficiency appeared to decrease the serum albumin fraction and increase the alpha and beta globulin fractions. The addition of cod liver oil to the tocopherol-deficient ration (B) accentuated the above changes while selenium supplementation (C) minimized the changes.

Erythrocytes from lot B, the cod liver oil supplemented group, hemolysed so readily that difficulty was encountered in obtaining non-hemolysed serum samples from this group. The presence of free hemoglobin in the sera interfered with evaluating the paper strips for beta and gamma globulin, especially the latter. As a result, the gamma globulin data for lots A and B are inconsistent. On the basis of the average gamma globulin values for lots C and D, however, gamma globulin was not affected by tocopherol deficiency.

The average serum glutamic-oxalacetic (SGOT) and glutamic-pyruvic transaminase (SGPT) data are presented in Table 25, page 75. Significant increases in both SGOT and SGPT values in lots A and B occurred by 118 days of age and persisted for the remainder of the experiment. Both selenium and alpha-tocopherol supplements in lots C and D respectively prevented elevation in the transaminase values for these groups.

The mean serum tocopherol values for each bleeding of the respective groups are shown in Table 26, page 75. At

Table 24.--Mean per cents for albumin and globulin fractions of serum from mink fed tocopherol-deficient ration (A) supplemented with 8% cod liver oil (B), 0.1 p.p.m. selenium (C) and 25 p.p.m. alpha-tocopherol (D).

Serum Fraction	Lot	Days of Age 74	88	102	118	132	164	Av.
Albumin	A	34.0	35.7	42.1	40.1	50.1	46.3	41.6
	±	3.7	2.2	1.6	2.0	0.8	0.7	
	B	37.4	37.6	39.6	45.4	-	-	39.0
	±	4.8	2.2	1.7	-			
	C	42.0	38.4	42.1	52.9	49.1	52.4	45.3
	±	0.5	2.4	1.0	1.0	0.8	2.4	
	D	40.3	43.1	40.5	55.6	53.7	53.3	48.8
	±	-	1.9	3.6	1.3	2.3	0.8	
Alpha Globulin	A	22.8	22.9	18.5	19.8	15.5	15.3	19.2
	±	2.4	2.7	1.1	3.5	1.7	2.8	
	B	21.9	20.7	17.9	25.9	-	-	20.4
	±	1.3	0.9	1.2	-			
	C	22.9	21.3	19.4	14.6	16.1	12.4	18.2
	±	1.0	1.2	0.8	0.7	1.7	0.9	
	D	21.5	24.1	17.4	15.7	14.0	15.9	17.7
	±	-	4.7	0.6	2.5	0.3	1.8	
Beta Globulin	A	24.1	23.5	23.6	24.7	19.2	21.7	22.8
	±	1.9	0.3	0.5	1.7	1.1	1.0	
	B	26.2	26.1	29.6	18.1	-	-	26.7
	±	0.4	1.7	0.9	-			
	C	22.1	21.9	22.7	20.8	19.7	17.1	21.0
	±	1.1	0.8	0.9	0.1	1.8	0.1	
	D	18.3	18.5	24.2	17.2	17.0	14.0	17.8
	±	-	1.1	2.1	0.4	1.3	1.0	
Gamma Globulin	A	18.9	17.8	15.8	15.3	15.2	16.7	16.4
	±	3.1	1.2	1.4	2.0	1.7	0.8	
	B	14.3	15.6	12.6	10.6	-	-	13.8
	±	4.1	1.0	1.7	-			
	C	12.9	18.3	15.7	11.6	15.1	18.1	15.3
	±	2.1	1.8	1.0	0.4	1.4	1.4	
	D	19.9	14.2	17.8	11.4	15.3	16.6	15.6
	±	-	1.7	2.2	1.6	1.2	0.9	

±Standard error.

Table 25.--Mean serum glutamic-oxalacetic and glutamic-pyruvic transaminase values in Sigma Frankel units for mink fed tocopherol-deficient ration (A) supplemented with 8% cod liver oil (B), 0.1 p.p.m. selenium (C) and 25 p.p.m. alpha-tocopherol (D).

Serum Transaminase	Lot	Days of Age						Av.
		74	88	102	118	132	157	
Glutamic-Oxalacetic	A	116	173*	117	349	356	309	237
		± 16	18	4	43	20	9	
	B	124	168*	146	375	401	400	280
		± -	21	26	73	44	-	
	C	108	163*	124	171	160	107	154
		± 26	23	8	20	6	28	
	D	150	120	124	176	142	143	143
		± -	36	4	12	18	30	
Glutamic-Pyruvic	A	63	73*	49	168	226	89	111
		± 6	8	4	80	9	-	
	B	33	66*	53	361	270	239	170
		± -	8	4	116	-	70	
	C	85	79*	59	62	73	44	67
		± 23	15	4	6	-	6	
	D	52	67	48	77	73	42	60
		± -	24	10	24	-	4	

*Layering hemolysis was present.
±Standard error.

Table 26.--Mean serum tocopherol values in micrograms/100 ml. serum from mink fed tocopherol-deficient ration (A) supplemented with 8% cod liver oil (B), 0.1 p.p.m. selenium (C) and 25 p.p.m. alpha-tocopherol (D).

Lot	Days of Age					
	74*	88**	102	118	132	157
A	85	41	35	75	14	--
±	7	12	13	14	6	
B	117	68	35	25	29	--
±	40	13	20	22	5	
C	130	96	27	26	17	9
±	10	12	2	4	4	3
D	164	213	464	536	1420	1221
±	-	19	14	19	230	307

*All animals had been on depletion ration 14 days.
**Animals had been on respective rations for 7 days.
±Standard error.

the first bleeding, when all the animals had been on the same basal tocopherol depletion ration for two weeks, the average was 120 micrograms/100 ml. serum and no layering hemolysis was present. By the succeeding period, when layering hemolysis was present in lots A, B and C, the average tocopherol value for all animals in these lots was 68 micrograms/100 ml. serum. There is little evidence from the serum tocopherol data that the 8% cod liver oil hastened tocopherol depletion under the conditions of the experiment. The tocopherol values in lots A, B and C continued to decline during the experiment while those for lot D, the tocopherol-supplemented group, continued to increase to the unexpectedly high level of 1.4 mg./100 ml. serum.

The pre-and post-Salmonella pullorum antigen injection titers are presented in Table 27, page 77. Little indication was present relative to an association between tocopherol deficiency and the ability of mink to produce antibody in response to Salmonella pullorum antigen.

Pathology

Tocopherol-deficient lot A.--These mink showed no deficiency symptoms prior to death or being sacrificed. Two mink at necropsy had gross myopathy of the internal intercostal muscles as illustrated previously in Figure 3. Histological examination revealed skeletal myopathy in the adductor muscles as well as the intercostal muscles. The myopathy observed was characteristic of phase two described in Experiment I and depicted in Figure 7.

Table 27.--Pre-and post-*Salmonella pullorum* antigen injection titers for mink fed tocopherol-deficient ration (A) supplemented with 8% cod liver oil (B), 0.1 p.p.m. selenium (C) and 25 p.p.m. tocopherol (D).

Lot	Mink No.	Pre-injection Titers			Post-injection Titers				
		1/5	1/10	1/20	1/20	1/40	1/80	1/160	1/320
A	1	-	-	-	+	±	-	-	-
	2	-	-	-	+	+	+	-	-
	3	+	+	-	+	+	±	-	-
	4	Fourth animal died during the study.							
B	5	-	-	-	+*	+	+	±	±
	6	+	-	-	±	+	+	-	-
	7	-	-	-	+*	+	+	±	±
	8	+	-	-	±	+	±	-	-
C	9	-	-	-	+	+	+	±	-
	10	-	-	-	+	+	-	-	-
	11	+	±	-	+	+	±	-	-
	12	+	-	-	+	+	±	±	-
D	13	±	-	-	+	+	±	-	-
	14	±	-	-	+	+	-	-	-
	15	-	-	-	+	±	-	-	-
	16	-	-	-	+	+	±	-	-

+positive agglutination
±questionable agglutination
-no agglutination
*serum from hemolyzed blood sample

Perilobular fatty infiltration plus centrolobular congestion and hemorrhages were the prominent pathological features in the liver. When compared to the normal portal triad (see Figure 16, page 80), the triads for the tocopherol-deficient mink were larger than normal and edematous (Figure 17, page 80).

Glomerular congestion, focal capillary congestion and pyknosis of the proximal and distal convoluted tubules were observed. Hemosiderin was prominent in the cytoplasm of the cells of some convoluted tubules (Figure 18, page 84).

The micro-droplets of non-acid-fast material were again observed in the interstitial spaces of the adipose tissue (see Figure 14, page 64). Varying degrees of the pneumonitis shown in Figure 15 on page 64 were present. Hemorrhagic foci were observed in the adrenal cortex and medulla.

Tocopherol-deficient cod liver oil-supplemented lot B.--Two of these mink had gross skeletal myopathy at necropsy. Histologically, the myopathy was more extensive than that observed in lot A. Many fibers, especially of the adductor muscles, were undergoing calcification in the presence of many sarcolemmal nuclei as in Figure 19 on page 82. On the basis of the classification presented in Experiment I, this myopathy was considered to be early phase three.

The adipose tissue of all mink in lot B was very yellow (Figure 22, page 84). Acid-fast pigment droplets, lymphocytes, and some macrophages were observed in the interstitial spaces as in Figure 23 on page 84.

Calcified foci were present in the cytoplasm of necrotic cells of proximal and distal convoluted tubules (see Figures 24 and 25 on page 85). The hepatic, adrenal and pulmonary lesions were present as described in lot A.

Tocopherol-deficient selenium-supplemented lot C.--While selenium supplementation of the tocopherol-deficient mink prevented the sudden deaths and gross myopathy observed in lots A and B, microscopic tocopherol-deficiency lesions were not completely prevented. Sacrificed mink from lot C had an early adductor myopathy characterized by an increase in the number of sarcolemmal nuclei as in Figure 6 on page 57.

The adipose tissue, when compared with the normal (Figure 20, page 83), contained interstitial evidence of a very early steatitis (Figure 21, page 83). Hepatic and renal congestion were noted. Lymphocytic foci were observed in the zona fasciculata and some degree of pneumonitis was present.

Tocopherol-supplemented lot D.--Twenty-five p.p.m. of alpha-tocopherol prevented all the symptomatic, clinical, gross and microscopic pathology associated with tocopherol deficiency in mink. One of the control mink, however, had a slight pneumonitis as observed in the previous lots.

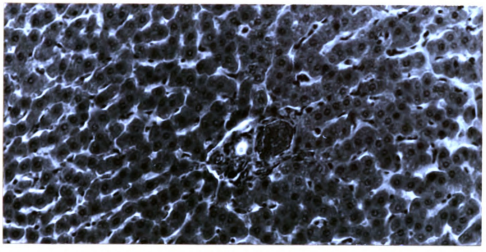


Figure 16.--Normal portal triad of mink. (x300)

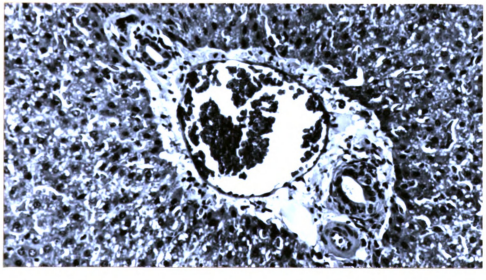


Figure 17.--Edematous portal triad from tocopherol-deficient mink. (x230)

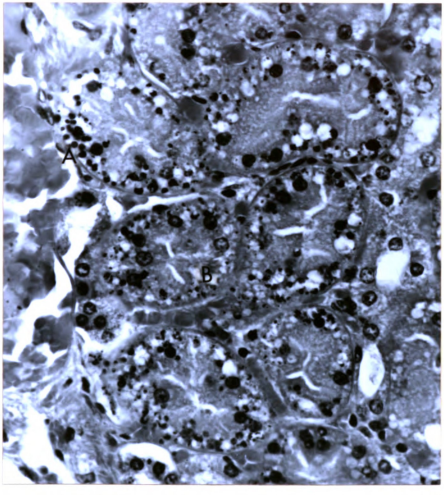


Figure 18.--Kidney of a tocopherol-deficient mink; A. Hemosiderosis; and B. Coagulation necrosis in convoluted tubules. (x620)

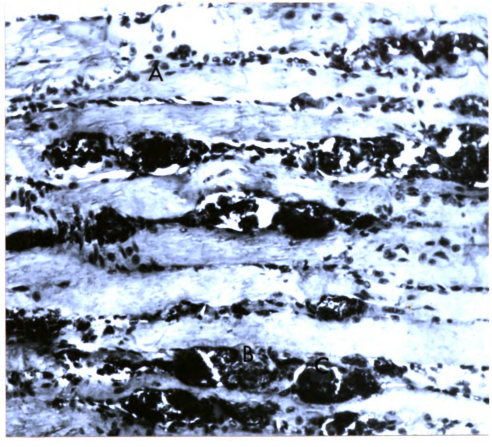


Figure 19.--Adductor of a tocopherol-deficient cod liver oil-supplemented mink; A. Sarcolemmal proliferation; B. Myofibrillar necrosis; and C. Early calcium deposition. (x350)

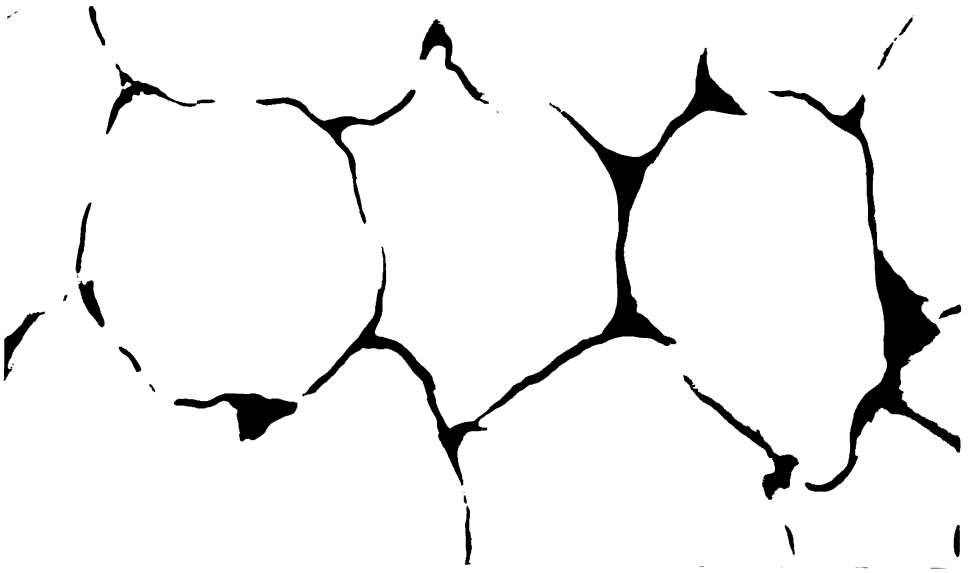


Figure 20.--Normal adipose tissue from tocopherol-supplemented mink. (x900)

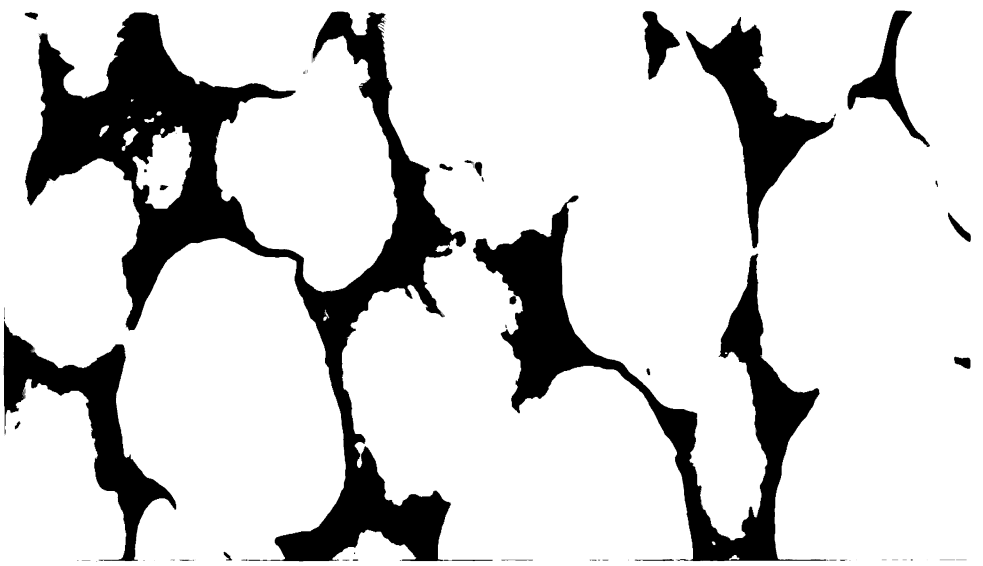


Figure 21.--Adipose tissue from tocopherol-deficient selenium-supplemented mink. Interstitial spaces contain amorphous, non-acid-fast material. (x860)

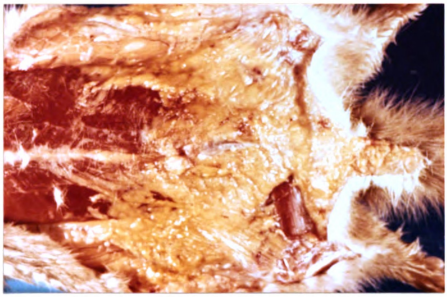


Figure 22.--Gross steatitis "yellow fat" of tocopherol-deficient cod liver oil-supplemented mink.

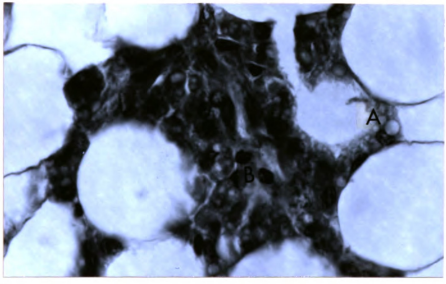


Figure 23.--Adipose tissue from tocopherol-deficient cod liver oil-supplemented mink; A. Acid-fast-pigment droplets; and B. Lymphocytes in the interstitial spaces. (Ziehl-Neelsen x860)

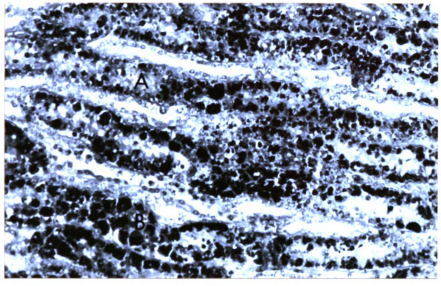


Figure 24.--Kidney of tocopherol-deficient cod liver oil-supplemented mink; A. Coagulation necrosis; and B. Calcification of the convoluted tubules in longitudinal section. (x250)

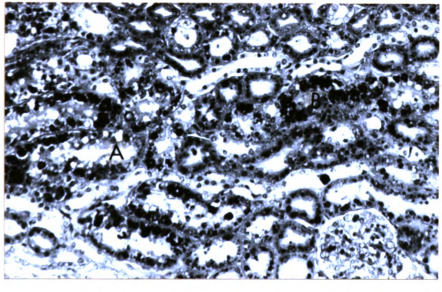


Figure 25.--Kidney of tocopherol-deficient cod liver oil-supplemented mink; A. Coagulation necrosis; and B. Calcification of the convoluted tubules in cross section. (x250)

Discussion

Mink Experiments I and II

Growth

Data obtained from these experiments provide little evidence that mink require tocopherol for growth. Although these experiments were conducted long enough for normally fed mink to attain over 80 per cent of their adult weight, two factors suggest that the experiments may not have been of sufficient length for growth differences to appear. First, Mackensie and Mackensie (1959) reported a tocopherol requirement for growth of both male and female rats maintained longer than four months on tocopherol-deficient rations. Second, slight weight losses were observed among the tocopherol-deficient mink, lots A, B and C, during the last weigh period of Experiment II.

Mortality

Of significance in the mortality information is the number of spontaneous deaths of tocopherol-deficient mink following such stress factors as being moved from the normal rearing cage to the less familiar but roomy metabolism cage or responding to an injection of Salmonella pullorum antigen. Explanation for these deaths cannot be made entirely on the basis of cardiac insufficiency because only two of the nine animals that died following such stresses had evidence of cardiac myopathy.

The observed adrenal pathology, however infrequent and inconsistent, supports the contention of other workers that the adrenal cortex may be the site of primary insufficiency during tocopherol deficiency. Raymondi (1958) demonstrated that the action of tocopherol is comparable to that of ACTH in that tocopherol deficiency influenced the zonae glomerulosa and fasciculata with cortical changes related to hyperemia. Hiisi-Brummer (1955) presented evidence that the stress of prolonged tocopherol deficiency results in a cortical dysplasia from a hyperfunction of the adrenal cortex. Histochemical studies are required to further examine the possible insufficiency of the adrenal gland during a tocopherol deficiency.

Deaths among the tocopherol-depleted mink in Experiment I were prevented when alpha-tocopherol and selenium supplementation was started. Therefore, in a semipurified ration containing 24 per cent molecularly distilled lard, adequate antioxidant activity to arrest and/or prevent fatal tocopherol deficiency lesions is provided by 25 p.p.m. of alpha-tocopherol or 1 p.p.m. of selenium as sodium selenite. The same tocopherol-sparing effects of selenium have been demonstrated by Schwarz et al. (1957) in the rat, by Eggert et al. (1957) in swine, by Dam (1957) in the chick and by Muth (1959) in sheep.

In Experiment II, 0.1 p.p.m. of selenium was also found adequate to prevent fatal tocopherol deficiency lesions.

Hematology

The relationship between the total leukocyte counts and mink age observed in Experiment I, and an earlier unpublished

study, was not demonstrated in Experiment II. However, the alterations in packed cell volumes and hemoglobin values during the course of both experiments indicate a relationship normally exists between these factors and mink age. This relationship was not indicated by Kubin and Mason (1948). These workers, however, did not indicate the ages of the mink from which their information was collected.

Although 25 p.p.m. of alpha-tocopherol in the dry diet maintained erythrocyte integrity in Experiments I and II, neither 1 p.p.m. or 0.1 p.p.m. of selenium used in Experiments I and II respectively was able to maintain the erythrocyte integrity in the tocopherol-deficient mink. This selenium-erythrocyte relationship has not been previously studied in the mink; however, these findings agree with Gitler et al. (1958) who used the rat and chick. In fact, from the results of an unpublished survey for compounds which might cause the layering type hemolysis in normal erythrocytes after a 48-hour refrigeration period in saline, sodium selenite was found effective. On this basis then, additional selenium in an already tocopherol-deficient ration would increase erythrocyte fragility rather than decrease the tendency for hemolysis.

The layering type hemolysis of mink erythrocytes appears to be a more practical indirect indicator of tocopherol deficiency than does the dialuric acid hemolysis test of Friedman et al. (1958). No doubt, with adequate modification for species, the dialuric acid test might be adaptable to the mink erythrocytes.

Serology

The serum protein data obtained from the alpha-tocopherol supplemented dark and brown mink from Experiments I and II respectively are considered normal. The alterations in the albumin and alpha globulin fractions, observed in the tocopherol-deficient mink sera during Experiment II and not observed during Experiment I, suggest that in the latter experiment, the tocopherol depletion period was insufficient to affect the serum protein fractions. Other investigators are not in agreement regarding the effects of tocopherol deficiency upon the serum protein fractions. Bottiglioni and Vannini (1957) found no serum protein alterations associated with tocopherol deficiency in the rat, while Keeler (1961) reported dystrophic sheep to have an increased alpha globulin fraction and decreased beta and gamma globulin fractions.

In Experiment I, when tocopherol depletion was started in mink at least three weeks younger than in Experiment II, it is important to note that spontaneous deaths occurred prior to significant changes in SGOT values. It is for this reason that the transaminase determinations are not considered as valuable aids for determining the presence of tocopherol deficiency myopathy in mink as Swingle et al. (1959) have reported them to be in sheep.

The SGOT and SGPT values for the control animals in each trial are in close agreement and again, in the absence of other data relative to the serum transaminases for mink,

the SGOT range of 103-176 and SGPT range of 42-90 with the respective averages of 136 and 60 Sigma Frankel units/ml. of serum are considered normal for mink receiving purified type rations.

The serum tocopherol data from Experiment I indicate that 25 p.p.m. of alpha-tocopherol in the semipurified ration containing 24 per cent molecularly distilled lard are adequate to maintain serum tocopherol levels above those associated with tocopherol deficiency myopathy. The requirement for tocopherol by the mink has not been previously estimated using purified type rations but the value of 25 p.p.m. in a diet containing a relatively saturated fat compares favorably with the range 10-20 mgs. of alpha-tocopherol/animal/day which Wilton (1959) indicated would prevent steatitis in mink fed a ration, the meat portion of which was frozen fish scraps.

The serum tocopherol data for Experiment II compare closely with those from Experiment I except for the unexpectedly high values in lot D by 156 and 171 days of age. These values indicate the mink were accumulating total body tocopherol from the dietary sources.

Serum tocopherol values of less than 50 micrograms/100 ml. of serum were associated with sudden deaths of non-selenium-supplemented mink in Experiment I. Based upon this experience, a high incidence of mortality was anticipated in Experiment II when the mink in lots A and B were 102 days old and had serum tocopherol values below 50 micrograms/100

ml. of serum; however, deaths were not frequent at this time. The fact that mink in Experiment II were three weeks older than those in Experiment I before tocopherol depletion began may account for the lower-than-anticipated incidence of mortality during Experiment II.

Information obtained from the antigen-antibody study, however limited, tends to indicate that a tocopherol deficiency does not impair the ability of mink to produce antibodies, at least to Salmonella pullorum antigen. This information lends support to the report of Axelrod and Pruzansky (1955) who reported that tocopherol-deficient chicks produced normal amounts of antibodies to porcine gamma globulin.

Urinalyses

Normal creatine excretion values for mink were not available for comparative purposes and the available creatinine data are not in agreement. Leoschke (1959) reported the normal 24-hour excretion of creatinine per kilogram of mink as 31 mgs. while Oldfield (1950) reported the figure of 15.5 mgs. The latter is within the range found in Experiment I. Neither the creatine or creatinine excretion data appear to be as satisfactory an indirect tocopherol depletion indicator as does the layering hemolysis test.

The specific gravity of mink urine was reported by Kubin and Mason (1948) to range between 1.018 and 1.036. The hydrometric values obtained in this study were usually above the upper limit of the above range. The unnatural type of

ration and the inherent fecal contamination problem may have affected the specific gravity values.

The surface tension values obtained in this study were approximately 4 dynes/cm. greater than the average of 42.7 which Leoschke (1959) reported for normal mink. Because only five animals, during Experiment I, were considered to have the chronic type of incontinence considered damaging to pelts and because of the fecal contamination problem, Dr. Leoschke's contention that urine from incontinent mink has a lower surface tension and thereby an increased affinity for the fur around the urethral orfice can neither be disputed nor supported.

Pathology

Skeletal muscle and adipose tissue were the most frequently affected tissues in experimental tocopherol deficiency in mink. Myopathy was observed, however, in the presence and absence of any form of steatitis described by Hartsough and Gorham (1949) and steatitis was observed in the absence of myopathy.

The internal intercostal and adductor muscles appeared to be predilection sites for tocopherol deficiency myopathy in mink. Extensive myopathy was often present without gross evidence of "white muscle" commonly associated with tocopherol deficiency. In such cases, the myopathy was histologically identified more readily in the adductor muscles than in the intercostal muscles. The skeletal myopathy, in general, followed the pattern of nutritional myopathy

described by Adams and Denny-Brown (1953). However, in mink, vacuolar degeneration appeared more characteristic of the early myopathy phase than was emphasized by Adams and Denny-Brown. Also, the internal nuclear rowing, described by West and Mason (1958) as a prominent feature of tocopherol deficiency myopathy in hamsters was not a common feature of the same myopathy in mink.

Gross cardiac myopathy, a condition which Benson (1959) reported in adult mink and called white heart disease, was observed only twice during these experiments. This incidence of tocopherol-deficiency cardiac myopathy is in contrast to that reported in lambs by Bacigalupo (1952). He indicated that right ventricular lesions were frequent in the experimentally-produced stiff-lamb syndrome.

The overall incidence of tocopherol deficiency myopathies in Experiment II was less than that observed in Experiment I. This is attributed to the fact that in Experiment I, the kits were weaned onto the tocopherol-deficient rations, while in Experiment II, kits were completely weaned and eating standard ranch rations before they were obtained for the experiment. Consequently, considerably more muscle development had occurred in the latter mink prior to starting them on the tocopherol depletion ration than had occurred in Experiment I.

With the variety of muscle lesions observed, it should be easy to formulate the steps in the pathogenesis of tocopherol deficiency myopathy, but the gamut of lesions is seldom observed in a single animal or a single muscle.

Swollen fibers and vacuolar degeneration are often present without signs of myophagic infiltration or focal calcification. Fibers may be differentially stained or even infiltrated with myophages without evidence of vacuolar degeneration present. And, individual calcified fibers can be present without any myopathy of the surrounding fibers or fasciculi.

When the internal rowing of skeletal muscle, described by West and Mason (1958), is present, it is believed to follow vacuolar degeneration, the nuclei accumulating in the vacuoles. Vacuolar degeneration, however, does not preclude internal rowing for internal nuclear rowing is seldom observed in tocopherol-deficient mink where vacuolar degeneration is a prominent characteristic.

A sequence for the pathogenesis of tocopherol deficiency myopathy in mink may be similar to the following:

1. Serum tocopherol values below 50 micrograms/100 ml. serum.
2. Swelling of individual fibers of the active voluntary muscles, especially the internal intercostals and the adductors.
3. Alteration of the pH of these swollen fibers indicated by differential staining characteristics.
4. Vacuolar degeneration and/or proliferation of sarcolemmal nuclei.
5. Fragmentation and lysis of the degenerate sarcoplasmic masses.
6. Attempted regeneration of the muscle by proliferation of myoblasts in intact endomysium.
7. Phagocytosis of the necrotic myofibrils and sarcolemmal cells.
8. Calcium deposition in the phagocytized or non-phagocytized sarcoplasmic debris.

9. Restoration of function to regenerated muscle fibers.

Unfortunately, the reasons why the decreased serum tocopherol starts this chain of events in the musculature remain more speculative than the above sequence would suggest.

Skeletal and cardiac myopathy in the absence of any form of steatitis was produced within 60 days in mink kits that were weaned onto the tocopherol-deficient and unsaturated fatty-acid-low basal rations used in these experiments. The situation wherein myopathy was present in the absence of steatitis was considered the uncomplicated tocopherol deficiency.

Mink depleted of tocopherol in the above manner and that survived a prolonged tocopherol deficiency (three months) developed the steatitis observed in Figure 14 on page 64. Micro-droplets of non-acid-fast material infiltrated the interstices of the adipose cells without changing the gross color or the physical characteristics of the fat.

Mink kits that survived a prolonged period of tocopherol depletion while being protected with sodium selenite showed evidence that the selenium afforded partial protection against the previous form of steatitis. However, another form of steatitis resulted which was characterized by the accumulation of amorphous material in the interstices of the fat (see Figure 21 on page 83). This steatitis appeared to be an earlier stage of the form previously described. The

evidence that selenium afforded partial protection against adipose tissue changes during tocopherol deficiency is not in full agreement with the report of Edwin et al. (1961) who found that selenium supplementation of necrogenic torula yeast diets, fed to rats, gave no protection against peroxidation of the body fats.

When mink kits were fed the basal tocopherol-deficient ration with an isocaloric addition of 8% cod liver oil, the steatitis described by Hartsough and Gorham (1949) and Gorham et al. (1951) was produced. As in Figure 23 on page 84, the interstices of the affected adipose tissue were infiltrated with acid-fast-pigment droplets, a few lymphocytes and some macrophages. The acid-fast-pigment rendered the fat yellow. Although there was a neutrophilia in these mink so affected, possibly resulting from the concurrent myopathy, the neutrophilic infiltration of the adipose tissue, characteristic of steatitis in cats (Cordy and Stillinger, 1953), was not observed.

The hepatic, renal and adrenal hemorrhages observed indicate a relationship exists between capillary permeability and tocopherol deficiency. Such evidence was provided by Grant (1961) who reported upon a microangiopathy associated with tocopherol deficiencies and yellow fat disease in swine. The angiopathy was identified by the presence of periodic-acid-Schiff positive material accumulating in the sub-endothelial area of small arterioles and capillaries, especially in the heart. The findings of Grant in this regard were not

confirmed in the mink experiments either histochemically or histopathologically.

Evidence has been presented by other researchers to indicate that the renal lesions previously attributed to tocopherol deficiency actually were the result of post-mortem autolysis which is more rapid in tocopherol-deficient animals than tocopherol-supplemented animals. Figures 24 and 25 on page 88, however, are considered evidence that ante-mortem renal lesions may be present following prolonged tocopherol deficiency, especially if the tocopherol depletion ration is relatively high in unsaturated fatty acids.

Although a pneumonitis, similar to the viral feline pneumonitis, was observed during these experiments in both the tocopherol-deficient and tocopherol-supplemented mink, the former were more severely affected. If this pneumonitis were proven to be of viral origin, the results of these experiments would indicate that tocopherol-deficient mink have a lower resistance to pneumonitis virus than do tocopherol-supplemented mink.

The pneumonitis may have served as the primary stress factor for the uncomplicated tocopherol-deficient animals and ultimately caused the sudden deaths that occurred among these mink. In fact, the venous distention and edema of the portal triads and the centro-lobular congestion in the liver, in conjunction with the pneumonitis, lend support to the theory that the sudden deaths among the tocopherol-deficient mink were caused primarily by hypoxia which in turn resulted in cardiac insufficiency.

SWINE EXPERIMENT

Materials and Methods

Fifteen Chester White-Yorkshire crossbred baby pigs were taken from their dams at four days of age and placed on the semipurified type ration (Table 28) prepared in the form of a synthetic milk.

Table 28.--Composition of tocopherol-deficient swine ration.

Ingredient	Per Cent
Vitamin free casein	10.0
Isolated soy assay protein*	12.0
Torula yeast*	20.0
Glucose (cerelose)	37.5
Molecularly distilled lard*	10.0
Solka-Floc*	5.0
Phillips and Hart Salt Mix (IV)*	4.0
Amino acid and vitamin supplements	0.5

*As described in Mink Experiments I and II.

The amino acid and vitamin supplements were prepared to furnish the constituents at the rates given in Table 29.

Table 29.--Amino acid and vitamin supplementation rates.

Component	Rate*	Component	Rate*
Arginine HCl	100.0	p-amino benzoic acid	3.0
Thiamine HCl	0.5	Folic acid	0.2
Riboflavin	1.0	Cyanocobalamin	0.16
Pyridoxine	0.5	Biotin	0.05
Calcium pantothenate	3.6	Vitamin A acetate	0.52
Nicotinic acid	5.0	Vitamin D_2	0.01
Choline chloride	200.0	Menadione	0.5
Inositol	25.0	Ascorbic acid	16.0

*mg./100 gm. solids.

The milk was prepared by suspending the dry ingredients, melted lard and fat-soluble vitamins in sufficient, constantly stirred 160° F. water to constitute 15 per cent total solids. The suspension was immediately homogenized in a two stage Manton Gaulon homogenizer at 500 and 2500 pounds pressure. After the mix was cooled in a standard spray-type milk cooler, the water-soluble vitamins were added.

The pigs rapidly learned to drink from a pan and were fed five times per day for the first two weeks and four times per day for the third week. Thereafter, they were gradually converted to the dry basal diet, fed three times per day and assigned to the experimental lots shown in Table 30.

Table 30.--Assignment of baby pigs to experimental lots.

Lot	Number of Pigs Per Lot	Ration
A	5	Basal tocopherol deficient.
B	4	Basal plus ethyl linoleate to replace an amount of lard equal to five per cent of the caloric content per unit weight.
C	4	Basal plus 100 p.p.m. alpha-tocopherol.

Allotting had been postponed because of a diarrhea problem which was fatal to two pigs prior to allotting and to one pig in lot B immediately following allotting.

Biweekly blood samples of 10-12 ml. were obtained from the anterior vena cava and all the hematological and

serological determinations described for the Mink Experiment II were made on the swine blood. Likewise, the necropsy and histological procedures used in the swine experiment were those described for the Mink Experiment II.

Near the termination of the swine experiment, a preliminary study was made to compare the responses of tocopherol-deficient and supplemented swine to the stress of an infection of transmissible gastroenteritis virus (T. G. E.). The T. G. E. virus was obtained from Purdue University in the form of homogenized infected intestines (lot 1206-9) harvested January 12, 1961. Three milliliters of the infected material was diluted to 20 ml. with sterile saline. Aliquots of this diluted material were administered orally, by means of a grain bolus, to two tocopherol-deficient and two tocopherol-supplemented pigs on the basis of body weight. The temperature of each animal was taken daily, and in addition, each animal was checked for evidence of anorexia, vomition and diarrhea. These observations were continued until all surviving animals appeared normal again.

Also near the termination of the swine experiment, the rapidity with which intramuscularly administered alpha-tocopherol was able to prevent the layering hemolysis (LH) in previously tocopherol-deficient swine was studied. Animals whose erythrocytes were positively demonstrating LH were given single intramuscular injections of alpha-tocopherol at the rate of one mg. per pound of body weight. Thereafter, the animals were bled daily to observe how soon LH ceased

following the tocopherol supplementation and how rapidly it returned. This repletion-depletion technique was used as a means of estimating the daily tocopherol requirement for swine under the conditions of this experiment.

Results

Growth

Uniformly poor growth was made by animals in each experimental group. An insidious diarrhea of unknown origin affected each group from about 7 to 21 days of age. Growth during this period was negligible and normal gains were never attained. No differences, however, were detected in the growth rates of the different lots which might be construed to be due to the effects of tocopherol deficiency.

Mortality

During the course of the experiment, six of the eight tocopherol-deficient pigs died suddenly while no tocopherol-supplemented pigs died. Table 31 presents the information pertaining to these deaths. With the exception of one, these deaths occurred sometime during the night and the dead animals were first observed at the next morning feeding. The second pig to succumb in lot B had a tympanites sufficient to rupture the abdominal wall and skin, permitting exteriorization of the small intestine. Gastric tympanites, in the second pig to succumb in lot A, caused gastric rupture and forced the gastric contents into the subcutaneous tissue as far posterior as the stifle area. It is possible that this death was a result of the stress of the T. G. E.

Table 31.--Observations pertaining to deaths of tocopherol-deficient swine.

Lot	Ration	Days on Expt.	Associated Observations
A	Basal tocopherol-deficient	80	Sudden death.
A	" " "	130	Sudden death 15 hours after exposure to T. G.E. virus. Rapid post-mortem tympanites.
A	" " "	131	Sudden death.
A	" " "	140	Sudden death and rapid post-mortem tympanites.
B	Tocopherol-deficient, ethyl linoleate-supplemented	50	Sudden death and rapid post-mortem tympanites.
B	Tocopherol-deficient, ethyl linoleate-supplemented	56	Sudden death and rapid post-mortem tympanites.

infection, however, a second tocopherol-deficient animal so exposed survived and elicited only a minor anorexic response to the virus.

Hematology

Alterations attributable to tocopherol deficiency were not observed in the packed cell volumes or hemoglobin values among the experimental groups. However, a variable response to the early diarrhea problem among the groups was reflected by a slight range in the average white cell counts not correlated with tocopherol deficiency. The data from the white cell counts, packed cell volumes and hemoglobin determinations are summarized in Table 32, page 104.

Table 32.--Mean white cell counts, packed cell volumes and hemoglobin values for swine fed tocopherol-deficient ration (A), supplemented with 5% ethyl linoleate (B) and 100 p.p.m. alpha-tocopherol (C).

Lot	White cells/cmm	Per cent packed cell volumes	Gms. hemoglobin/ 100 ml. blood
A	14,976 ± 2,070	37.7 1.1	11.4 0.2
B	11,438 ± 1,890	38.2 1.6	11.7 0.3
C	13,130 ± 1,400	38.3 1.1	11.5 0.2

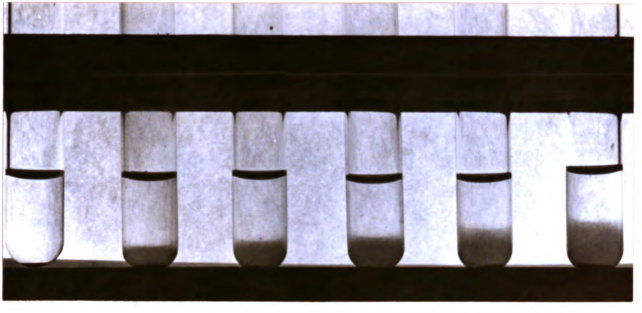
±Standard error.

The average differential counts for each lot for the duration of the experiment are presented in Table 33. The counts were not affected by the presence or absence of tocopherol in the ration. The lymphocyte : neutrophil ratio was 64:34.

Table 33.--Mean differential counts for swine fed a tocopherol-deficient ration (A) supplemented with 5% ethyl linoleate (B) and 100 p.p.m. alpha-tocopherol (C).

Leukocytes	Lots A	B	C
Neutrophils			
Segmented	33	32	35
Nonsegmented	1	>1	1
Lymphocytes	64	65	62
Monocytes	1	1	2
Eosinophils	1	1	1
Basophils	>1	1	>1

The 48-hour layering type hemolysis of erythrocytes from the tocopherol-deficient swine of lot A occurred when the animals were 77 days old and continued for the duration of the experiment (See Figure 26 on page 105). Erythrocytes from the



A B C D E F

Figure 26.--Gradations of the 48-hour refrigerated hemolysis in 0.7 per cent saline. A. Non-hemolysed erythrocytes from a tocopherol-supplemented pig are visible in bottom of tube. B. to F. Lysed fractions of erythrocytes from tocopherol-deficient swine are concentrated (layered) near bottoms of tubes.

two animals that died in lot B never elicited the layering phenomenon and the surviving pig in lot B was 105 days old before its erythrocytes showed LH. Erythrocytes from the tocopherol-supplemented swine never elicited the response.

Near the termination of the experiment a lot A tocopherol-deficient pig whose erythrocytes were demonstrating the layering hemolysis, was given intramuscularly 1 mg. of alpha-tocopherol acetate/pound of body weight and bled daily for the next 11 days. The erythrocytes taken from this pig 24 hours after the injection of tocopherol failed to elicit the layering hemolysis. Thereafter, erythrocytes taken daily from this animal did not elicit the hemolysis phenomenon until the eighth day post-injection after which the hemolysis persisted. From this repletion-depletion technique, a tocopherol requirement value of 125 micrograms/pound of body weight/day was obtained.

As in Mink Experiment II, ethoxyquin (Santoquin*), a commercial antioxidant, was added at the rate of 100 p.p.m. to the tocopherol-deficient ration of a lot A pig whose erythrocytes showed the layering hemolysis. In the seven-day trial, during which time the animal was bled daily, the antioxidant was ineffective in preventing the hemolysis phenomenon. The data obtained from the dialuric acid hemolysis determinations are summarized in Table 34 on page 107. The first significant elevation in the dialuric acid values occurred in lot A when the pigs were 73 days old and 14 days

*Monsanto Chemical Company, St. Louis, Missouri.

Table 34.--Average dialuric acid hemolysis values (per cent) for erythrocytes from swine fed tocopherol deficient ration (A) supplemented with 5% ethyl linoleate (B) and 100 p.p.m. alpha-tocopherol (C).

Lot	Days of Age											
	21	28	35	42	49	56	63	70	77	84	98	105
A	-	-	-	6.9	1.8	-	64.3	-	27.9*	-	14.5	25.2
B	1.5	0.6		5.1		4.4		16.0		-		- *
C	-	0.8		1.8		0.1		0.7		1.8		-

*Layering hemolysis present.

ahead of the onset of the layering hemolysis for this lot. When the remaining animal in lot B was 70 days old, there was an indication that it had an increased dialuric acid value. Unfortunately, no later valid dialuric acid determinations were obtained from this lot to compare with the onset of layering hemolysis which occurred at 105 days of age.

The data from the paper electrophoresis analyses of the serum protein fractions are presented in Table 35, page 108. The gamma globulin was consistently lower in lot A than in lots B or C. However, because the same change relative to gamma globulin did not occur in lot B, the alterations in this serum fraction were not considered associated with the presence or absence of tocopherol in the ration. The other fractions were not significantly altered by treatment.

Elevations in the serum glutamic-oxalacetic and glutamic-pyruvic transaminase values occurred in lot A simultaneously with the onset of the layering hemolysis when the animals

Table 35.--Mean per cents for albumin and globulin fractions of serum from swine fed tocopherol deficient ration (A) supplemented with 5% ethyl linoleate (B) and 100 p.p.m. alpha-tocopherol (C).

Serum Fraction	Lot	Days of Age 14	21	28	35	42	49	56	63	70	77	84	91	105	126	Expt. Av.
Albumin	A		31.6		36.2	31.9	36.3		36.7		35.1		39.6	32.3	33.7	34.8
	±		2.6		2.7	1.4	2.5		2.1		2.0		2.6	1.8	2.1	
	B	27.3	23.9	34.1		31.4		38.6		38.4		35.1		35.3		33.0
	±	-	0.4	2.8		1.9		1.4		-		-		-		
	C	26.7	26.0	34.2		29.2		40.9		44.7		32.0		33.0		33.3
	±	6.6	1.1	0.7		0.3		0.8		3.9		1.7		2.4		
Alpha Globulin	A		32.7		30.6	35.6	28.8		34.9		29.6		28.4	30.2	27.2	30.9
	±		1.5		0.8	4.5	1.1		3.9		1.3		2.9	2.8	1.4	
	B	18.7	27.0	29.2		28.8		31.0		32.4		28.4		30.0		28.2
	±	-	0.8	1.3		0.9		1.4		-		-		-		
	C	27.3	27.9	22.9		32.9		25.2		26.8		29.6		28.7		27.7
	±	2.6	1.8	0.4		2.0		2.1		0.6		2.8		0.9		
Beta Globulin	A		16.7		19.5	20.1	19.5		19.6		19.7		19.9	21.0	21.8	19.7
	±		1.0		1.4	1.9	0.9		0.4		0.8		0.9	1.5	1.0	
	B	20.7	20.3	20.0		19.8		17.6		16.4		19.9		15.7		18.8
	±	-	1.1	4.2		2.3		0.7		-		-		-		
	C	21.8	20.1	20.5		24.7		18.4		14.6		20.3		15.9		19.5
	±	2.4	1.8	0.5		1.7		0.6		1.3		2.1		1.9		
Gamma Globulin	A		19.3		13.8	12.6	15.5		8.8		15.6		12.2	16.6	17.4	14.6
	±		1.2		1.1	1.2	2.5		1.1		1.0		1.6	1.4	0.4	
	B	33.3	30.4	16.9		20.0		12.9		12.9		16.8		19.0		20.3
	±	-	2.1	0.3		7.0		0.7		-		-		-		
	C	24.2	26.0	22.4		13.4		15.8		13.9		17.7		22.4		19.5
	±	6.7	2.5	0.9		4.1		1.6		3.3		2.0		3.5		

±Standard error.

were 77 days of age. In the ethyl linoleate-supplemented tocopherol-deficient group (lot B), the SGOT values were first elevated after 84 days of age and the SGPT values were not significantly elevated until 105 days of age. At this age, the layering hemolysis was also observed in lot B. The average serum transaminase values for each bleeding period of the respective groups are given in Table 37 on page 110.

The data from the serum tocopherol determinations made during this experiment are summarized in Table 36.

Table 36.--Mean serum tocopherol values (micrograms/ml.) for swine receiving tocopherol-deficient ration (A) supplemented with 5% ethyl linoleate (B) and 100 p.p.m. alpha-tocopherol(C).

Lot	Days of Age													
	14	21	28	35	42	49	56	63	70	77	84	91	105	126
A		134		138	54	172		60*		66**		56	42	46
±		5		10	-	26		4		22		15	10	16
B	109	180	180		74		50		85*		39		70**	
±	11	21	34		17		6		-		-		-	
C	220	152	240		216		221		226		245		260	
±	-	60	27		20		34		16		12		17	

*Onset of elevated dialuric acid hemolysis values.
**Onset of layering hemolysis.

Illogically high total tocopherol values for lot A at 49 days of age were noted. Had this value been close to the 42 day value, the tocopherol depletion rates for the two lots would have been very similar. According to these data, ethyl linoleate supplementation of lot B did not hasten tocopherol depletion. In lot A, excepting day 49 data, the layering hemolysis occurred after 35 days of serum tocopherol values

Table 37.--Mean serum glutamic-oxalacetic and glutamic-pyruvic transaminase values in Sigma Frankel units/ml. serum from swine fed tocopherol-deficient ration (A) supplemented with 5% ethyl linoleate (B) and 100 p.p.m. alpha-tocopherol (C).

Serum Transaminase	Lot	Days of Age													
		14	21	28	35	42	49	56	63	70	77	84	91	105	126
Glutamic-Oxalacetic	A		31		Chyle	44	39		46		162		106	115	110
	±		1		Turbidity	-	2		3		13		15	5	13
	B	34	45	39		44		46		34		79		270	
	±	-	1	-		3		1		-		-		-	
	C	35	48	41		42		50		37		39		38	
	±	-	2	3		3		2		2		1		11	
Glutamic-Pyruvic	A		23		Chyle	39	36		52		90		75	81	97
	±		1		Turbidity	1	2		11		16		21	9	10
	B	32	34	40		43		37		35		52		114	
	±	-	3	-		7		1		-		-		-	
	C	-	36	41		32		44		36		46		29	
	±		1	1		2		3		2		8		4	

±Standard error.

less than 66 micrograms/100 ml. In lot B, an average of 63 micrograms of tocopherol/100 ml. serum had been present for more than 60 days before the layering hemolysis phenomenon appeared.

In the antigen-antibody response study conducted near the termination of this experiment with three tocopherol-deficient and two tocopherol-supplemented swine, there was an indication that the deficient pigs elicited a greater antibody response than did the controls. The evidence is presented in Table 38.

Table 38.--Pre- and post-Salmonella pullorum antigen injection titers for tocopherol-deficient and supplemented swine.

Tocopherol Status	Anim. No.	Pre-injection Titers 1/5	1/10	1/20	1/40	Post-injection Titers 1/160	1/320	1/640	1/1280
Deficient	1	+	+	-	-	+	+	+	-
	2	+	±	±	-	+	+	±	-
	3	+	±	-	-	+	+	+	±
Supplemented	1	+	+	-	-	+	±	-	-
	2	+	±	-	-	+	±	-	-

+Positive agglutination.
±Questionable agglutination.
-No agglutination.

The titer for the tocopherol-deficient swine appeared to be between 1/640 and 1/1280, while the titer for the supplemented swine appeared to be between 1/320 and 1/640.

The results of the preliminary study to compare the effects of the stress of a T. G. E. infection on tocopherol-deficient and supplemented animals are presented in Table 39, page 112. One tocopherol-deficient animal (NB6) succumbed

Table 39.--Summary of responses of four-month-old tocopherol-deficient and supplemented swine to an exposure to transmissible gastroenteritis virus (T. G. E.).

Observations	Date	Tocopherol-Deficient NB5	Tocopherol-Deficient NB6	Tocopherol-Supplemented WB2	Tocopherol-Supplemented WB6
	9/28				
Temp.		103.2	102.7	102.8	102.7
Stool		Normal	Normal	Normal	Normal
LH		+	+	+	+
	2:00 p.m.--.5 cc. diluted 1206-9 T. G. E. virus administered/pound body weight.				
	9/29				
Temp.		103.5	Dead	102.5	103.6
Anorexia		++		-	+++
Diarrhea		-		-	-
Vomition		±		±	±
	9/30				
Temp.		103.5		103.4	102.0
Anorexia		+		++	+++
Diarrhea		++		-	+++
	10/1				
Temp.		101.7		103.3	103.0
Anorexia		-		-	+
Diarrhea		-		-	+++
LH		+		-	-
	10/2				
Temp.		103.0		103.8	102.3
Anorexia		-		-	-
Diarrhea		-		+	+++
	10/3				
Temp.		101.4		102.8	102.5
Diarrhea		-		+++	+++
	10/4				
Diarrhea		-		++	+++
	10/4				
Temp.		103.8		100.	102.0
Diarrhea		-		+	++
	10/6				
Temp.		103.6		102.4	103.0
Diarrhea		-		-	+
	10/7				
Diarrhea		-		-	-

-Absent
±Questionable
+Mild
++Moderate
+++Severe

sometime within 15 hours after the T. G. E. virus was administered. An acute gastritis (Figure 27 on page 116) was noted in this pig. The other tocopherol-deficient pig, NB5, on the other hand, reacted mildly to the infection and recovered 3 days after the virus was administered. The tocopherol-supplemented animals elicited a greater enteric response following exposure to the virus than did the surviving tocopherol-deficient animal. Unfortunately, sufficient tocopherol-deficient animals were not available to repeat this study for more conclusive data.

Pathology

Symptoms.--With the exception of two animals which had a pronounced cyanosis on occasion, especially of the ears, at about 90 days of age, no other symptoms associated with a tocopherol deficiency were observed in either lot A or B.

Gross lesions.--The most consistent gross pathology observed in the tocopherol-deficient swine was the hepatic involvement as shown in Figure 28 on page 117. The livers appeared to have a perilobular to generalized fatty infiltration with questionable necrotic areas on the non-diaphragmatic surfaces. One liver had raised white 1 mm. nodules scattered over its diaphragmatic surface. Bilateral edema and crepitant swellings were observed in the posterior quarters and axillary regions of animals that died in both lots A and B. Two animals that died in lot A had ecchymotic to suffusion type hemorrhages in the gracilis muscle (Figure 29, page 118) and flank area (Figure 30, page 118).

Microscopic lesions.--Extensive myopathy was not a characteristic of the tocopherol-deficient swine that died or were sacrificed in this experiment; however, when myopathy was observed, it involved the adductor and gracilis muscles similarly. The myopathy in lots A and B was characterized by swollen fibers, interfascicular hemorrhage (Figure 31, page 119), edema of the endomysial spaces (Figure 32 on page 119), central nuclei in hyalinized fibers (Figure 33 on page 120), internal nuclear rowing, fragmentation and lysis of the muscle fibers (Figure 34, page 120), and myoblast proliferation (Figure 35, page 121). The latter was especially noticed in the sacrificed ethyl linoleate-supplemented swine.

The myocardium of the tocopherol-deficient animals appeared to have longer and more prominent chains of internal nuclei than was considered normal. Also, numerous bizarre nuclei were evident. Unlike the plump, foamy Purkinje fibers of normal swine (Figure 36, page 122), the Purkinje fibers of tocopherol-deficient swine were shrunken and atrophic as in Figures 37 and 38 on page 122.

The hepatic lesions of lots A and B were not unlike one another and consisted principally of centrolobular hemorrhage as shown in Figure 39, page 123. Hemosiderosis was frequently observed as a result of the hemorrhage and the interlobular septae were often edematous.

Renal lesions in both tocopherol-deficient lots were limited to medullary peritubular edema, cortical peri-

tubular hemorrhage and hemosiderosis of the convoluted tubule epithelium. Congestion and petechial hemorrhages were frequently observed in the adrenal cortex. On one occasion, what appeared to be vacuolar degeneration was noted in the zona glomerulosa of a tocopherol-deficient pig. The normal and degenerative glomerulosa zonae are illustrated in Figures 40 and 41 on page 124.

Less frequently observed lesions which were considered associated with tocopherol deficiency included congestion and hemorrhage of the testes and a perivascular edema of the cerebrum. In the ethyl linoleate-supplemented group, a nonpigmented steatitis was observed that was characterized by a segmented appearance of the fat cells, Figure 42, page 125. Pulmonary lesions were seen in swine from each experimental lot. Pulmonary congestion and interalveolar serous exudation (Figure 43, page 126) were observed in the tocopherol-deficient swine that died suddenly. The pneumonitis shown in Figure 44, page 126, was found in the tocopherol-supplemented and deficient swine; however, it was more extensive in the latter group.

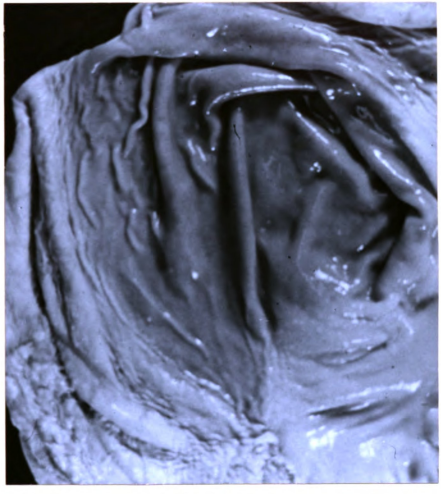


Figure 27.--Acute gastritis of a pig exposed to transmissible gastroenteritis virus.

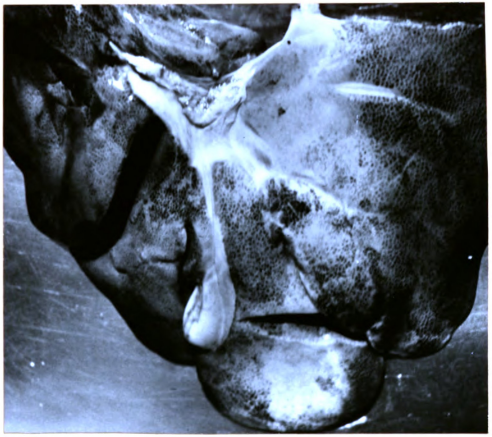


Figure 26.--Perilobular and generalized fatty infiltration of the liver from a tocopherol-deficient pig.

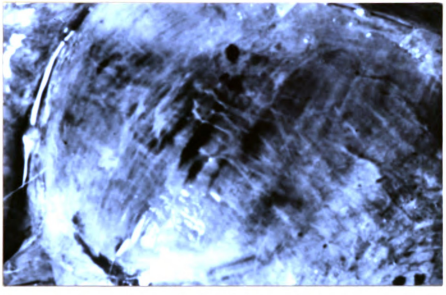


Figure 29.--Suffusion hemorrhages in a gracilis from a tocopherol-deficient pig.

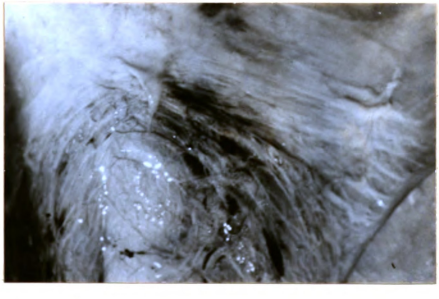


Figure 30.--Subcutaneous and fascial hemorrhages of a tocopherol-deficient pig.

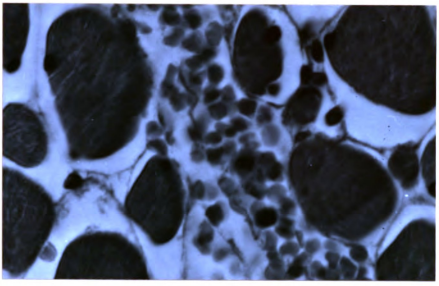


Figure 31.--Hemorrhage in a gracilis from a tocopherol-deficient pig. (x1000)

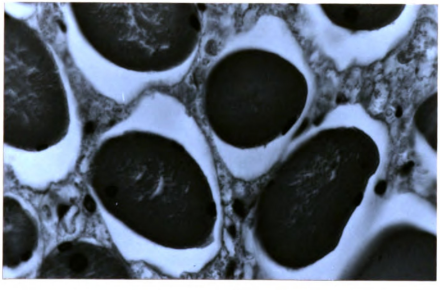


Figure 32.--Endomysial edema in an adductor from a tocopherol-deficient pig. (x1000)

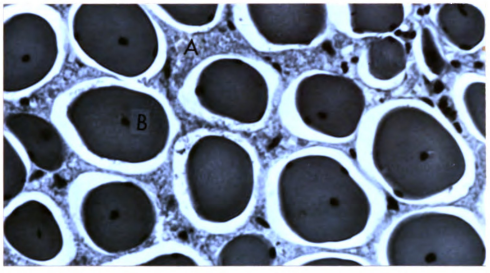


Figure 33.--Adductor from a tocopherol-deficient pig. A. Endomysial edema; B. Internal nuclei. (x450)

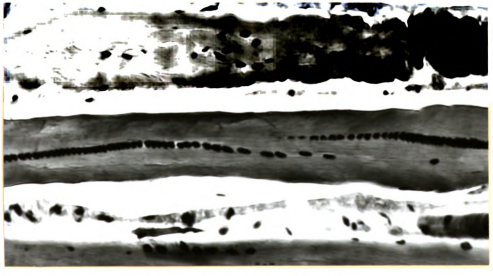


Figure 34.--Internal nuclear rowing in a fiber of an adductor from a tocopherol-deficient pig. (x390)

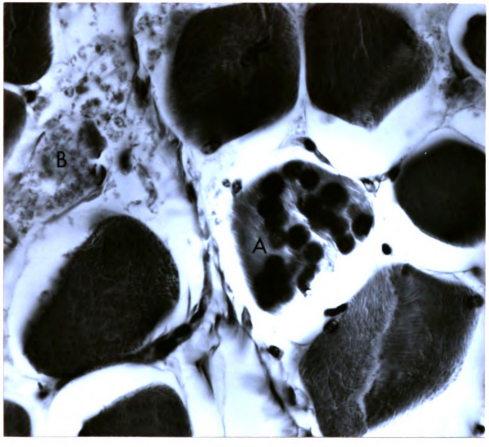


Figure 35.--Adductor from a tocopherol-deficient pig. A. Myoblastic proliferation; B. Endomysial edema. (x1050)

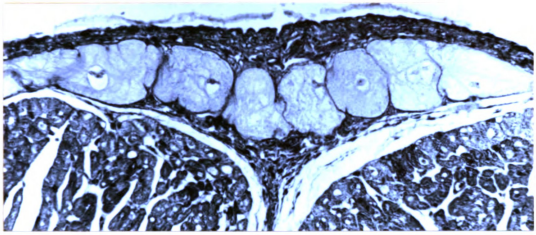
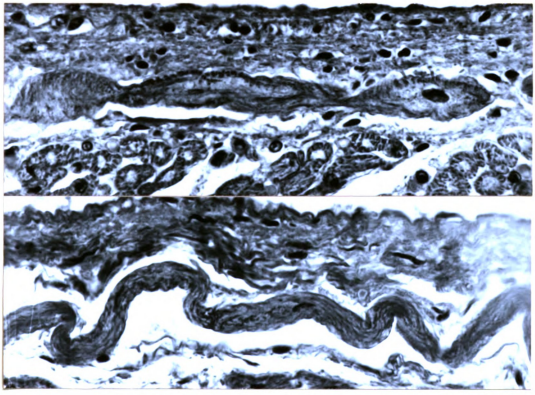


Figure 36.--Purkinje fibers from a tocopherol-supplemented pig. (x260)



Figures 37 and 38.--Purkinje fibers from tocopherol-deficient swine. (x700)

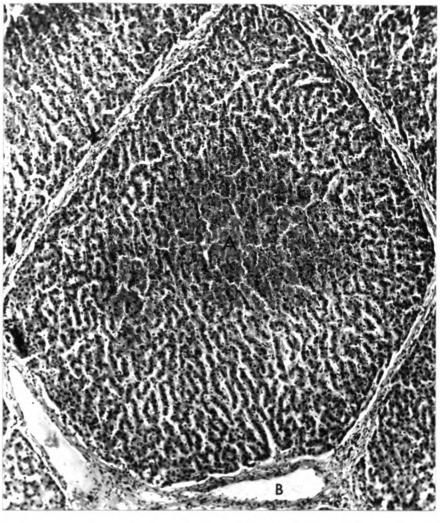


Figure 39.--Liver from a tocopherol-deficient pig. A. Centrolobular hemorrhage; and B. Interlobular septal edema. (x110)

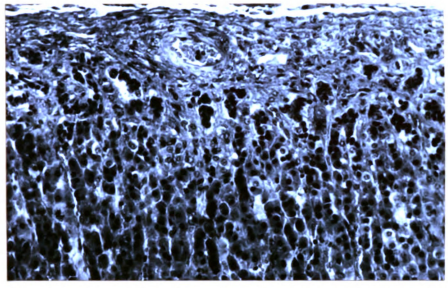


Figure 40.--Adrenal gland from a tocopherol-supplemented pig. (x260)

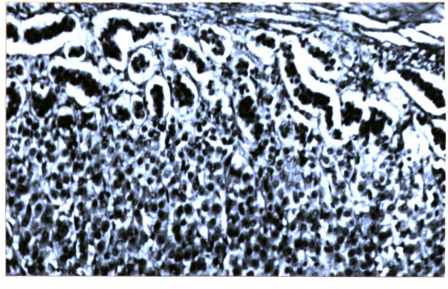


Figure 41.--Vacuolar degeneration of the zona glomerulosa from a tocopherol-deficient pig. (x260)

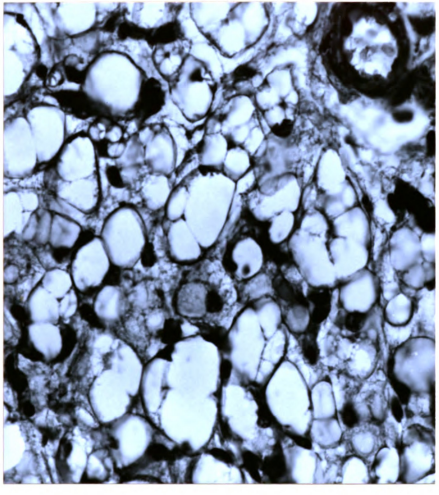


Figure 42.--Adipose tissue from a tocopherol-deficient pig. Note segmented appearance of the fat cells. (x710)

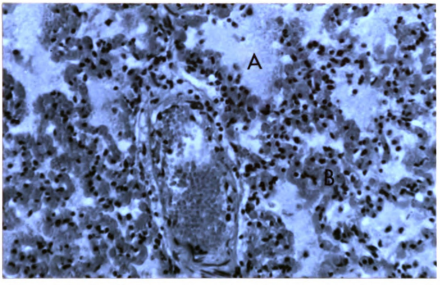


Figure 43.--Lung from a tocopherol-deficient pig. A. Serous, alveolar exudate; and B. Interalveolar pulmonary congestion. (x260)

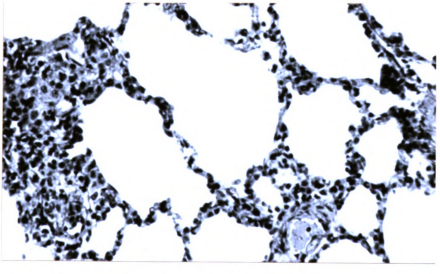


Figure 44.--Pneumonitis of a tocopherol-deficient pig. (x320)

Discussion

Growth

Although poor gains were made by the swine in this experiment, there were no differences among the lots which could be attributed to tocopherol deficiency. This finding concurs with the results of Pelligrini (1958) who used a similar semi-purified type ration containing torula yeast as the principal protein. Also, Dammers *et al.* (1958), who studied tocopherol deficiency in swine fed a more conventional ration, found no differences in growth attributable to a tocopherol deficiency.

Mortality

The sudden deaths and the rapidly developing post-mortem tympanites are considered significant among the mortality data. Evidence is available to the effect that at least two of these deaths, which were followed by the rapid post-mortem tympanites, were due to an overwhelming stress factor of saprophytic clostridial organisms. Large colonies of clostridium-like rods were found in the liver of one pig that succumbed in lot A and *Cl. perfringens* was was cultured from the intestine of another severely bloated lot A animal with hemorrhages and crepitating swellings in the subcutaneous tissues of the flank area.

Of the animals sacrificed in each group, only *Salmonella newport*, *Salmonella oranienburg* and *Proteus vulgaris* were

isolated from the intestine. Bacteria were not isolated from other organs. The highly concentrated rations being consumed might have predisposed these swine to deaths from clostridial infections. However, the resistance to such infections demonstrated by the tocopherol-supplemented lot is another indication of the inability of the tocopherol-deficient swine to withstand stress--in this case, the stress of saprophytic organisms.

Hematology

With the exception of the low number of band neutrophils, the total white and differential counts are within the normal range for swine given by Calhoun and Smith (1958). Likewise, the packed cell volume and hemoglobin values for each lot are within the ranges considered normal for swine by Miller et al. (1961a). The absence of a tocopherol effect upon these hematological factors supports the findings of Pelligrini (1958) and Borgman (1959) who also found these factors unaltered during a tocopherol deficiency in swine and rabbits respectively.

The layering hemolysis phenomenon required 77 days to elicit in swine. However, its occurrence coincided with the first elevated transaminase values and with serum tocopherol values below 65 micrograms/100 ml. serum.

Although the first elevated dialuric acid values for swine occurred two weeks prior to the layering hemolysis phenomenon, the latter is considered a more reliable indirect indicator of tocopherol deficiency than the dialuric acid

test. This is because the hemolytic action of dialuric acid is inhibited by contact with such inert laboratory materials as polyethylene. Forbes and Draper (1958) were unable to demonstrate an increased susceptibility of tocopherol-deficient swine erythrocytes to dialuric acid hemolysis. Two possible reasons for this are considered here. Either their experiment was not continued for a sufficient length of time or all the precautions for the dialuric acid test, as outlined by Friedman (1958), were not strictly observed.

Serology

Comparing the serum protein electrophoretic patterns obtained in this study with those for normal swine obtained by Miller et al. (1961), the serum albumin is lower by approximately 10 per cent, alpha and beta globulin fractions are within the normal range and gamma globulin is 10 per cent higher than normal. The high gamma globulin values may be a reflection of a response to the severe diarrhea problem encountered in the initial phase of this experiment. The absence of an association between the electrophoretic patterns of serum proteins and tocopherol deficiency is in agreement with the work of Bottiglioni (1957) in the rat, but it is in contrast to the report of Keeler (1961) who found dystrophic sheep to have an increased alpha-globulin fraction and decreased beta and gamma globulin fractions.

Cornelius and associates (1959) reported normal serum glutamic-oxalacetic and glutamic-pyruvic transaminase values for swine as 31.1±14.1 and 27.3±7.8 respectively. Wretlind

et al. (1959) gave normal SGOT and SGPT values for swine as 28.03±15.39 and 20.37±5.54 respectively. In comparison to these values considered normal, the pre-layering hemolysis SGOT and SGPT values obtained during this experiment are elevated with means of 40.9±1.6 and 37.4±2.1 for SGOT and SGPT respectively.

Finding elevated post-layering hemolysis transaminase values for the tocopherol-deficient swine in this experiment supports the work of Orstadius et al. (1959) and Lannek et al. (1961) who demonstrated elevated SGOT and SGPT values in field cases of both toxic liver dystrophy and muscular dystrophy of swine. Based on the extent of the pathology found in the muscles and liver of these tocopherol-deficient swine, the transaminase determinations appear to be as sensitive myopathy indicators for swine as Kutler and Marble (1958) have found them to be in lambs and calves.

It is apparent that 100 p.p.m. of alpha-tocopherol in semi-purified type rations are sufficient to maintain the tocopherol level in swine serum near 225 micrograms/100 ml. of serum. This amount is adequate to maintain the integrity of erythrocytes and prevent lesions of muscle, adipose tissue and liver associated with tocopherol deficiency in swine. These tocopherol deficiency lesions in swine can be anticipated when total serum tocopherol values fall below 65 micrograms/100 ml.

The available data, however limited, pertaining to the serum tocopherol values for swine appear to be in agreement

with the data obtained in this experiment. Leat (1961) reported plasma tocopherol values of 26-60 micrograms/100 ml. for swine fed less than .84 mgs. of mixed tocopherols/100 grams of meal and values of 350 to 450 micrograms for swine fed 10 mg. of alpha-tocopherol succinate/100 grams of meal. Bratzler et al. (1950) reported tocopherol values of 248, 668 and 594 micrograms/100 ml. of plasma from swine fed concentrates supplemented with 3, 55 and 110 mg. of mixed tocopherols/kg. of live weight respectively. These workers, therefore, drew the conclusions that plasma tocopherol levels did not indicate the level of tocopherol supplementation. Unfortunately, Obel (1953) gave no serum tocopherol data in her extensive study of hepatosis diaetetica.

The results of the preliminary studies relative to the responses of tocopherol-deficient and supplemented swine to T. G. E. virus and Salmonella pullorum antigen are somewhat parallel. Tocopherol-supplemented swine appeared to have lower Salmonella pullorum antibody titers than did the tocopherol-deficient swine following the series of S. pullorum antigen injections. Possibly because of associated reasons, tocopherol-supplemented swine showed a more severe enteric response (diarrhea) than did the surviving tocopherol-deficient pig. (Although one tocopherol-deficient pig died following exposure to the T. G. E. virus, this death occurred too soon--about 15 hours--after the T. G. E. was administered to have been directly caused by the virus.) These results are not in agreement with the report of Axelrod and Pruzansky

(1955) who indicated that antibody production is not altered during tocopherol deficiency, at least in the chick.

Pathology

The cyanosis observed in this experiment is considered a manifestation of a stage of tocopherol depletion and was reported by Obel (1953) as a part of the clinical picture in spontaneous hepatosis diaetetica.

The adequacy of the ration for the sulfur-containing amino acids is considered responsible for the relative absence of hepatic necrosis in the animals that died on the tocopherol-deficient rations. These rations were designed to meet the requirement of swine for DL methionine as reported by the National Research Council (.6% of the diet on a dry weight basis) yet have the limiting amino acid in the sulfur-containing amino acid group.

With specific reference to internal nuclear rowing of skeletal muscle, the myopathy observed in swine was more characteristic of that described by West and Mason (1958) in the cheek pouch of the tocopherol-deficient hamster than has been described for other species.

The hemorrhagic myositis, hepatitis, nephritis and orchitis observed are considered related to capillary and arteriolar permeability. As reported in the mink experiments, Grant (1961) has demonstrated a microangiopathy (MAP), characterized by the sub-endothelial accumulation of periodic-acid-Schiff positive material in tocopherol-deficient swine. However, sub-endothelial, PAS positive material was not

demonstrated in tocopherol-deficient swine on this experiment.

The Purkinje fiber degeneration observed in swine has been reported by Maplesden and Loosli (1960) to occur in the tocopherol-deficient calf; however, they did not illustrate their observations nor elaborate further on the pathology.

The tocopherol deficiency produced in swine from lot A was not associated with steatitis and was therefore considered uncomplicated. In swine from lot B, the isocaloric addition of 5 per cent ethyl linoleate provided insufficient unsaturated fatty acid to complicate the tocopherol deficiency with an acid-fast-pigmented steatitis but did result in an intermediate form of steatitis not previously illustrated.

SUMMARY

Experimental tocopherol deficiency was produced in mink and swine fed semipurified type rations. Development of the tocopherol deficiency was evaluated in terms of growth rates, symptomatology, biweekly hematological and serological studies, gross pathology and histopathology. The effects of tocopherol deficiency upon antibody production were determined by comparing the Salmonella pullorum antibody titers of tocopherol-deficient and supplemented animals following a series of Salmonella pullorum antigen injections. The tocopherol-depleting effects of cod liver oil (mink) and ethyl linoleate (swine), the tocopherol-sparing effects of selenium and ethoxyquin, and the species requirement for tocopherol were measured by erythrocyte fragility tests and histologic techniques.

Alterations in growth rates or consistent physical symptoms were not characteristic of tocopherol deficiency in mink or swine; however, sudden deaths were frequent among the tocopherol-deficient animals. The deaths among the deficient mink usually followed exposure to a stress factor.

The most significant hematological alteration that coincided with tocopherol depletion was an increased erythrocyte fragility that was indicated in both species by a 48-hour layered hemolysis in refrigerated saline. The increased fragility was also detected in swine by the dialuric acid hemolysis test. An absolute neutrophilia was associated with

tocopherol-deficient mink that had steatitis. Packed cell volumes and hemoglobin concentrations increased with the age of mink and were unaffected by tocopherol deficiency in either mink or swine.

Prolonged tocopherol deficiency of mink (Experiment II) resulted in decreased serum albumin and increased alpha and beta globulin fractions while the serum protein fractions were not altered by tocopherol deficiency in swine. Elevated serum glutamic-oxalacetic and glutamic-pyruvic transaminase values and serum tocopherol values between 50 and 70 micrograms/100 ml. were associated with tocopherol deficiency lesions.

Grossly, internal intercostal and adductor myopathy was common in tocopherol-deficient mink while subcutaneous edema, hemorrhagic myositis and a perilobular to generalized fatty infiltration of the liver were noted in the tocopherol-deficient swine.

Histologically, the skeletal myopathy of tocopherol-deficient mink consisted of swollen, differentially stained fibers, vacuolar degeneration, sarcolemmal and myoblastic proliferation and calcification of the non-phagocytized myofibrillae. Also associated with the deficiency in mink were calcified necrotic myocarditis, centrolobular hepatic hemorrhage, coagulation necrosis with calcification of the convoluted tubules and calcified necrotic foci in the adrenal cortex.

Microscopic lesions characteristic of tocopherol deficiency in swine were centrolobular hepatic hemorrhage,

endomysial edema, hemorrhagic myositis, a slight myoblastic proliferation, hyalinized adductor fibers containing rowed internal nuclei and Purkinje fiber degeneration. Sarcolemmal proliferation and calcification of the myofibrillae were not observed in swine while the internal nuclear rowing of skeletal muscle was not characteristic of tocopherol deficiency in mink.

Tocopherol deficiency did not impair or enhance the antibody response of mink to Salmonella pullorum antigen; however, tocopherol-deficient swine demonstrated a greater ability to produce Salmonella pullorum antibody than did tocopherol-supplemented swine.

Isocaloric supplementation of 8% cod liver oil to the tocopherol-deficient ration fed to mink caused the deposition of a yellow, acid-fast pigment in the interstices of the adipose tissue. An isocaloric supplement of 5% ethyl linoleate to the tocopherol-deficient ration fed to swine, on the other hand, did not cause the acid-fast-pigmented steatitis. Neither supplement hastened tocopherol depletion as measured by biweekly serum tocopherol values.

Selenium supplementation at the rates of 0.1 and 1 p.p.m. prevented fatal tocopherol deficiency lesions in mink. Neither selenium nor ethoxyquin was effective in preventing the 48-hour hemolysis phenomenon in mink or swine. Alpha-tocopherol supplementation of the basal mink ration at the rate of 25 p.p.m. and the swine ration at 100 p.p.m. was adequate to prevent all lesions associated with a tocopherol deficiency.

REFERENCES

Adams, R. D., Denny-Brown, D. and Pearson, C. M. 1953. Diseases of muscle. New York: Paul B. Hoeber, Inc.

Adamstone, F. B., Krider, J. L. and James, M. F. 1949. Response of swine to vitamine E deficient rations. Annals of New York Acad. of Sci., 52:260-268.

Agduhr, E. 1926. Changes in the organism caused by cod-liver oil added to the food. Aeta. Paediatr., 6: 165-179.

Alfinslater, R. B., Hansen, H. W. and Shull, R. L. 1961. Relationship of dietary linoleate to tocopherol. Fed. Proc., 20:366.

Andersson, P. 1960. Nutritional muscular dystrophy in cattle with special reference to the functional state of the thyroid. Acta Pathologica et Microbiologica Scandinavica Supplementum 134, 48:5-91.

Augustinsson, K.B., Grant, C.A., Olsson, B. and Thafvelin, B. 1960. Blood plasma esterases in pigs with induced dietetic and toxic hepatic lesions. Zentralblatt fur Veterinarmedizin, 8:729-744.

Axelrod, A. E. and Pruzansky, J. 1955. The role of the vitamins in antibody production. Vitamins and Hormones, 13:1-27.

Bacigalupo, R. A. 1952. The effects of vitamin E deficiency in the lamb. Michigan State College: Ph.D. Thesis.

Bauer, C. D. and Berg, C. P. 1943. The amino acids required for growth in mice and the availability of their optical isomers. J. Nutrition, 26:51-63.

Bay, F. and Vøgt-Moller, P. 1934. Continued studies of treatment of sterility in cows and breeding sows with wheat germ oil (vitamin E). Vet. J., 90:288-290.

Bender, A. D., Schottelius, D. D. and Schottelius, B. A. 1959. Effect of vitamin E deficiency on protein composition of guinea pig skeletal muscle. Proc. Soc. Exp. Biol. Med., 102:362-364.

Benson, R. F. 1959. New insight into causes and effective treatment of mink diseases. The Black Fox Magazine and Modern Mink Breeder, 42:8-10.

Bicknell, R. and Prescott, F. 1948. The vitamins in medicine. London: William Heinemann Medical Books Ltd.

Bieri, J. G. 1961. The nature of the action of selenium in replacing vitamin E. Am. J. of Clin. Nutrition, 9, Part 2:89-96.

Blincoe, C. and Dye, W. B. 1958. Serum transaminase in white muscle disease. J. An. Sci., 17:224-226.

Blincoe, E. and Marble, D. W. 1960. Blood enzyme interrelationships in white muscle disease. Am. J. Vet. Res., 21:866-869.

Bonetti, E., Aloisi, M. and Merucci, P. 1952. Modificazioni nelle proteine muscolari contrattili del coniglio in corso di avitaminosi E. Experientia, 8:69-71.

Borgman, R. F. 1959. Avitaminosis E and nutritional muscular dystrophy in relation to induced stresses. Kansas State University: Ph.D. Thesis.

Bottiglioni, E. and Vannini, P. 1957. Changes in the serum-protein picture in vitamin E-deficient or tocopherol-acetate treated rats. Boll. e mem. soc. toscoumbro-emiliana med. interna., 7:115-122.

Bouman, J. and Slater, E. C. 1957. The possible role of alpha tocopherol in the respiratory chain. The identification and quantitative determination of alpha-tocopherol in respiratory chain preparation. Biochim. Biophys. Acta., 26:624-633.

Breirem, K. 1952. Feeding stuffs from fish and other marine animals. Norske Landbruk, 59:139-161.

Brinkhous, K. M. and Warner, E. D. 1941. Muscular dystrophy in biliary fistula dogs: possible relationship to vitamin E deficiency. Am. J. Path., 17:81-86.

Bro-Rasmussen, F. and Hjarde, W. 1957. Determination of alpha tocopherol by chromatography on secondary magnesium phosphate. Acta Chem. Scand., 11:34-43.

Brown, F. 1953. Occurrence of vitamin E in cod and other fish-liver oils. Nature, 171:790-91.

Bunyan, J., Green, J., Edwin, E.E., and Diplock, A. T. 1960. Studies on vitamin E. 3. The relative activities of tocopherols and some other substances *in vivo* and *in vitro* against dialuric acid induced hemolysis of erythrocytes. Biochem. J., 75:460-467.

Burr, G. O. 1945. The role of various substances in stabilizing animal tissues. Quartermaster Corps Manual, QMC 17-7, 141-145.

Butturini, U. 1949. Clinical and experimental studies on vitamin E. Annals of N. Y. Acad. Sci., 52:397-405.

Calhoun, M. L. and Smith, E. M. 1958. Hematology. Diseases of Swine. H. W. Dunne, Ed., Ames, Iowa: Iowa State College Press, 37-57.

Carpenter, L. E. 1949-1950. The nutritive value of wheat germ oil in swine diets. Hormel Inst., U. of Minnesota, 29-33.

Christensen, F. and Dam, H. 1951. Inhibitory effect of dietary methylene blue on dialuric acid hemolysis of erythrocytes from vitamin E deficient rats. Acta. Pharmacol. et Toxicol., 7:167-170.

Coffin, E. L. 1953. Manual of veterinary clinical pathology. Ithaca, N. Y.: Comstock Publishing Co., Inc.

Cordy, D. R. and Stillinger, C. J. 1953. Steatitis ("yellow fat disease") in kittens. North Am. Vet., 37:714-716.

Cornelius, C. E., Bishop, J., Switzer, J. and Rhode, E. 1959. Serum and tissue transaminase activities in domestic animals. Cornell Vet., 49:116-126.

Corwin, L. M. and Schwarz, N. 1959. An effect of vitamin E on the regulation of succinate oxidation in rat liver mitochondria. J. Biol. Chem., 234:191-197.

Corwin, L. M. 1960. Maintenance of sulfhydryl groups by alpha-tocopherol. Abstr. Fifth Inter. Congress on Nutrition, 24-25.

Crider, Q. E., Alaupovic, P. and Johnson, B. C. 1961. On the function and metabolism of vitamin E. III. Vitamin e. and antioxidants in the nutrition of the rat. J. of Nutrition, 73:64-70.

Dacie, J. V., Vaughn, J. M., and Oxon, D. M. 1938. The fragility of red blood cells: Its measurement and significance. J. Path. & Bact., 46:341-356.

Daft, F. S., Endicott, K. M., Ashburn, L. L. and Sebrell, W. H. 1943. Vitamin E deficiency in rats given succinyl sulfathiazole in purified diets. Proc. Soc. Expt. Biol. and Med., 53:130-131.

Dalgaard-Mikkelsen, S., Momberg-Jorgensen, H. C. and Petersen, F. H. 1958. Prevention of yellow fat of minks. Beretn. Forsøgslab. København No. 308.

Dam, H. 1944. Studies on vitamin E deficiency in chicks. J. Nutrition, 27:193-211.

Dam, H. and Granados, H. 1951. The influence of certain substances on massive hepatic necrosis and lung hemorrhages in rats fed low-protein, vitamin E deficient diets. Acta. Pharmacol. et Toxicol., 7:181-188.

Dam, H., Krause, L, Prange, I. and Sondergaard, E. 1951. Substances affording a partial protection against certain vitamin E deficiency symptoms. Acta. Physiolo. Scand., 22:299-310.

Dam, H. 1957. Influence of antioxidants and redox substances on signs of vitamin E deficiency. Pharmaco. Rev., 9: 1-16.

Dam, H. and Sondergaard, E. 1957. Prophylactic effect of selenium dioxide against degeneration (white striation) of muscle in chicks. Experientia, 13:494.

Dammers, J., Stolk, K. and van Wieringen, G. 1958. De Betekenis van Vitamine E voor Mestvarkens. Versl. Landbouwk. Onderz. 64, 5:1-18.

Davis, C. L. and Gorham, J. R. 1954. The pathology of experimental and natural cases of "yellow fat" disease in swine. Am. J. Vet. Res., 15:55-59.

Dinning, J. S. and Day, P. L. 1957. Tissue concentration of nucleic acids and creatine in the vitamin E deficient monkey. J. of Nutrition, 63:393-397.

Diplock, A. T., Green, J., Edwin, E. E. and Bunyan, J. 1960. Studies on vitamin E. 4. The simultaneous determinations of tocopherols, ubiquinones and ubichromenols (substance SC) in animal tissues: A reconsideration of the Keilin-Hartree heart preparations. Biochem. J., 76:563-571.

Dodd, D. C., Blakely, A. A., Thronbury, R. S. and Dewes, H. F. 1960. Muscle degeneration and yellow fat disease in foals. New Zealand Vet. J., 8:45-50.

Dodd, D. C. and Newling, P. E. 1960. Muscle degeneration and liver necrosis in the pig: report of a natural outbreak. New Zealand Vet. J., 8:95-98.

Drake, C., Grant, A. B. and Hartley, W. J. 1960. Selenium and animal health. 2. The effect of selenium on unthrifty weaned lambs. New Zealand Vet. J., 8:7-10.

Draper, H. H. 1959. Biopotency of non-tocopherol compounds in vitamin E deficiency diseases. Proc. Soc. Exp. Biol. Med., 102:737-9.

Duggan, D. E. 1959. Spectrofluorometric determination of tocopherols. Arch. Biochem. Biophys., 84:116-122.

Duncan, W. R. H., Garton, G. A., McDonald, I. and Smith, W. 1960. Observations on tocopherol absorption by pigs. Brit. J. Nutr., 14:371-377.

Edwin, E. E., Diplock, A. T., Bunyan, J. and Green, J. 1960. Studies on vitamin E. 1. The determination of tocopherol in animal tissues. Biochem. J., 75:450-456.

Edwin, E. E., Bunyan, J., Diplock, A. T. and Green, J. 1961. The role of tocopherol, selenium and antioxidants in the rat. Nature, 189:747-748.

Eggert, R. G., Patterson, E., Akers, W. T. and Stokstad, E. L. R. 1957. The role of vitamin E and selenium in the nutrition of the pig. J. Animal Sci., 16:1037.

Evans, H. M. and Bishop, K. S. 1922. On the existence of a hitherto unrecognized dietary factor essential for reproduction. Science, 56:650-657.

Filer, L. J., Rumery, R. E., and Mason, K. E. 1947. Pigmentation of adipose tissue as influenced by unsaturated fatty acids in vitamin E deficient rats. Anat. Record., 97:387.

Forbes, R. M. and Draper, H. H. 1957. Production and study of vitamin E deficiency in the baby pig. J. Animal Sci., 16:1037.

Friedman, L., Weiss, W., Whurry, F. and Kline, O. L. 1958. Bioassay of vitamin E by the dialuric acid hemolysis method. J. of Nutrition, 65:143-160.

Garton, G. A. and Duncan, W. R. H. 1954. Dietary fat and body fat; the composition of the back fats of pigs fed on a diet of cod liver oil and lard. Biochem. J., 57:120-125.

Garton, G. A. and Naftalin, J. M. 1953. Produced exudative diathesis in swine on E low rations containing C10. Vet. Rec., 65:262.

Garton, G. A., Duncan, W. R. H. and Madsen, K. A. 1958. Observations on feeding pigs on low-fat diet with and without supplementary tocopherol. Brit. J. of Nutr., 12:97-105.

Gedigk, P. and Fischer, R. 1959. On the origin of lipopigments in muscle fibers: Studies in experimental vitamin E deficiency on rats and on organs of man. Virchow Arch. Path. Anat., 332:431-68.

Gitler, C., Sunde, M. L. and Baumann, C. A. 1958. Effect of certain necrosis preventing factors on hemolysis in vitamin E deficient rats and chicks. J. of Nutrition, 65:397-407.

Goettsch, M. and Pappenheimer, A. M. 1931. Nutritional muscular dystrophy in the guinea pig and rabbit. J. Exp. Med., 54:145-165.

Gorham, J. R., Baker, G. A. and Boe, N. 1951. Observations on the etiology of yellow fat disease in mink. Vet. Med., 46:100-102.

Gorham, J. R., Boe, M. and Baker, G. A. 1951. Experimental "yellow fat" disease in pigs. Cornell Vet., 41:332-338.

Grant, C. A. 1961. Morphological and aetiological studies of dietetic microangiopathy in pigs ("mulberry heart"). Acta Vet. Scand. Suppl. 3, 2:1-105.

Green, J., Edwin, E. E., Bunyan, J. and Diplock, A. T. 1960. Studies on vitamin E. 2. Reversal of respiratory decline in nutritional liver necrosis by intraportal administration of tocopherols and other substances. Biochem. J., 75:456-460.

Green, J., Edwin, E. E., Diplock, A. T. and Bunyan, J. 1961. Role of selenium in relation to ubiquinone in the rat. Nature, 189:748-749.

Hartsough, G. R. and Gorham, J. R. 1949. Steatitis (yellow fat) in mink. Vet. Med., 44:345-346.

Hiisi-Brummer, L. 1955. Histological and histochemical alterations of the mouse adrenal cortex caused by tocopherol (vitamin E) deficiency. Ann. Acad. Sci. Fennicae. Ser. A., 5:1-59.

Hilditch, T. P. 1956. The chemical constitution of natural fats, third ed. New York: John Wiley and Sons.

Hill, E. G., Warmanen, E. L., Hayes, H. and Holman, R. T. 1957. Effects of essential fatty acid deficiency in young swine. Proc. Soc. Exp. Biol. and Med., 95:274-278.

Hjarre, A. 1951. So-called toxic liver dystrophy in pigs. Tijdscht. Diergeneesk, 76:409-414.

Horn, Z., Bogsch, S. and Altmann, O. 1955. Action of vitamin E on carbohydrate metabolism. Z. ges. inn. Med. u. ihre Grenzgebiete, 10:140-5.

Horwitt, M. K., Harvey, C. C., Century, B., Navazio, F. and Witting, L. 1960. Tocopherol deficiency in man. Abstr. Fifth Inter. Congress on Nutrition, 23.

Horwitt, M. K., Harvey, C. C., Century, B. and Witting, L. A. 1961. Effect of polyunsaturated lipids on tocopherol requirements. J. Am. Dietetic Assoc., 38:231-235.

Houchin, O. B. and Mattill, H. A. 1942. The oxygen consumption, creatine and chloride content of muscles from vitamin E deficient animals as influenced by feeding alpha tocopherol. J. Biol. Chem., 146:301-307.

Hove, E. L. and Harris, P. L. 1947. Relative activity of the tocopherols in curing muscular dystrophy in rabbits. J. of Nutrition, 33:95-106.

Hove, E. L. 1953. The relation of pyridine toxicity in rats to dietary vitamin E. J. of Nutrition, 50:361-371.

Hove, E. L. 1955. Anti-vitamin E stress factors as related to lipid peroxides. Am. J. Clin. Nutrition, 3:328-36.

Hove, E. L. and Seibold, H. R. 1955. Liver necrosis and altered fat composition in vitamin E-deficient swine. J. of Nutrition., 56:173-186.

Jolly, R. D. 1960. A preliminary experiment of the effect of selenium on the growth rate of calves. New Zealand Vet. J., 8:13.

Keeler, R. F. and Young, S. 1961. An electrophoretic analysis of protein extracts from normal and dystrophic ovine muscle. Biochem. J., 81:93-98

Keith, T. B. and Schneider, A. P. 1957. Muscular dystrophy in calves and lambs. I. The relation of environmental conditions to incidence. J. Am. Vet. Med. Assn., 131:519-522.

Kokatnur, M. G., Seiichi, O., Kummerow, F. A. and Scott, H. M. 1960. Effect of long-chain keto-acids on encephalomalacia in chicks. Pro. Soc. Expt. Biol. and Med., 104:170-171.

Kubin, R. and Mason, M. M. 1948. Normal blood and urine values for mink. Cornell Vet., 38:79-85.

Kuttler, K. L. and Marble, D. 1958. Relationship of SGOT to naturally occurring and artificially induced white muscle disease in calves and lambs. Am. J. Vet. Res., 19:632-636.

Lalor, R. J., Leoschke, W. L. and Elvehjem, C. A. 1951. Yellow fat in mink. J. of Nutrition, 45:183-188.

Lam, K. W., Riegl, M. and Olson, R. E. 1961. Biosynthesis of selenocoenzyme A in the rat. Fed. Proc., 21:229A.

Lannek, N., Lindberg, P., Nilsson, G., Nordstrom, G. and Orstadius, K. 1961. Production of vitamin E-deficiency and muscular dystrophy in pigs. Res. Vet. Sci., 2:67-72.

Lawrie, R. A. 1960. Post mortem glycolysis in normal and exudative longissimus dorsi muscles of the pig in relation to so-called white muscle disease. J. Comp. Path. and Therap., 70:273-295.

Leat, W. M. F. 1961. Studies on pigs reared on diets low in tocopherol and essential fatty acids. Brit. J. Nutr., 15:259-270.

Leekley, J. R. and Cabell, C. A. 1959. Effect of butylated-hydroxy-toluene, diphenyl-para-phenylenediamine and other additives on reproduction and steatitis in mink fed fish diets. J. An. Sci., 18:1534-1535.

Leoschke, W. L. 1959. Bringing facts into focus on wet belly knowledge. U. S. Fur Rancher, 13-17, May.

MacDonald, A. M., Blaxter, K. L., Watts, P. S. and Wood, W. A. 1952. The nutrition of the young Ayrshire calf. 10. Histopathology of muscular dystrophy and its relations to muscle chemistry. Brit. J. Nutr., 6:164-169.

—Machlin, L. J. and Gordon, R. S. 1960. Etiology of muscular dystrophy, exudative diathesis and encephalomalacia in the chicken. Abstr. Fifth Inter. Congress on Nutrition, 19.

Mackenzie, J. B. and Mackenzie, C. G. 1959. The effect of alpha-tocopherol, alpha-tocopherylhydroquinone and their esters on experimental muscular dystrophy in the rat. J. of Nutrition, 67:223-235.

Madsen, L. L. 1936. The comparative effects of cod liver oil, cod liver oil concentrate, lard and cottonseed oil in a synthetic diet on the development of nutritional muscular dystrophy. J. of Nutrition, 11:471-494.

Maplesden, D. C. and Loosli, J. K. 1960. Nutritional muscular dystrophy in calves. II. Addition of selenium and tocopherol to a basal, dystrophogenic diet containing cod liver oil. J. Dairy Science, 43:645-653.

Marvin, H. N., Dinning, J. S. and Day, P. 1960. Erythrocyte survival in vitamin E-deficient monkeys. Proc. Soc. Exp. Biol. and Med., 105:473-475.

Mason, K. E. and Rao, B. H. 1960. Antisterility and anti-vitamin K activity of alpha-tocopherolhydroquinone. Abstr. Fifth. Inter. Congress on Nutrition, 24.

McDermid, A. M. and Ott, G. L. 1947. "Yellow fat" as observed in mink. J. A. V. M. A., 110:34-35.

Melville, R. S. and Hummel, J. P. 1951. Creatine and glycocyamine metabolism in rabbits in vitamin E deficiency. J. Biol. Chem., 1:383-389.

Menschik, Z. 1960. Vitamin E and associated dietary factors influencing reticular and elastic fibers of connective tissue. Abstr. Fifth Inter. Congress of Nutrition, 24.

Mertz, W. and Schwarz, K. 1955. Impaired intravenous glucose tolerance as an early sign of dietary necrotic liver degeneration. Arch. Biochem. Biophys., 58:504-506.

Miller, E. R., Ullrey, D. E., Ackerman, I., Schmidt, D. A., Hoefer, J. A. and R. W. Luecke. 1961. Swine hematology from birth to maturity. I. Serum proteins. J. An. Sci., 20:31-35.

Miller, E. R., Ullrey, D. E., Ackerman, I., Schmidt, D. A., Luecke, R. W. and Hoefer, J. A. 1961a. Swine hematology from birth to maturity. II. Erythrocyte population, size and hemoglobin concentration. J. An. Sci., 20:890-897.

Moore, T. and Sharman, I. M. 1959. Nutritional studies of isoniazid in rats deficient in vitamin E. Am. Rev. of Respiratory Diseases, 80:223-231.

Morgulis, S. and Spencer, H. C. 1936. A study of the dietary factors concerned in nutritional muscular dystrophy. J. of Nutrition, 11:573-592.

Muth, O. H., Oldfield, J. E., Schubert, J. R. and Remmert, L. F. 1959. White muscle disease (myopathy) in lambs and calves. VI. Effects of selenium and vitamin E on lambs. Am. J. Vet. Res., 21:231-234.

Naftalin, J. M. and Howie, J. W. 1949. Hepatic changes in young pigs reared in a cold and damp environment. J. Path. Bact., 61:319-328.

Nishida, T., Tsuchiyama, H., Inoue, M. and Kummerow, F. A. 1960. Effect of intravenous injection of oxidized methyl esters of unsaturated fatty acids on chick encephalomalacia. Proc. Soc. Exp. Biol. and Med., 105:308-312.

Obel, A. L. 1953. Studies on the morphology and etiology of so-called toxic liver dystrophy (hepatosis diaetetica) in swine. Acta Path. Micro. Scand. Suppl., 94:1-87.

Oldfield, J. E. 1950. A study of nitrogen metabolism with special reference to mink. Manuscript, Dept. of An. Husb., University of British Columbia.

Orstadius, K., Wretlind, B., Lindberg, P., Nordström, G. and Lannek, N. 1959. Plasma transaminase and transferase activities in pigs affected with muscular and liver dystrophy. Sonderdruck aus Zentralblatt für Veterinärmedizin, 6:971-980.

Oser, B. L. and Oser, M. 1956. Inhibitory effect of feed grade DPPD (diphenyl-p-phenylenediamine) on parturition in rats. J. Agr. Food Chem., 4:796-797.

Pellegrini, L. 1958. A study of vitamin E deficiency in pigs fed a torula yeast diet. Ph.D. Thesis, University of Minnesota.

Phillips, P. H. and Hart, E. G. 1935. The effect of organic dietary constituents upon chronic fluoride toxicosis in the rat. J. Biol. Chem., 109:657-663.

Pindborg, J. J. 1949. Role of the intestinal flora in the development of vitamin E deficiency. Nature, 164:493.

Pollard, C. J. and Bieri, J. G. 1959. Studies of the biological function of vitamin E. I. Tocopherol and reduced diphosphopyridine nucleotide-cytochrome c reductase. Biochim. Biophys. Acta., 34:420-430.

Quaife, M. L., Scrimshaw, N. S. and Lowry, O. H. 1949. A micromethod for assay of total tocopherols in blood serum. J. Biol. Chem., 180:1229-1235.

Quortrup, E. R., Gorham, J. R. and Davis, C. L. 1948. Non suppurative panniculitis (yellow fat) in mink. Vet. Med., 43:228-230.

Raymondi, G. 1958. The influence of vitamin E on the adrenal cortex. Gazz. intern. med. e chir., 63:899-908.

Redfearn, E. R. and Punphrey, A. M. 1960. The kinetics of ubiquinone reactions in heart-muscle preparations. Biochem. J., 76:64-71.

Reitman, S. and Frankel, S. 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminase. Am. J. Clin. Path., 28:56-63.

Robinson, K. L. and Coey, W. E. 1951. A brown discoloration of pig fat and vitamin E deficiency. Nature, 168:997-998.

Roderuck, C. E. 1949. Analysis of certain components of skeletal muscle during vitamin E deficiency. J. Biol. Chem., 181:11-16.

Rose, C. S. and György, P. 1952. Specificity of hemolytic reaction in vitamin E deficient erythrocytes. J. Physiol., 168:414-420.

Rosenfeld, I. 1960. Effect of selenium on methionine formation in vivo and in vitro. Fed. Proc., 19:4.

Rosenfeld, I. 1961. Biosynthesis of seleno-compounds from inorganic selenium by the sheep. Fed. Proc., 20:10.

Rousseau, J. E., Dicks, M. W., Teichman, R., Helmboldt, C. F., Bacon, E. L., Prouty, R. M., Dolge, K. L., Eaton, H. D., Jungherr, E. L. and Beall, G. 1957. Relationships between plasma, liver and dietary tocopherol in calves, lambs, and pigs. J. An. Sci., 16:612-622.

Schottelius, B. A., Schottelius, D. D. and Bender, A. D. 1959. Effect of vitamin E on myoglobin content of cavia skeletal muscle. Proc. Soc. Exp. Biol. Med., 102:581-583.

Schwarz, K. and Foltz, C. M. 1957. Selenium as an integral part of Factor 3 against dietary necrotic liver degeneration. J. Am. Chem. Soc., 79:3292-3293.

Schwarz, K., Stesney, J. and Foltz, C. M. 1959. Relation between selenium traces in l-cystine and protection against dietary liver necrosis. Metabolism, 8:88-90.

Schwarz, K. 1960. Factor 3, selenium and vitamin E. Nutrition Reviews, 18(7):193-197.

Schwarz, K. 1961. A possible site of action for vitamin E in intermediary metabolism. Am. J. Clin. Nutr., 9, Part 2:71-75.

Semenova, L. P. 1958. Changes in neuromuscular endings in vitamin E deficient rats. Doklady Akad. Nauk., 120:1162-1164.

Sharman, L. M. and Moore, R. 1958. The partial vitamin E activity of NN diphenyl-para-phenylenediamine in the rat. Biochem. J., 69:61-2.

Sharman, G. A. M., Blaxter, K. L. and Wilson, R. S. 1959. Prevention of enzootic muscular dystrophy by selenium administration. Vet. Rec., 71:536.

Sharman, I. M. and Richards, P. J. 1960. Commercial breads as sources of vitamin E for rats as determined by the hemolysis test. Brit. J. Nutr., 14:85-89.

Stafseth, H. J., Stockton, J. J. and Newman, J. P. 1959. Laboratory manual for immunology. Minneapolis: Burgess Publishing Company.

Swingle, K. F., Young, S. and Dang, J. C. 1959. Relationship of SGOT to nutritional muscular dystrophy in lambs. J. Vet. Res., 20:75-77.

Tedeschi, G. G. and De Cicco, A. 1953. Antivitamin E activity of o-cresol esters. 3a Giornata sci., Consiglio nazl. richerche. Convegno vitamine, Milan, 644-648.

Tedeschi, G. G. and De Cicco, A. 1954. Antivitamin E action of thymol, carvacrol and guiacol. Boll. soc. ital. biol. sper., 30:727-729.

Teleford, I. R., Wiswell, O. B. and Smith, E. L. 1954. Tocopherol prophylaxis in multiple exposure to hypoxia. Proc. Soc. Exptl. Biol. and Med., 87:162-164.

Victor, J. 1934. Metabolic and irritability changes in nutritional myopathy of rabbits and ducks. Am. J. Physiol., 108:229-236.

Watts, B. M., Cunha, T. J. and Major, R. 1948. Effect of feeding and injecting hogs with tocopherols on the susceptibility of pork fat to rancidity. Oil and Soap, 23:254-257.

West, W. T. and Mason, K. E. 1958. Histopathology of muscular dystrophy in the vitamin E deficient hamster. Am. J. Anat., 102:323-363.

White, A. A. and Hess, W. C. 1957. Some alterations in serum enzymes in progressive muscular dystrophy. Proc. Soc. Exptl. Biol. Med., 94:541-544.

Wilton, G. S. 1958. Steatitis on mink farms in Alberta. Can. J. Comp. Med. Vet. Sci., 22:204-244.

Wretlind, B., Orstadius, K. and Lindberg, P. 1959. Transaminase and transferase activities in blood plasma and in tissues of normal pigs. Sonderdruck aus Zentralblatt für Veterinarmedizin, 10:963-970.

Yang, C., Dialameh, G. H. and Olson, R. E. 1959. Selenium and CoA biosynthesis. Fed. Proc., 18:553.

Zaehringer, M. V., Bring, S. V., Rickard, C. A. and Lehrer, W. R., Jr. 1959. Effect of tocopherol supplementation of swine rations on the storage life of frozen pork. Food Technol., 13:313-317.

Zalkin, H. and Tappel, A. L. 1960. Mechanism of vitamin E action. IV. Lipide peroxidation in the vitamin E deficient rabbit. Arch. Biochem. Biophys., 88:113-117.

[redacted] JAN 17 '64

JUL 15 4

[redacted]