THE PATHOLOGY OF VITAMIN E-SELENIUM DEFICIENCY IN SWINE AS INFLUENCED BY EXERCISE AND CONFINEMENT

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KENNETH M. AYERS

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ABSTRACT

THE PATHOLOGY OF VITAMIN E-SELENIUM DEFICIENCY IN SWINE AS INFLUENCED BY EXERCISE AND CONFINEMENT

By

Kenneth M. Ayers

Eight pigs were used to study the pathology of a vitamin E-selenium deficiency, as influenced by exercise. The basal vitamin E-selenium deficient diet was a purified ration containing adequate amounts of other nutrients. It was composed of soya protein, cerelose, and cod liver oil. The protein content of the ration was decreased during the experiment, and a stress agent, silver acetate, was fed to all pigs during the last 9 days of the experiment.

Pigs fed the basal ration died suddenly after an average of 63 days, and exhibited cardiac degeneration, liver necrosis, and muscular dystrophy. Exercise did not significantly influence the clinical signs or severity of the lesions. Pigs fed the basal ration and subjected to exercise did display subendocardial hemorrhages, however, whereas the other pigs did not. The administration of vitamin E and selenium provided complete protection against the lesions noted.

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Kenneth M. Ayers

A THESIS

Submitted to
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Dedicated to

My Lovely Wife

and Daughter

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Finally, the author gratefully acknowledges the support given him and the sacrifices made by his wife Jane.

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INTRODUCTION AND OBJECTIVES

Vitamin E and selenium have been intensively studied in recent years with respect to their importance in the nutrition of man and animals. There have been numerous publications pertaining to sources, requirements, and the metabolism of these nutrients. Clinical diseases have been reported in most species of animals, which respond to the administration of vitamin E and/or selenium. The description of these diseases, however, pertains to field problems in which the amounts of vitamin E and selenium are undetermined. Information on the nature of lesions in animals fed deficient amounts of these nutrients, and subjected to the stress of exercise, is limited.

The objectives of this experiment were as follows: (1) to evaluate the influence of exercise on swine fed a vitamin E-selenium deficient diet; (2) to determine the effect of strict confinement on pigs fed a vitamin E-selenium deficient diet; and (3) to evaluate the effect of dietary "stress" in pigs fed a vitamin E-selenium deficient diet.

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LITERATURE REVIEW

Vitamin E and selenium have been the subject of a great deal of research in recent years. Many different aspects of the deficiency syndrome have been studied and reported upon. This review pertains primarily to the special pathology of vitamin E and selenium deficiency in swine.

Vitamin E

History. Evans and Bishop (1922) discovered the importance of vitamin E in nutrition. They reported that female rats maintained on a diet using casein as a source of protein had a high incidence of reproductive failure. The condition could be prevented by feeding fresh lettuce, dried alfalfa, or high levels of butterfat. Vitamins A, D, or C were ineffective in preventing the disorder. In later work they isolated from wheat germ oil, an alcohol, which possessed all of the properties of vitamin E (Evans *et al.*, 1936).

G. M. Calhoun, a professor of Greek at Berkeley, and a friend of Evans, suggested that the new vitamin be named tocopherol. The word is derived from the Greek *tocos*, meaning childbirth, and *phero*, meaning to bear or carry. The suffix *"ol"* indicates the compound is an alcohol.

Tocopherol is composed of a basic chromane ring, on which methyl groups are located at various points (Vasington *et al.*, 1960). There are 7 methyl derivatives, of which alpha-tocopherol is the most abundant and the most active.

Absorption and Distribution. The mechanism of absorption of vitamin E appears to be similar to the other fat soluble vitamins (Wiss *et al.*, 1962). Its absorption is probably linked with fat and is facilitated by the bile. The gastrointestinal lymphatics may play a role in absorption (Roels, 1967). Absorption appears to be incomplete, as large amounts are excreted in the feces.

The administration of tocopherol in the free form rapidly results in high blood values. When administered in the acetate form, absorption is much slower, probably as the result of partial saponification of the compound in the intestine. The intact ester is then partially absorbed and hydrolyzed in the tissues (Wiss *et al.*, 1962).

The heart, lungs, spleen, liver, and mammary gland are sites of major concentrations of vitamin E (Mason, 1942). Wiss *et al.* (1962) reported that much of the tissue vitamin E is concentrated in the mitochondria and microsomes.

Metabolic Role. The exact nature of the metabolic role of vitamin E is unknown. Much of the research to determine its role has been concerned with the ability of tocopherol to prevent the peroxidation of certain essential compounds in the body, such as fatty acids, other vitamins, and enzymes. The antioxidant theory is based on this ability, and Tappel (1962) is one of the theory's most ardent supporters. He suggests that vitamin E acts solely as a biological lipid antioxidant. If tocopherol does function as a lipid antioxidant, then other antioxidants should be able to replace vitamin E in preventing the deficiency. Diphenyl-P-phenylene-diamine (DPPD), an antioxidant structurally unrelated to tocopherol, can replace vitamin E in preventing encephalomalacia in chicks (Singsen *et al.*, 1955). Crider (1961) later reported that DPPD will prevent fetal resorption in rats fed a vitamin E deficient diet.

The presence of fat in the diet, especially polyunsaturated fats, plays an important role in the production of many of the vitamin E deficiency syndromes. Singsen *et al.* (1955) mentioned that a simple vitamin E deficiency per se does not cause encephalomalacia in the chick. The addition of unsaturated fats such as cod liver oil to a vitamin E deficient diet will produce the disease, however.

The second part of Tappel's theory suggests that the fat undergoes peroxidation, and that the resulting lipid peroxides damage the lipid component of lysosomal, microsomal, and mitochondrial membranes. Tappel has measured increased lipid peroxide levels in the tissues of vitamin E deficient animals.

If the lysosomal membranes were damaged, and their proteolytic enzymes were subsequently released, these enzymes could conceivably cause tissue damage by autolytic digestion. Bunyan *et al.* (1967a,b), working in England, described increased tissue levels of these hydrolases in vitamin E deficient chicks exhibiting muscular dystrophy and exudative diathesis. He has also reported increased levels in vitamin E deficient rats displaying testicular degeneration.

If vitamin E acts as a lipid antioxidant, a deficiency of the nutrient would cause decreased levels of unsaturated fats in the tissues and increased tissue levels of lipid peroxides. Bunyan *et al.* (1967c) has reported that vitamin E deficiency does not depress total polyunsaturated fatty acid levels in rat kidney, heart, spleen, brain, adrenal, and adipose tissue during experimental periods of up to 13 months. It has also been reported that adipose tissue levels of lipid peroxides in vitamin E deficient rats remain low unless large amounts of unsaturated fats are fed for long periods of time (Bunyan *et al.*, 1968). Bunyan *et al.* (1967a) reported that there was no rise in lipid peroxides

in dystrophic chick breast muscle, cerebellum, cerebrum, or adipose tissue. Similarly, lipid peroxide levels in chicks with exudative diathesis were not altered. In contrast to Tappel, these British workers concluded that vitamin E is not concerned with lipid peroxidation *in vivo*.

Other workers have suggested that vitamin E may be involved in ion transport and cellular respiration. A discussion of these hypotheses is included in the section on dietary hepatic necrosis.

Selenium

History. Prior to the 1950's, selenium was thought to be important only as a toxic element found in the soil of certain parts of the United States. In 1957, it was reported to be interrelated with vitamin E and effective in preventing many of the syndromes attributed to vitamin E deficiency (Schwarz, 1962). A year later, Muth (1963) reported that 0.1 ppm selenium protected lambs and calves against white muscle disease. Selenium at a level of 0.3 ppm was reported to prevent exudative diathesis in chicks (Patterson *et al.*, 1957).

Tissue Distribution and Excretion. Wright and Bell (1966) reported that an oral dose of radioactive labeled selenium produces peak plasma and whole blood concentrations 12 hours after administration. Less than 20% was present in the circulation 4 hours after an intravenous dose. Oral administration results in 15% of the dose being excreted in the feces and 7.5% being excreted in the urine. Following an intravenous dose, 21% is excreted in the urine and 2.7% is excreted in the feces. The greatest concentration of selenium following intravenous administration is in the kidneys.

Jones and Godwin (1963) found a preferential concentration of selenium in the nuclei of most tissues.

McConnell (1963) reported that selenium can pass the placental barrier in dogs. He also found that 90% of the selenium in a lactating bitch's milk is bound to protein.

Metabolic Role. Olcott *et al.* (1961) suggested that selenomethionine may act as a lipid antioxidant *in vivo*. Hamilton and Tappel (1963) mentioned that selenium was 500 times more active as an antioxidant than alpha-tocopherol. They also reported that there did not appear to be any synergism between the two nutrients. They concluded that selenium acts independently in reacting with free radical intermediates of lipid peroxidation, thus breaking the chain reaction.

Schwarz (1958b) suggested that selenium and vitamin E were each essential to the proper function of two separate, but equivalent, metabolic pathways. Either one of these pathways was sufficient to maintain an animal's health.

Vitamin E Deficiency in Swine

The gross and microscopic lesions of vitamin E deficiency in the pig may assume any one or a combination of many forms. The important syndromes include: "yellow fat" (Davis and Gorham, 1954), liver necrosis (Hove and Seibold, 1955), muscular dystrophy (Marr *et al.*, 1956), anemia (Nafstad, 1965), mulberry heart disease (Lamont *et al.*, 1950), cardiac and extracardiac vascular degeneration (Grant, 1961).

Mulberry Heart Disease

Natural Occurrence. Lamont (1950), in Ireland, was the first to describe a naturally occurring syndrome, which he called mulberry heart disease (MHD). The myocardium of affected pigs was deep purple, and there were subepicardial hemorrhages of varying size scattered over its surface. Harding (1960), working in England, described the microscopic appearance of naturally occurring MHD. He observed areas of congestion and hemorrhage in the myocardium. In addition, there was pyknosis of the muscle nuclei, hyaline degeneration, and loss of cross striations in the myocardial fibers.

Donnelly (1969), working in Ireland, has described three separate clinical types of MHD. Type I occurs in 80% of all clinical cases, and is characterized by sudden death. Type II occurs in 17% of affected pigs. Prior to death, skin pallor, cyanosis, palpebral edema, respiratory distress, and ataxia are observed. Type III is characterized only by a short period of ataxia before death.

A Swedish worker, Grant (1961), has made an extensive study of naturally occurring MHD. Sudden death was the most common clinical sign reported. Gross lesions included subepicardial petechiae and ecchymoses, subendocardial hemorrhages, mottling of the myocardium, hydrothorax, and ascites. Histopathologic examination revealed congestion, hemorrhage, and myocardial degeneration. Sections stained by the periodic acid-Schiff method demonstrated an accumulation of PAS-positive material in the walls of capillaries, precapillary arterioles, and arterioles. Since the latter was the primary lesion, he named this disease "microangiopathy" (MAP). Extracardiac MAP was found in the lungs, skin, kidneys, stomach, skeletal muscle, liver, and adrenal capsule. He concluded that MHD and MAP are one disease.

Experimental Mulberry Heart Disease. Grant (1961) produced MHD (MAP) with various experimental diets, and has demonstrated the protective effect of selenium and tocopherol. Pigs fed a diet consisting of a semi-synthetic soya meal, with cod liver oil, developed MAP. Pigs fed the same diet to which sodium selenite (0.2 mg./kg. of ration) was added were fully protected. Pigs fed grain and high levels of corn oil developed MAP. Tocopherol supplementation effectively suppressed the development of MAP in pigs fed this diet.

Dietary Hepatic Necrosis

Quin and Shoeman (1933), in the United States, made one of the earliest reports of what was probably dietary hepatic necrosis (DHN). They investigated an outbreak in a herd of swine in Iowa and described the livers as being covered with spots of hemorrhage. They were unable to determine the cause of the outbreak.

Daft *et al.* (1942) reported the occurrence of DHN in rats fed a casein-base diet. The addition of methionine or cystine in sufficient amounts prevented the disease.

Dietary hepatic necrosis has been produced in swine fed a vitamin E deficient diet containing cod liver oil (Hove and Seibold, 1955).

Michel *et al.* (1969), using a fat-free torula yeast diet, produced DHN in swine. Supplementation with methionine or low levels of vitamin E did not completely prevent the disease. High levels of vitamin E, additional protein, or selenium offered complete protection.

The basic biochemical disturbance in dietary hepatic necrosis is unknown. Schwarz (1962) has reported that liver in the latent phase of DHN cannot maintain normal respiratory activity *in vitro*. He has named this phenomenon respiratory decline. He theorizes that the cause of this

decline is a blockage of sensitive sulfhydryl sites, by trace element(s), on an enzyme necessary for respiration in mitochondria. The origin of the trace element(s) is unknown.

McLean (1963) suggests that the basic lesion is faulty ion transport. Normal rat liver slices lose potassium in cold saline solution and reaccumulate potassium when placed in warm, oxygenated Ringer's solution. Liver slices from rats fed a vitamin E deficient diet are unable to reaccumulate the potassium lost during cooling. In addition, oxygen uptake in the cooled liver slices decreases. The addition of vitamin E to the diet abolishes these defects, whereas selenium does not. If liver slices from vitamin E deficient rats are incubated as soon as they are cut, the ionic composition does not change, and oxygen uptake does not decrease. McLean suggests that the defects in vitamin E deficient tissues, that are caused *in vitro* by the stress of cooling, may be produced *in vitro* by some other stress. Vitamin E deficient tissues may not be able to recover from situations that stress the ion transport mechanism.

Muscular Dystrophy

Metzger and Hagan (1927), at Cornell, were the first to report muscular dystrophy (MD) in animals. They investigated an outbreak that occurred in a flock of sheep, but were unable to determine the cause. Marr *et al.* (1956) reported 3 outbreaks of MD in sheep in the north of Scotland.

Diet plays an all-important role in the production of the disease. Century and Horwitt (1960) have reported that the addition of 7% cod liver oil, 7% linseed oil, or 15% corn oil to a vitamin E deficient diet will produce the disease readily in rats. The addition of 15% coconut oil or 0.2% corn oil failed to produce the disease.

A diet using purified soya meal as a source of protein has been used to produce muscular dystrophy in swine (Ewan *et al.*, 1969).

Young *et al.* (1961) reported that the disease in lambs is not related simply to the length of time their dams were fed a diet known to cause muscular dystrophy, but was dependent on the stage of gestation or lactation during which it was fed. The greatest incidence of MD in lambs occurred when the dystrophogenic ration was fed to ewes during late gestation and early lactation.

High levels of sulphur in the diet may negate the protective effect of selenium and increase the incidence of muscular dystrophy (Hintz and Hogue, 1964).

The amount of activity the muscle is subjected to appears to play an important role in the production of the disease. Pappenheimer and Goettsch (1940) performed unilateral sciatic nerve sections on young rats nursing dams fed a vitamin E deficient diet. Muscular dystrophy was later found in the gastrocnemius of the leg with the intact nerve. The gastrocnemius of the leg on which a nerve section had been performed displayed only those changes associated with nerve section.

Young and Keeler (1962) immobilized the left foreleg of lambs born to ewes fed a diet known to cause muscular dystrophy. Muscular dystrophy was later observed, but the muscles of the left foreleg were unaffected.

Serum Enzymes

Serum enzyme determinations are useful as an aid in the diagnosis of many diseases, and are often of value in prognosis.

Serum glutamic oxaloacetic transaminase (SGOT) levels have been used to aid in the diagnosis of muscular dystrophy. The enzyme is found in most body cells, but its concentration is highest in muscle (Medway *et*

al., 1969). Blincoe and Marble (1960) have reported marked increases of SGOT in sheep with white muscle disease.

Serum lactic acid dehydrogenase (LDH) levels are often used in man, in conjunction with SGOT determinations, to aid in the diagnosis of acute myocardial infarction (Medway *et al.*, 1969). Blincoe and Marble (1960) have reported that LDH values are markedly elevated in sheep with white muscle disease.

MATERIALS AND METHODS

Experimental Animals and Design

A single litter of 6 male and 2 female Yorkshire-cross pigs was used. They were purchased from a local farmer and were 12 days old when the experiment began. Prior to the start of the experiment, the pigs were identified by ear-notching. They were weighed at the beginning and end of the experiment, and at intervals during its course. Their final weights were determined at the time of death or as soon after death as possible. The pigs were observed twice daily for signs of illness. The general experimental design is given in Table 2.

Housing

Pigs 1 through 6 were maintained on a concrete-floored 4' x 12' pen. The pen was in a heated, isolated room. Pigs 7 and 8 were maintained in 1.5' x 2' metal metabolism cages.

Method of Exercise

Pigs 1 through 4 were exercised daily for 30 minutes on a motor driven treadmill. The circumference of the treadmill was 90 inches and, when in operation, it revolved at a speed of 25 rpm. The total distance traveled by each pig in the 30-minute exercise period was approximately 1 mile. Pigs 5 and 6 were allowed to move freely within their pen. Movement was restricted in Pigs 7 and 8 by the confines of the cages.

Rations and Feeding Practices

All pigs were fed twice daily. Those in individual cages were fed and watered in separate bowls. Water was supplied to the penned pigs by an automatic device. The caged animals were watered individually. They emptied their bowls frequently and were often without water during part of the night.

The feed was prepared in a Hobart commercial mixer,* in amounts calculated to last 1 week. The prepared feed was stored in polyethylene bags at room temperature.

During the last 9 days of the experiment, silver acetate was added to the basal ration as a 0.15% (weight/volume) solution.

The basal ration and the time length each variation was fed are given in Table 1.

Supplements

The supplements used in this experiment included: vitamin E (aqueous) and selenium. One hundred I.U. of aqueous vitamin E and 0.2 mg. of sodium selenite were administered to Pigs 1 and 2 by intramuscular injection, 3 times weekly.

Serum Enzyme Determinations

Blood samples were collected from the anterior vena cava at weekly intervals during the course of the experiment. Terminal samples were collected prior to euthanasia of the supplemented animals and as soon after death as possible in the unsupplemented pigs. The samples were then centrifuged, and the serum was withdrawn and frozen. They were thawed

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*Hobart Manufacturing Company, Troy, Ohio.

Table 1. Basal ration

Day 1 to 41		Day 42 to 48		Day 49 to 67	
Purified Soya Meal*	25%	Purified Soya Meal	10%	Purified Soya Meal	5%
Cerelose	60%	Cerelose	79%	Cerelose	80%
Cod liver oil	5%	Cod liver oil	5%	Cod liver oil	5%
Cellulose	5%	Cellulose	5%	Cellulose	5%
Mineral Mix**	4%	Mineral Mix	4%	Mineral Mix	4%
Vitamin Mix***	25 ml./ kg. ration	Vitamin Mix	25 ml./ kg. ration	Vitamin Mix	25 ml./ kg. ration

*General Biochemicals, Chagrin Falls, Ohio. Approximately 82% protein.

**Mineral Mix:

Calcium carbonate	26.8760%
Calcium phosphate monobasic	8.5110%
Cupric sulphate	0.0268%
Ferric citrate	2.4620%
Magnesium sulphate	9.1380%
Manganese sulphate	0.0313%
Potassium iodide	0.0716%
Potassium phosphate dibasic	37.8500%
Sodium chloride	15.0060%
Zinc chloride	0.0223%
Cobalt chloride	0.0050%
	100.0000%

***Vitamin Mix:

Thiamine mononitrate	0.300 gm.
Riboflavin	0.600 gm.
Pyridoxine	0.200 gm.
Calcium pantothenate	3.000 gm.
Niacin	4.000 gm.
Para-amino-benzoic acid	1.300 gm.
Biotin	0.005 gm.
Folic acid	0.026 gm.
Inositol	13.000 gm.
Ascorbic acid	8.000 gm.
Choline chloride	130.000 gm.
Vitamin B_{12} (0.1% triturated)	10.000 gm.
Vitamin D_2 (500,000 I.U./gm.)	0.044 gm.
Menadione sodium bisulfite	0.400 gm.
Absolute ethyl alcohol	250.000 ml.
Distilled water q.s.	2500.000 ml.

later and lactic acid dehydrogenase* and serum glutamic oxaloacetic transaminase* values were determined.

Pathology

The number of days each pig was maintained on the experimental regimen is given in Table 2. The animals were necropsied at the end of the indicated time period.

The supplemented pigs were euthanatized by electrocution. A careful gross necropsy was performed on each pig. Tissues collected for microscopic study included: stomach, duodenum, jejunum, ileum, spiral colon, lung, heart, tongue, intercostal muscle, diaphragm, and masseter muscle. Sections were also taken from the superficial gluteal, quadriceps femoris, longissimus dorsi muscles, adrenal, kidney, urinary bladder, liver, gallbladder, ovary, testicle, brain, and skin. Blood and tissue (liver) samples were collected and frozen for future analysis of vitamin E and selenium content.

The tissue specimens for histopathologic examination were fixed in 10% neutral buffered formalin. They were processed in an automatic processing machine,** sectioned at 6 μ, and stained with hematoxylin and eosin and by the periodic acid-Schiff method, as described in the Armed Forces *Manual of Histologic and Special Staining Technics* (1960).

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*Sigma Kit No. 505, Sigma Chemical Company, St. Louis, Mo.

**Autotechnicon, Technicon Company, Chauncey, New York.

Table 2. Experimental design

Pig No.	Treatment	Exercise	Days on Experiment
1	Basal + E-Se	Treadmill*	67†
2	Basal + E-Se	Treadmill*	64†
3	Basal	Treadmill*	64††
4	Basal	Treadmill*	63††
5	Basal	Penned**	66††
6	Basal	Penned**	61††
7	Basal	Caged***	63††
8	Basal	Caged***	65††

*30 minutes/day

**Free movement in pen

***Metabolism cages

†Euthanatized

††Died during the course of the experiment

RESULTS

The animals ate well during the greater part of the study period. When the protein level of the ration was lowered to 5%, however, their appetites decreased. Pigs 1 through 6 gained weight rapidly, were well muscled, and had smooth, glossy hair coats. Pigs 7 and 8 gained very slowly and had very dull and rough hair coats. The total and average daily weight gains are given in Table 3. During the last week of the experiment, all pigs developed a diarrhea. Bacteriologic cultures of the kidney, lungs, and spleen were negative for pathogens in Pigs 1, 2, 3, 4, 6, and 7. Cultures of the ileum of the above animals were also negative for pathogens. In Pig 5, there was a heavy overgrowth of all organs and ileum with *Proteus sp.* In the viscera of Pig 8, there was a moderate growth of *Proteus vulgaris* and a nonhemolytic *Streptococcus sp.* *Salmonella sp.* were isolated from the ileum of this pig. It is presently being serotyped.

The supplemented animals had no difficulty running on the treadmill at any time during the experiment. About midway in the study period, Pigs 3 and 4 began to tire after approximately 20 minutes of exercise. After a short period of rest they would appear normal. During the last week of the experiment, Pig 3 would collapse and refuse to rise after approximately 20 to 25 minutes of exercise. After a short period of rest it would appear normal.

Death occurred suddenly in the unsupplemented pigs. The pig would appear normal at the evening feeding and be found dead the next morning.

Table 3. Total and average daily weight gain

Pig No.	Treatment	Starting Wt. (kg.)	Total Wt. Gain (kg.)	Days on Experiment	Avg. Daily gain (kg.)
1	Basal + E-Se (treadmill)	5.20	17.05	67	.26
2	Basal + E-Se (treadmill)	2.04	4.23	64	.07
3	Basal (treadmill)	5.59	12.59	64	.20
4	Basal (treadmill)	3.00	12.90	63	.21
5	Basal (penned)	5.21	12.49	66	.19
6	Basal (penned)	3.18	14.54	61	.24
7	Basal (caged)	3.63	6.82	63	.11
8	Basal (caged)	4.36	8.82	65	.14

Pigs 3 and 5 were observed just prior to death. They displayed ataxia, respiratory distress, and collapse just prior to death.

Serum Enzymes

Nonhemolyzed blood samples were difficult to obtain. Serum glutamic oxaloacetic transaminase (SGOT) and lactic acid dehydrogenase (LDH) values were not determined in those samples exhibiting hemolysis. The SGOT values were within normal limits in the supplemented pigs. The terminal SGOT levels in Pigs 3, 4, 7, and 8 were markedly elevated. The SGOT was slightly elevated in the last blood sample taken from Pig 6 before its death. The weekly SGOT values are given in Table 4.

The LDH values were very high and variable in all animals. These values are given in Table 5.

Pathology

Grossly there was mottling of the myocardium, liver necrosis, generalized venous congestion, edema of the gallbladder, hydrothorax, and ascites. Histopathologic examination revealed cardiac myopathy, muscular dystrophy, and pulmonary lesions. The following describes the general gross and histopathologic appearance of the lesions.

Cardiac Lesions

Gross. The hearts of the supplemented pigs were normal on gross examination. In those animals not supplemented with vitamin E and selenium, the right ventricle appeared collapsed and thin on cut section. The surface of the myocardium was mottled with pale and dark red areas (Figure 1). These extended through the entire thickness of the myocardium. There were numerous petechiae on the epicardial surface of the right ventricle of

Table 4. Weekly SGOT values (Sigma-Frankel units)

Pig No.	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Terminal
1	34	H	H	H	22	54	28
2	30	H	H	H	H	30	H
3	18	H	H	H	30	36	250
4	26	50	H	H	30	60	240
5	18	50	H	H	22	36	H
6	32	H	H	H	20	60	H
7	34	22	H	H	H	H	220
8	44	H	36	54	134	224	240

H = hemolysis

Table 5. Weekly LDH values (I.U.)

Pig No.	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Terminal
1	900	H	H	H	1400	1440	H
2	750	H	H	H	H	1390	1720
3	1180	H	H	H	1240	1720	1840
4	1280	1540	H	H	1280	1660	1840
5	1320	1500	1400	H	1180	1030	H
6	1560	H	H	H	1440	1780	H
7	1160	1180	H	H	H	H	1820
8	1560	H	1180	1280	1800	1800	1840

H = hemolysis

Pig 8. Numerous subendocardial hemorrhages were found in the left ventricle of Pigs 3, 4, 5, and 6 (Figure 2). They caused the overlying endocardium to bulge and appeared to extend into the myocardium a few mm.

Microscopic. There was rowing of the sarcolemmal nuclei and slight congestion of the myocardium of the pigs supplemented with vitamin E and selenium.

The dark red areas observed grossly in the myocardium of the unsupplemented animals were extremely congested microscopically. There was also hemorrhage between the muscle fibers in these areas. Those areas that were pale grossly displayed slight congestion and little hemorrhage between the muscle fibers. The subendocardial extravasations caused the overlying endocardium to bulge, and the hemorrhage extended into the myocardium, separating the muscle fibers slightly (Figure 3). The hemorrhage also caused separation and displacement of the Purkinje fibers in the area. There was a dramatic increase in the number of sarcolemmal nuclei, and many appeared pyknotic. The majority of the nuclei were arranged in linear rows. The sarcoplasm of many of the muscle fibers appeared to be undergoing a granular form of degeneration. Within a muscle cell, there would be many small, spherical, slightly eosinophilic granules surrounding the nucleus. There were many focal areas in which individual fibers or groups of fibers were undergoing fragmentation and myolysis, and in these areas there were aggregations of sarcolemmal nuclei (Figure 4). The most severe lesions were observed in Pig 8. Very little of the myocardium was unaffected. In addition to the changes listed above, there were many focal areas of calcification and fibrosis.

Figure 1. Mottling of the myocardium and flaccid appearance of right ventricle. Pig fed basal ration.

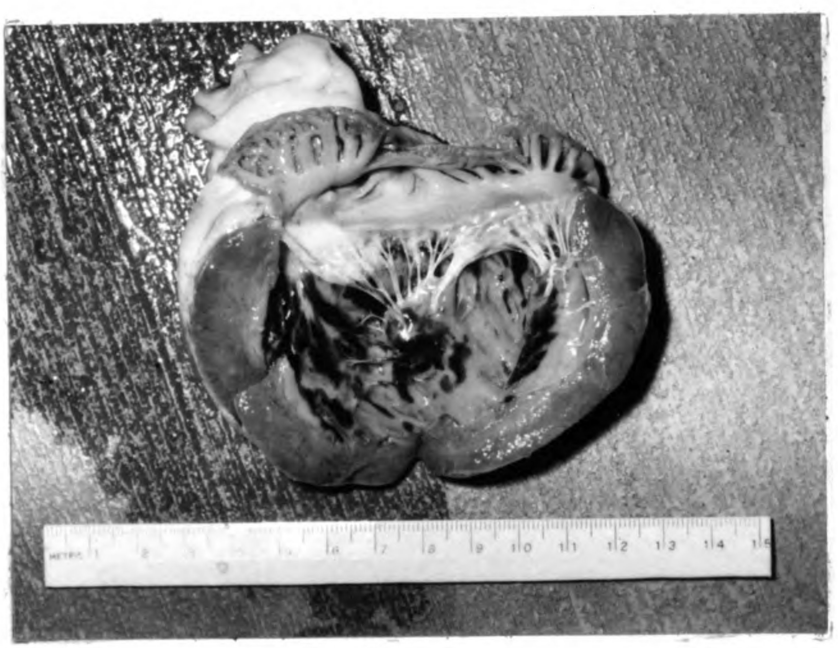

Figure 2. Subendocardial hemorrhage. Pig fed basal ration and exercised on treadmill.

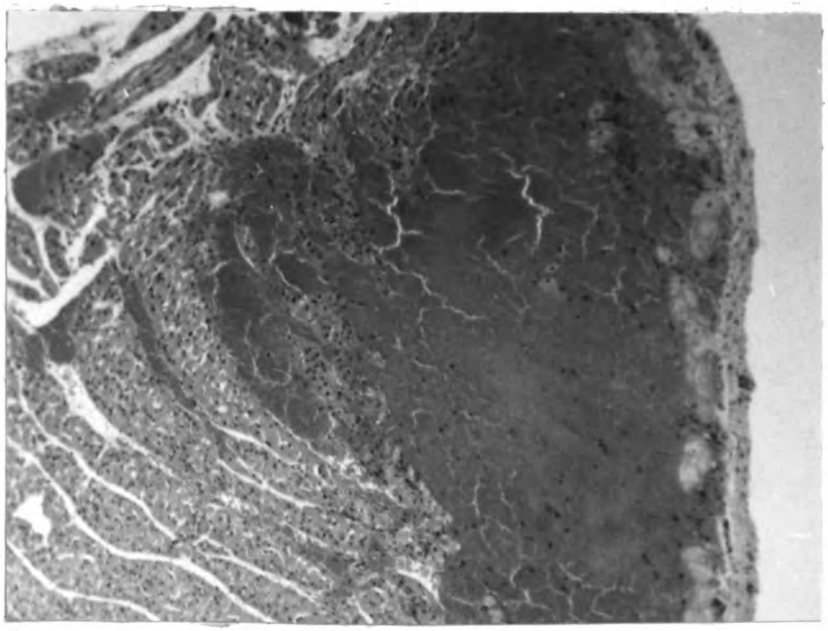

Figure 3. Subendocardial hemorrhage. Pig fed basal ration and exercised on treadmill. Hematoxylin and eosin stain. x 400.

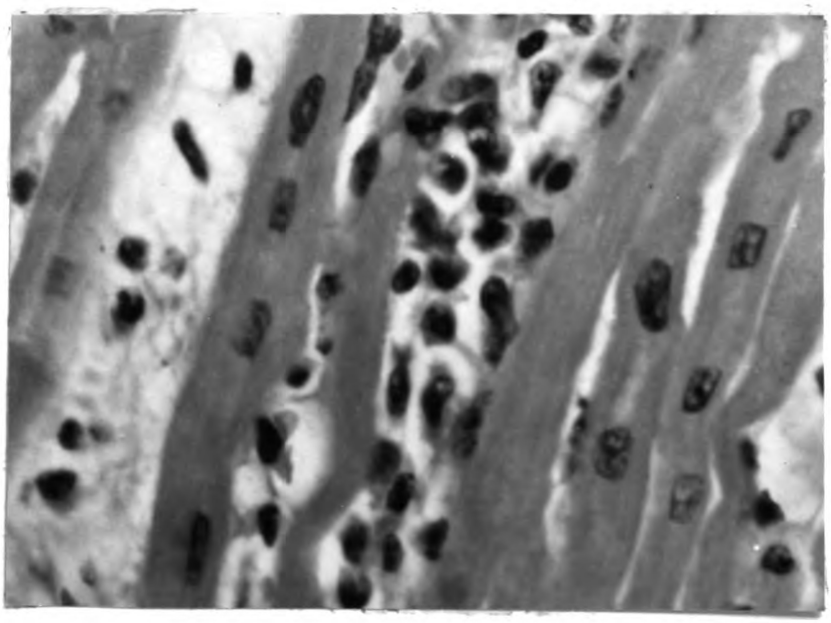

Figure 4. Cardiac myopathy. Note the local increase in muscle nuclei. Pig fed basal ration. Limited exercise. Hematoxylin and eosin stain. x 400.

Sections stained by the periodic acid-Schiff method did not display the microangiopathy described by Grant (1961).

Hepatic Necrosis

Gross. The livers of those animals supplemented with vitamin E and selenium were normal. The livers from the nonsupplemented pigs were enlarged and friable. There were numerous red spots of varying size scattered over the surface and in the parenchyma. There were 4 fractures, each approximately 5 cm. long, on the surface of the liver of Pig 7 (Figure 5). There were large blood clots adhering to the fractured areas.

Microscopic. In the livers from the animals supplemented with vitamin E and selenium, the cytoplasm of the hepatocytes failed to stain with hematoxylin and eosin. The lesion was not due to fat content, as an oil red O stain was negative. Suitably fixed tissues were not available for glycogen stains, but the lesion was suggestive of hydropic degeneration.

In the livers from the nonsupplemented group, some lobules displayed only a vacuolar degeneration of the hepatocytes and congestion of the sinusoids. In other lobules, there was centrilobular necrosis and pooling of blood around the central vein (Figure 6). Hepatocytes peripheral to this were undergoing a vacuolar degeneration and the sinusoids were congested. In many lobules, the architecture was completely obliterated by necrosis of the hepatocytes and a filling of the lobule with blood. The necrosis was limited by the interlobular connective tissue. In addition to these changes, there was a moderate increase in the interlobular connective tissue in Pigs 7 and 8.

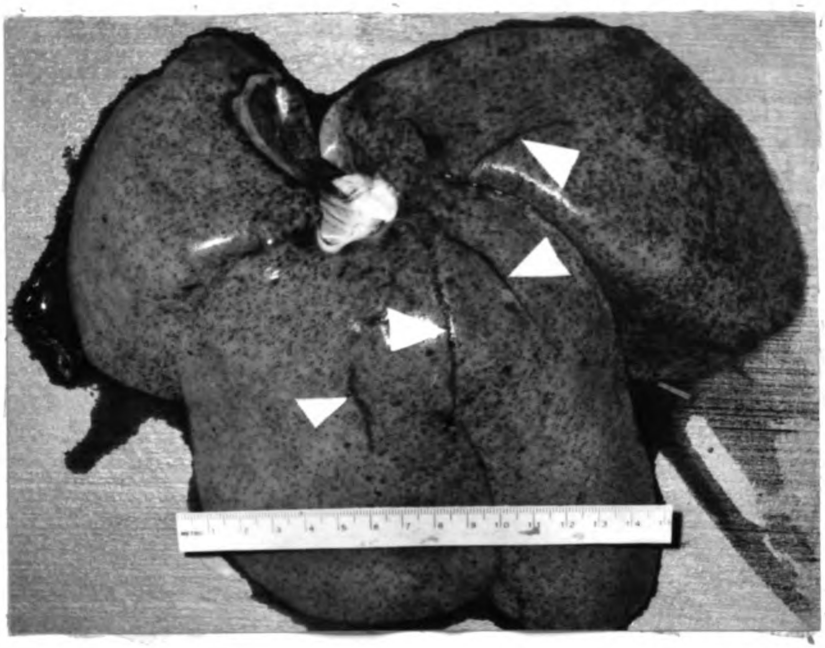

Figure 5. Hepatic necrosis. Pig fed basal ration. Arrows point to fractures.

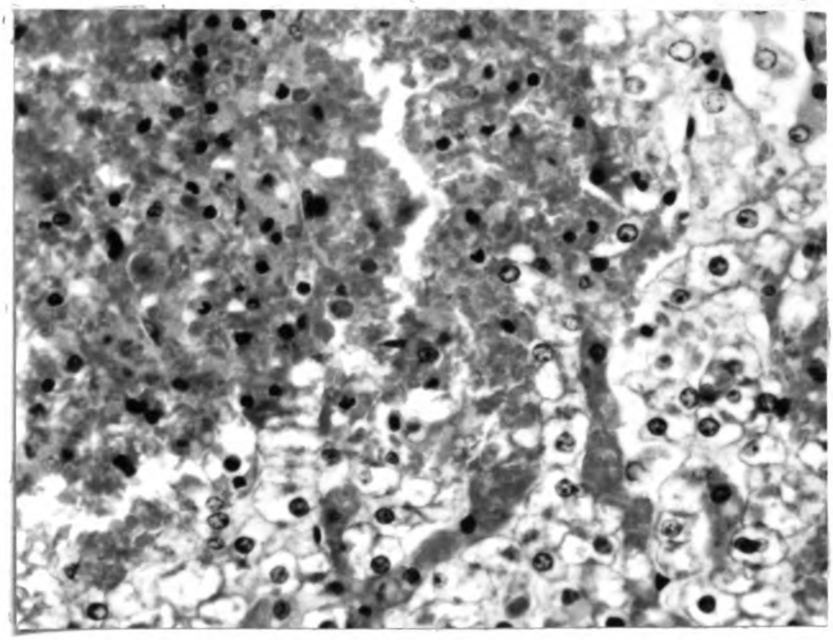

Figure 6. Hepatic necrosis. Note pyknotic nuclei, hemorrhage and hydropic degeneration. Pig fed basal ration. Hematoxylin and eosin stain. x 400.

Muscular Dystrophy

Gross. There were no gross lesions of muscular dystrophy in the supplemented animals. The skeletal muscles of Pigs 3, 5, and 8 were slightly pale.

Microscopic . There was no apparent pattern of involvement in the muscles of the nonsupplemented animals. In some sections, only edema and separation of the fibers were observed. In other sections, there was an increase in the number of sarcolemmal nuclei, nuclear rowing, and focal areas of myolysis. In these areas, there were aggregations of sarcolemmal nuclei (Figure 7).

Additional Findings

Ascites and Hydrothorax. There were no significant collections of fluid in the thorax or abdomen of the supplemented animals. There were 15 to 20 ml. of serosanguineous pleural fluid in Pigs 3, 4, 5, 6, 7, and 8. Approximately 20 ml. of serosanguineous fluid were found in the peritoneal space of Pigs 4, 6, and 8. There were approximately 60 ml. of serosanguineous fluid in the peritoneal space of Pigs 3 and 5. There were 110 ml. of sanguineous fluid in the peritoneal space of Pig 7.

Generalized Venous Congestion. In the nonsupplemented animals, there was moderate to severe congestion grossly and microscopically in the following organs: brain, posterior 2/3 of the small intestine, spleen, spiral colon, lungs, kidneys, adrenal, liver, and stomach.

Edema and Hemorrhage of the Gallbladder. The gallbladders from the supplemented animals were normal grossly and microscopically. Those from the nonsupplemented animals were opaque and distended with bile on gross

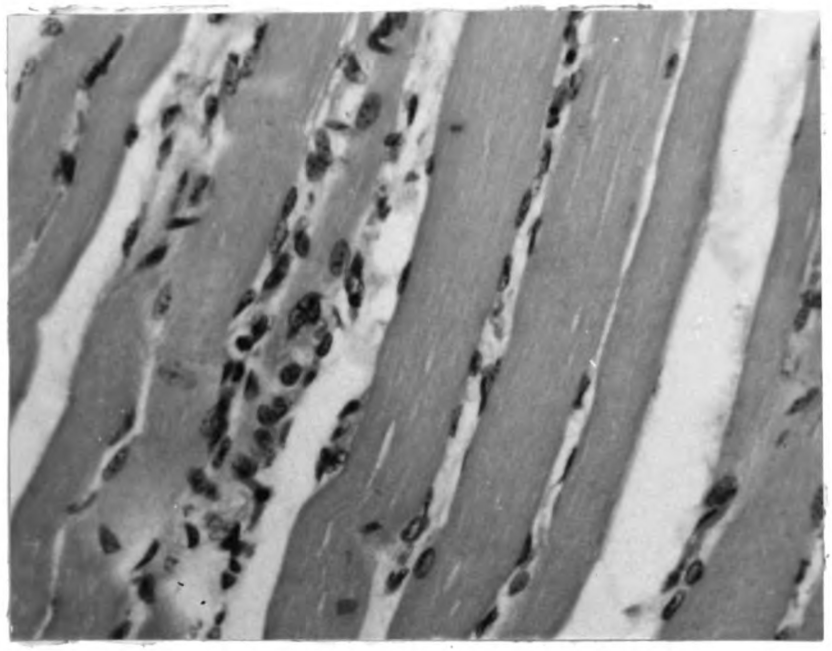

Figure 7. Muscular dystrophy. Note local increase in muscle nuclei. Pig fed basal ration. Limited exercise. Hematoxylin and eosin stain. x 400.

examination. The bile was very viscous, the wall of the structure was thickened on cut section, and there was a line of hemorrhage at the gallbladder-liver junction. Microscopically, the wall was very edematous.

Pulmonary Lesions. On gross examination, the lungs of all pigs exhibited some degree of pathologic alteration. In general, there was emphysema, congestion, focal areas of atelectasis and, in some pigs, consolidation. Microscopically, there were alternating areas of emphysema and atelectasis and severe congestion. In Pigs 3, 4, 5, 6 and 7 there was pulmonary edema and inflammatory infiltrates consisting of primarily lymphocytes.

DISCUSSION

Clinical Signs and Mortality

Clinical signs were not a consistent feature of this experiment. This is in agreement with the reports in the literature. The diarrhea observed in Pigs 1, 2, 3, 4, 6, and 7 is difficult to explain, as all organs and the ileum were negative for pathogens by bacteriologic culture. In Pig 8, the diarrhea was very likely due to the *Salmonella* that were present. The *Salmonella* organism may have played a role in the pathogenesis of the diarrhea in Pig 5, but this would be difficult to prove, as the only organism found was *Proteus sp.* A heavy overgrowth of one organism may mask the presence of another, however.

The tiring observed in Pigs 3 and 4 when exercised may have been due to a weakness in the skeletal muscles or the result of the decreased output of a failing heart.

Sudden death, and the signs of ataxia, respiratory distress, and collapse observed in Pigs 3 and 5 prior to death, suggest that acute circulatory failure was the most probable cause of death in all animals. The fractures of the liver in Pig 7 and the large quantity of bloody fluid in its abdomen, indicate that in this animal a decrease in blood pressure may have played a secondary role in its death. The cardiac myopathy would almost certainly interfere with muscular contraction, while subendocardial hemorrhage, by separating and displacing the Purkinje fibers, would probably induce conduction defects. The condition of the liver must also be considered in determining the cause of death. Acute,

massive liver necrosis would interfere with the liver's ability to perform a variety of vital functions.

Protein Content of Ration

The basal ration containing 25% protein was fed to all pigs for 41 days. At the end of this time, all pigs appeared clinically normal. It was thought that perhaps the high protein level may have been exerting a protective effect. Consequently, it was decided to lower the protein level to 5%. At this point all pigs had been eating well. When the protein level in the ration was decreased to 5%, however, there was a decrease in appetite. It was difficult to determine if supplementation with vitamin E and selenium affected appetite as both the supplemented and nonsupplemented pigs ate from the same pan.

The low protein level in the ration may also have played a role in the pathogenesis of the ascites and hydrothorax observed in these animals.

Growth

Supplementation with vitamin E and selenium did not appear to enhance the growth rate. This supports the work of Ewan *et al.* (1969). The slowest growth rate was observed in an animal supplemented with vitamin E and selenium. This animal, however, was the "runt" of the litter, in that its weight at the beginning of the experiment was approximately half that of its siblings. Growth rate also seemed to be depressed in the nonsupplemented animals that were housed in metabolism cages. There was an approximately 50% reduction in average daily gain in these pigs. The "stress" of close confinement may have been a factor in suppressing their growth rate.

Effect of Silver Acetate

Dietary liver necrosis has been observed in rats fed a vitamin E deficient diet, to which silver acetate was added (Diplock *et al.*, 1967). The effect of silver acetate in this experiment is difficult to evaluate. The day after it was first added to the ration, the first death occurred. The lesions in this animal were quite severe, and it would seem unlikely that the silver exerted any major influence in their development. The effect of silver acetate in the remainder of the pigs is impossible to evaluate, as all were being fed silver in the basal ration.

Serum Enzymes

Serum enzyme determinations were hampered by the difficulty in obtaining nonhemolyzed blood samples. This increased susceptibility to hemolysis was observed in all animals at various times. This suggests that vitamin E-selenium deficiency increases the fragility of the erythrocyte and makes it more susceptible to hemolysis. In support of this finding, Fitch (1968) has reported increased susceptibility to hemolysis in erythrocytes of primates fed a vitamin E deficient diet. Regardless of its pathogenesis, any degree of hemolysis will interfere with SGOT and LDH analyses. The markedly elevated SGOT values in the non-supplemented pigs were probably the result of cardiac and skeletal myopathy, as well as liver necrosis. Blincoe and Marble (1960) have reported elevated SGOT values in sheep with white muscle disease. The SGOT values in Pigs 3, 4, 6, and 7 increased quite late in the experiment. In contrast, there was a more gradual increase in Pig 8, which suggests that the lesions in this animal were of a more chronic nature. The microscopic appearance of the heart and liver verified this finding.

The extremely high LDH values observed in all pigs may have been due to trace hemolysis that could not be detected visually.

Method of Exercise

Certain deficiencies in the design of the treadmill were encountered during the latter part of the experiment. The animals survived longer than was expected and, as a result, were almost too large to exercise on the treadmill. They were able to stop the treadmill at will, but gentle coaxing prevented this. Perhaps a more powerful motor would have prevented this problem.

Since the pigs were exercised daily, the possibility must be considered that they became conditioned to the exercise. In future experiments, it might be wise to wait until the latter stage of the experimental period before exercising the animals.

Pathology

Cardiac Lesions. The results of this experiment suggest that supplementation with vitamin E and selenium affords pigs complete protection against the lesions produced in nonsupplemented animals. Grant (1961), in a series of experiments, reached the same conclusion. The results also suggest that some form of exercise enhances the development of subendocardial hemorrhage. There was very little difference in the severity of lesions between those pigs that were exercised on the treadmill and those given limited exercise. This suggests that perhaps the amount of exercise the pigs were given on the treadmill was insufficient to cause any major difference in severity of lesions. The chronic nature of the lesions in Pig 8 suggests that perhaps close confinement may be a greater stress than exercise.

Hepatic Necrosis. The results of this study suggest that supplementation with vitamin E and selenium completely protects pigs against hepatic necrosis of dietary origin. Michel *et al.* (1969) has reported that high levels of vitamin E or selenium will prevent hepatic necrosis in pigs fed a vitamin E deficient diet. A very characteristic feature of the lesion in this experiment was the limitation of the necrosis by the interlobular septa. Chronic changes do occur, as is indicated by the proliferation of connective tissue in the interlobular septa of Pigs 7 and 8.

Hydropic Degeneration of the Liver. The failure of the hepatocytes of all livers to stain with hematoxylin and eosin, oil red O, or by the periodic acid-Schiff method, suggests that this lesion was hydropic degeneration. This lesion may have been the result of the low protein level in the ration during the latter part of the experiment. This is in agreement with the report of Hove and Seibold (1955). The addition of silver acetate to the ration may also have played a role in the pathogenesis of this alteration.

Muscular Dystrophy. The results of this experiment suggest that supplementation with vitamin E and selenium also fully protects pigs against nutritional muscular dystrophy. The degree of muscular dystrophy in the experimental animals was not severe. Other workers have reported muscular dystrophy in swine fed a vitamin E deficient diet in which soya bean meal was the source of protein (Ewan *et al.*, 1969).

There did not appear to be any difference in severity of lesions between the pigs that were exercised on the treadmill and those given limited exercise. Perhaps the amount of exercise the treadmill group was subjected to was insufficient to produce any major difference in severity. The pigs housed in metabolism cages had about the same degree

of muscular dystrophy as those that were exercised. This also suggests that close confinement may be as great a "stress" as exercise.

These results suggest that the basal ration may not be as dystrophogenic as other rations reported in the literature.

SUMMARY

Eight pigs were used to study the pathology of a vitamin E-selenium deficiency, as influenced by exercise. The basal vitamin E-selenium deficient diet was a purified ration containing adequate amounts of other nutrients. It was composed of soya protein, cerelose, and cod liver oil. The protein content of the ration was decreased during the experiment, and a stress agent, silver acetate, was fed to all pigs during the last 9 days of the experiment.

Pigs fed the basal ration died suddenly after an average of 63 days and exhibited cardiac degeneration, liver necrosis, and muscular dystrophy. Exercise did not significantly influence the clinical signs or severity of the lesions. Pigs fed the basal ration and subjected to exercise did display subendocardial hemorrhages, however, whereas the other pigs did not. The administration of vitamin E and selenium provided complete protection against the lesions noted.

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VITA

Kenneth M. Ayers was born in Waukegan, Illinois, on 5 August, 1939. He is a graduate of Pasadena Senior High School of Pasadena, Texas. He completed his preveterinary and veterinary studies at Texas A & M University. He received his D.V.M. on 5 August, 1966.

Following graduation from veterinary school, the author entered the United States Air Force as a First Lieutenant in the Veterinary Corps. After completing his basic officer training at Sheppard AFB, Texas, he was assigned to Royal Air Force Station Bentwaters, near Woodbridge, England. He served there as Chief of Veterinary Services from 28 October, 1966, to 2 August, 1969. Dr. Ayers was promoted to the rank of Captain on 27 March, 1967.

On 29 September, 1967, Captain Ayers married the former Jane Mary Black of Felixstowe, Suffolk, England. They have a daughter, Misty Michele, aged 18 months, and are expecting the arrival of their second child in September 1970.

In the fall of 1969, the author entered the graduate school at Michigan State University, under the sponsorship of the Armed Forces Institute of Technology, to pursue a Master's degree in veterinary pathology. After graduation, he will begin an assignment at the Armed Forces Institute of Pathology in Washington, D.C.