AVIAN MALARIA IN THE VITAMIN E-SELENIUM DEFICIENT DUCK

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By

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ABSTRACT

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A total of 44 white Pekin ducks was used in 2 experiments to evaluate the influence of *Plasmodium lophurae* and *Plasmodium spartani* infection in ducks fed an adequate or a vitamin E-selenium deficient diet. The ducks fed the vitamin E-selenium deficient diets developed clinical signs as early as 9 days on the diet. The most evident clinical signs were a general muscular weakness in vitamin E-selenium deficient ducks and an anemia in the infected ducks. Prominent lesions in this research included: skeletal myopathy (vitamin E deficiency), smooth muscle myopathy (selenium deficiency), and nephrosis, splenomegaly, and anemia (malaria). Because of focal areas of mineralization with skeletal and gizzard myopathy and severe myocarditis found in tissues of several infected, vitamin E deficient ducks, it would appear that vitamin E deficiency lesions may be made more severe by malaria. The plasma alpha tocopherol values decreased significantly ($P < .05$) while the serum glutamic-oxalacetic transaminase (SGOT) values were elevated in vitamin E deficient, malarious ducks. In vitamin E-selenium deficient duck erythrocytes, dialuric acid hemolysis proved to be sensitive and reliable only for the assessment of vitamin E status and not malaria. Furthermore, it appeared that selenium in the presence of vitamin E deficient erythrocytes provided partial protection against *in vitro* dialuric acid hemolysis.

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INTRODUCTION

A voluminous amount of general information is available on vitamin E, such as requirements, sources, biochemistry, storage in the body, clinical signs of deficiency, and chemical and histological lesions in standard texts and bibliographic references, such as *Vitamins and Hormones* (1962) and *Vitamin E* (1949). In general the importance of vitamin E in animal nutrition and health has increased in recent years. This emphasis has been attributed to numerous causes such as confinement rearing, increased growth rates, and infection.

In addition there appears to be a paucity of information on the interrelationship between vitamin E deficiency and infectious disease. In order to evaluate this interrelationship a study was made on the effects of a malarial infection in the vitamin E-selenium deficient white Pekin duck. Furthermore, there were 2 specific objectives for this study. First, major emphasis was to determine pertinent clinical signs and biochemical and histopathologic changes of vitamin E-selenium deficiency in the duck. Secondly, the influence of superimposed malarial infection on vitamin E-selenium deficient ducks was to be evaluated.

REVIEW OF LITERATURE

Brief Historical Background

Even though vitamin E was discovered about 50 years ago (Evans and Bishop, 1922), quite a few years went by before its importance in the field of nutrition and health received due recognition. Past experimental work pertinent to this research has demonstrated that major manifestations of tocopherol deficiency in animals include: (a) exudative diathesis, (b) encephalomalacia, (c) adipose tissue discoloration, (d) incisor depigmentation, (e) reproductive failure, (f) liver necrosis, (g) lung hemorrhage, (h) erythrocyte fragility, (i) anemia, and (j) muscular degeneration. A deficiency of vitamin E has also been associated with sterility in laboratory animals (Emerson and Evans, 1939) and with an influence on membrane permeability (Dam and Glavind, 1939). More recently a number of symposia and conferences have been devoted to vitamin E's role in human and animal nutrition (Association of Vitamin Chemists, 1969; Harry Steenbock Symposium, 1969; and the Fourth International Symposium on Vitamin E, 1970). These meetings all emphasized the importance of understanding how vitamin E functions in the animal organism and issued a challenge to elucidate its metabolic role.

Theories of Alpha Tocopherol Activity

Autoxidation. Zalkin and Tappel (1960) suggested that vitamin E was a biological antioxidant by functioning solely to stabilize cellular unsaturated lipids against oxidative deterioration. Tappel (1965)

reported that autoxidation of lipid is a fundamental cellular process occurring randomly and perhaps uncontrollably in the absence of vitamin E and other antioxidants. In his autoxidation theory Tappel stated that free radicals are formed during peroxidation and are highly toxic to the following subcellular compounds: enzymes, mitochondrial lipids, cytochromes, vitamin E, carotenoids, and sulfhydryl compounds. Madsen, McCay and Maynard (1935) found that cod liver oil, rich in unsaturated fatty acids, accelerated the onset of muscular dystrophy in vitamin E deficient rabbits and guinea pigs. This finding was the first evidence for the existence of biological autoxidation. In contrast, Blaxter, Brown and MacDonald (1953) found no depression of tocopherol in the liver, muscles, adipose tissue or serum of cod liver oil-induced dystrophic calves. Fitch and Dinning (1963) reported that signs of tocopherol deficiency in the monkey were not directly related to the amounts of dietary unsaturated fatty acids. Feeding rats high dietary levels of cod liver oil, Green *et al.* (1967) found no increased destruction of tocopherol in any tissue.

Enzymatic Activity. In contrast to the autoxidant theory of Tappel and associates, Nason, Donaldson and Lehman (1957) implicated vitamin E as an active compound of the terminal respiratory chain in mammalian skeletal and cardiac muscle. They concluded that vitamin E may function as a cofactor in cytochrome C reductase activity in both the nicotinamide adenine dinucleotide (NAD) and succinic oxidase systems. This point of view was not supported by the more recent work of Pollard and Bieri (1959). Schwarz (1965) stated that selenium and vitamin E may possibly have a biochemical role in the mitochondria.

Biological Membrane Stability. In producing exudative diathesis in vitamin E deficient chickens, Dam and Glavind (1939) noticed an increase in capillary permeability. Grant (1961) observed microangiopathy characterized by endothelial swelling, mural necrosis, and hyaline degeneration of the capillaries in tocopherol deficient swine. Lucy and Dingle (1964) advocated that vitamin E acts as a biological membrane stabilizer by preventing swelling and subsequent hemolysis of red blood cells in the presence of excess vitamin A. Using *in vivo* methods, Jacob and Lux (1968) found membrane phospholipids and thiols of vitamin E deficient rats were degradable by hydrogen peroxide. The membrane stability theory is upheld in part by the electron microscopy work of Porta, De La Iglesia and Hartroft (1969), who observed the plasma membrane of the hepatic cells to frequently rupture along the sinusoidal border in rat hepatic necrosis caused by vitamin E deficiency.

Pathology of the Vitamin E-Deficient Duck, Turkey and Dog

Avitaminosis E of ducklings is characterized by ducks walking awkwardly with their feet turned in. Later they may be found sprawled flat on the floor of their pens. Pappenheimer and Goettsch (1934) reported that vitamin E deficiency lesions of the duck were confined solely to the skeletal muscles. Microscopically, the lesions consisted of widespread hyaline necrosis of muscle fibers with edema and heterophilic cellular infiltration. In addition muscle creatine was lower than normal; creatine values of deficient birds ranged from 88 mg. to 310 mg. per 100 gm. fresh tissue compared to the normal mean value of 445 mg. In contrast to their findings of encephalomalacia in the vitamin E deficient chick, Pappenheimer and Goettsch found that the central nervous system of the deficient duck

was not affected when they were fed the same basic ration. Rigdon (1966) described findings of contracture, Zenker's necrosis, torticollis, and a pendulous crop in widespread hereditary myopathy of the white Pekin duck. DL-Alpha tocopherol given orally at 50 mg. per kg. of body weight did not prevent widespread myopathy. Rigdon (1962) also reported that amyloidosis occurred in cirrhotic livers concomitantly with spontaneous muscular dystrophy of the white Pekin duck. There have been no reports of ducks being susceptible to exudative diathesis (Dam and Glavind, 1939) which experimentally is readily produced in chicks fed diets based on torula yeast and which in field cases is suspected to be caused by a selenium deficiency (Thompson, 1953; Salisbury *et al.*, 1962).

Jungherr and Pappenheimer (1937) stated that the only vitamin E deficiency lesion in the turkey was severe myopathy of gizzard smooth muscle. According to Biester and Schwarte (1952), in most cases these lesions are recognizable grossly as being characterized by irregular, grayish plaques beneath the transparent serous covering even though the external conformation and tonus of the gizzard appeared normal. In reporting a selenium responsive gizzard myopathy in young poults, Scott *et al.* (1967) stated the earliest histological lesions consisted of hyaline bodies in the gizzard muscle cell cytoplasm and of interstitial edema with heterophilic cellular infiltration. With increasing chronicity the degenerating parenchyma was replaced with densely packed fibrous connective tissue. Furthermore, they pointed out that .18 ppm selenium in the presence of vitamin E (11 I.U. per kg. of feed) or .28 ppm selenium in the absence of added vitamin E prevented gizzard myopathy completely. Scott *et al.* (1967) suggested that the severity of gizzard and heart myopathy of young poults is not dependent upon dietary polyunsaturated fatty acids.

In dogs a "brown bowel" syndrome characterized by a dark colored serosal surface of the intestinal muscularis has been reported (Cordes, Mosher and Brown, 1966; Hayes, Rousseau and Hegsted, 1970). Their histological examination of smooth muscle revealed a fine granular pigment at the perinuclear poles of the lesser affected cells. Furthermore, they found that larger aggregates, usually acid-fast and fluorescent, were found in macrophages when smooth muscle cells were more severely affected. Pigment-laden macrophages were also seen in the mesenteric lymph nodes.

Erythrocyte Hemolysis in the Vitamin E-Deficient Animal

Dialuric Acid Hemolysis. Rose and Gyorgy (1949, 1952) reported that dialuric acid caused hemolysis of red blood cells from vitamin E deficient rats. When they observed an increase in peroxides using the thiobarbituric acid test, Tsen and Collier (1960) concluded that dialuric acid caused hemolysis by peroxidizing lipid membranes of red blood cells deficient in vitamin E or saturated with PUFA (polyunsaturated fatty acids). Based on this phenomenon, Friedman *et al.* (1958) described a biological assay method for vitamin E. In this method the percentage of hemolysis was correlated to the amount of alpha tocopherol injected or fed orally to deficient animals. Using the dialuric acid method, Gitler, Sunde and Baumann (1958) found the hemolysis of red blood cells from deficient chicks exceeded the hemolysis of red blood cells from tocopherol supplemented birds. They also observed a range of *in vitro* hemolysis (40 to 60%) in the deficient chick which was lower than the range of *in vitro* hemolysis (85 to 98%) they reported in the tocopherol deficient rats. Using canine blood, Hayes and Rousseau (1970) observed an inverse relationship between dialuric acid hemolysis and plasma tocopherol when plasma vitamin E values fell below 500 µg. per 100 ml. of blood.

Saline Hemolysis. The layering hemolysis test, a saline hemolysis method, has been successfully used on mink and equine red blood cells (Stowe *et al.*, 1962; Stowe, 1969). Muytjens (1956) found little hemolysis of tocopherol deficient chick red blood cells using saline but observed significant hemolysis using dialuric acid.

The Possible Interrelationship of Vitamin E and Malaria Infection

Zaiman (1940) reported that in rats fed 2500 *Trichinella spiralis* larvae fewer worms were recovered from muscles of vitamin E deficient animals than from control animals. Mason and Bergel (1955) observed that vitamin E deficient diets promoted the growth of human leprosy bacilli in hamsters and rats not normally susceptible to the disease. Dewitt (1957) reported that adult *Schistosoma mansoni* organisms were greater in number but sexually less mature in mice fed a vitamin E deficient diet. However, the net pathogenic effect of these organisms was less compared to infected control animals because the production of large numbers of eggs normally responsible for tissue damage did not occur.

In addition to the lack of data on vitamin E's role in infectious processes, there is a paucity of general information regarding the role of nutrients in malarial infection. Trager (1943) observed that biotin deficient ducks and chickens developed much more severe infections with *Plasmodium lophurae* than did nondeficient, infected animals. On the other hand, Seeler and Ott (1944) concluded that riboflavin deficient chicks were more resistant to *Plasmodium lophurae* infection since the infected, deficient birds had a lower parasitized erythrocyte count compared to supplemented, infected animals. Maegraith (1948) noted that human malarious hearts had microscopic lesions of fragmentation and fatty

degeneration of cardiac muscle fibers. More recently, Cenedella *et al.* (1968, 1969) observed that intraerythrocytic *Plasmodium berghei* synthesized high levels of phospholipids utilizing primarily oleic acid derived free from the plasma and possibly from the hydrolysis of host lipid membranes by use of parasitic phospholipases. Although fragmenting, degenerative cardiac muscle fibers and disrupted lipid membranes have been observed in vitamin E deficiency (Scott *et al.*, 1967; Porta *et al.*, 1968), and since there are no substantial reports of vitamin E-malaria interaction, the possibility of vitamin E having a protective effect in the course of malarial infection would be most interesting to investigate.

A voluminous amount of information is available on vitamin E. However, very little applies to animal health. Furthermore, there is a need for more evidence regarding vitamin E's specific role in infectious disease processes: Research to obtain additional information on vitamin E-selenium's role in animal health would be most helpful in efficient livestock production and fundamental biomedicine.

MATERIALS AND METHODS

General Plan

A total of 44 ducks was used in 2 separate experiments. In Experiments I and II 20 and 24 ducks, respectively, were on trial. The experiments were designed to study the effects of varying levels of vitamin E and/or selenium on the growing duck and the subsequent influence of acute avian malarial infection on the vitamin E-selenium deficient duck. The experimental plan was as follows:

Experiment I: Relationship Between Constant Level of Vitamin E and Selenium in the Diet and Malarial Infection

Group No.	No. Ducks	Treatment
1 (control)	5	Basal diet supplemented with 50 I.U./kg. of vitamin E and .2 ppm of selenium
2 (control)	5	Basal diet only
3 (infected)	5	Basal diet supplemented with 50 I.U./kg. of vitamin E and .2 ppm of selenium
4 (infected)	5	Basal diet only

Experiment II: Relationship Between Varying Levels of Vitamin E and Selenium in the Diet and Malarial Infection

Group No.	No. Ducks	Treatment
1 (infected)	4	Basal diet only
2 (infected)	4	Basal diet supplemented with .2 ppm of selenium
3 (infected)	4	Basal diet supplemented with 25 I.U./kg. of vitamin E and .1 ppm of selenium

Group No.	No. Ducks	Treatment
4 (infected)	4	Basal diet supplemented with 50 I.U./kg. of vitamin E and .05 ppm of selenium
5 (infected)	4	Basal diet supplemented with 100 I.U./kg. of vitamin E
6 (infected)	4	Basal diet supplemented with 100 I.U./kg. of vitamin E and .2 ppm of selenium

All the ducks in Groups 3 and 4, Experiment I, were infected with the avian malarial parasite *Plasmodium lophurae* on experimental Day 7 (7 days of feeding the experimental diet) while all the ducks in Groups 1 through 6, Experiment II, were infected with *Plasmodium spartani* on experimental Day 14.

Animals, Housing and Diets

The ducklings used were 14 days old at the onset of experimentation, were of the white Pekin breed,* and were a mixture of both sexes. They were grouped according to initial weight and sex so as to achieve group populations of similar constitution.

Once removed from their brooder at 14 days of age, groups of 5 and 4 ducklings in Experiments I and II, respectively, were placed in wire bottomed, steel cages and were fed the experimental diet. During the periods of malarial infection, the infected and noninfected ducklings were kept in separate rooms with similar environmental conditions.

Prior to the onset of experimentation the ducklings were fed Chick Startena** until they reached 2 weeks of age, at which time they were fed the experimental diet. The diet was primarily a torula yeast, cod liver

*Ridgway Hatchery, La Rue, Ohio.

**Ralston-Purina, St. Louis, Missouri.

oil-based ration and has not been used in previous duck vitamin E deficiency studies, although a comparable diet has been used as a rat ration (Porta *et al.*, 1968). Furthermore, since the basal ration was relatively free of vitamin E and selenium, any vitamin E and/or selenium that the ducklings received was obtained for all practical purposes by supplementation. The ingredients of the basal ration are given in Table 1. In both experiments the ducklings were fed *ad libitum* throughout the experiment with the ration fed dry and separately from the water supply.

Methods and Techniques

Collection of Blood. Periodic samples of heparinized whole blood were taken from the ducks by means of withdrawal from the recurrent-tarsal vein.

Dialuric Acid Hemolysis Method. The *in vitro* dialuric acid method of Rose and Gyorgy (1949, 1952) using the modifications of Gitler, Sunde and Baumann (1958) was performed on the duck red blood cells.

Plasma Alpha Tocopherol Determination. In order to detect plasma alpha tocopherol using .4 ml. of blood plasma, the micromethod of Fabianek *et al.* (1968) was employed. This photometric procedure required the use of the Beckman DU Spectrophotometer* with a setting of 536 mμ for maximum absorbance.

Muscle Creatine Determination. Muscle creatine values for the control birds in Experiment I only were found by employing the Folin creatine

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*DU Spectrophotometer, Beckman Instruments, Inc., Fullerton, Calif.

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Table 1. Basal diet for Experiments I and II

Ingredients	% Diet
Torula yeast[1]	44.0
Cerelose[2]	41.3
Cod liver oil[3]	5.0
Cellulose[4]	3.0
DL-Methionine[5]	0.5
Glycine[6]	0.2
Mineral Mix*[7]	6.0
Vitamin Mix**[8]	20 ml./kg.

*Salts N (Fox and Briggs, 1960).

**Vitamin mix contained: thiamine mononitrate, .36 gm.; riboflavin, .8 gm.; cyanocobalamin, .002 gm.; calcium pantothenate, 2 gm.; niacin, 5 gm.; choline chloride, 130 gm.; vitamin D_2, 20,000 I.U.; menadione sodium bisulfide, .7 gm.; pyridoxine, .7 gm.; biotin, .020 gm.; folic acid, .15 gm.; absolute ethanol, 200 ml.; and distilled water, q.s. 2000 ml.

[1]Torula yeast, St. Regis, Rhinelander, Wisc.

[2]Cerelose, Corn Products Company, Englewood Cliffs, N.J.

[3]Cod liver oil, Whitmoyer Laboratories, Myerstown, Pa.

[4]Cellulose, General Biochemicals, Chagrin Falls, Ohio.

[5]DL-Methionine, Nutritional Biochemicals Corporation, Cleveland, Ohio.

[6]Glycine, Nutritional Biochemicals Corporation, Cleveland, Ohio

[7](Briggs) Salts N, General Biochemicals, Chagrin Falls, Ohio.

[8]Vitamins, Merck and Co., Inc., Rahway, N.J.

procedure (1914) with the modifications suggested by Rose, Helmer and Chanutin (1927).

Total Erythrocyte Count. In order to obtain the total erythrocyte count, the red blood cells in heparinized whole blood were diluted to a 1:50,000 dilution and were counted electronically by the Model B Coulter Counter.*

Serum Glutamic-Oxalacetic Transaminase (SGOT) Determination. The Trans Ac test,** employing the procedure of Babson *et al.* (1962), was used to determine SGOT values in Experiments I and II. In most cases, GOT values for plasma rather than serum samples were determined.

Histological Technique. At the time of necropsy, tissues routinely taken included the cerebellum, cerebrum, liver, spleen, kidneys, lungs, adrenals, gizzard, small intestine, pancreas, heart, and skeletal muscles. These tissues, collected in Bouin's preservative, were then prepared and stained in accordance with the procedures described in the Armed Forces Institute of Pathology's *Manual of Histologic and Special Staining Technics* (1957).

Experimental Procedure

Experiment I: Constant Level of Vitamin E and Selenium in the Diet and Malarial Infection. Twenty 2-week-old ducklings were divided into 4 groups, 2 of which were fed the deficient-purified diet and the other 2 of which received the supplemented-purified diet. At 0, 5 and 14 days of the experiment, the ducks were weighed, 2-ml. blood samples were collected,

*Model B, Coulter Counter, Coulter Electronics, Hialeah, Fla.

**SGOT (Trans Ac method), General Diagnostics, Morris Plains, N.J.

and the biochemical procedures mentioned previously were performed. In accordance with the protocol, after 7 days on trial birds in Groups 3 and 4 were taken to a separate room and injected intravenously with *Plasmodium lophurae*-infected erythrocytes at a lethal dose of 1×10^8 parasitized duck red blood cells. After 9 days on trial (2 days post-infection for Groups 3 and 4) all the birds in the experiment were weighed and re-bled for the previously mentioned biochemical parameters. Weighing and bleeding was once again performed on all ducks that had not succumbed to the infection at Day 14 of the experiment. After 28 days all remaining ducks were euthanatized. At death tissues were collected from each duck for histopathological studies.

Experiment II: Varying Levels of Vitamin E and Selenium in the Diet and Malarial Infection. Twenty-four 2-week-old ducklings were divided equally into 6 groups and, as given in the experimental plan, were fed varying levels of vitamin E and/or selenium. At the onset of the trial and after 14 experimental days, all the birds were weighed and bled in order to evaluate the previously mentioned biochemical parameters. On trial Day 14 all the birds in the experiment were injected with *Plasmodium spartani*-infected erythrocytes at a lethal dose of 1×10^8 parasitized red blood cells. The birds were weighed and once again bled on trial Day 17 (3 days postinfection). The experiment concluded by trial Day 22, when the last duck died. As was the case in the first experiment, tissue was collected from each bird for histopathological evaluation.

RESULTS

General Observations

At the onset of each experiment, the ducks readily consumed the purified diet after having previously eaten a commercial diet. The purified diet promoted some feather picking possibly because it was quite powdery and adhered to the oily duck feathers. The most evident clinical sign for vitamin E-selenium deficiency was skeletal muscular weakness. This condition was seen as early as 9 days after the start of experimentation. In addition, the most striking clinical malarial sign, anemia, was evident by 3 to 4 days after infection.

Total Body Weight and Average Daily Gain

In Experiment I all 4 groups of ducks gained weight for the first 5 days (Table 2). While Group 1 (the control-supplemented ducks) continued to grow, the other 3 groups decreased in the rate of growth after Day 9 (Figure 1). By experimental Day 14, Group 2 (the nonsupplemented ducks) stopped growing, whereas Group 3 (the supplemented-infected birds) were losing weight. Furthermore, all birds in Group 4 (the nonsupplemented, infected group) had the slowest rate of growth and all but 1 were dead by experimental Day 14.

In the second experiment by Day 17 when all 6 groups of ducks were affected by malaria infection, only Group 1, the basal-fed ducks, had a significantly lower ($P < .05$) average daily weight gain (Table 3). All the other groups of ducks which were supplemented with vitamin E and/or selenium gained weight during the experiment.

Table 2. Experiment I: Average total body weight (kg.)

Group No.	Treatment	Time (days) 0	5	9	14
1	Basal + E + Se	.621	.723	.846	.933
2	Basal	.604	.689	.893	.873
3	Basal + E + Se + Malaria*	.652	.737	.829	.750
4	Basal + Malaria*	.615	.741	.755	---

*Malaria infection initiated on experimental Day 7.

Table 3. Experiment II: Average total body weight and average daily weight gain (kg.) of ducks infected and fed various levels of vitamin E and selenium

Group No.	Treatment	Initial Wt.	Final Wt.	No. of Days	Avg. Daily Gain
1	Basal + Malaria*	.507	.808	17	.017
2	Basal + .2 Se + Malaria	.528	1.071	17	.032
3	Basal + 25 E + .1 Se + Malaria	.485	1.124	17	.032
4	Basal + 50 E + 0.5 Se + Malaria	.481	1.093	17	.036
5	Basal + 100 E + Malaria	.484	1.088	17	.036
6	Basal + 100 E + .2 Se + Malaria	.476	1.021	17	.032

*Malaria infection was initiated on experimental Day 14.
Vitamin E units I.U./kg. of feed; selenium ppm.

Packed Cell Volume (PCV)

When the ducks in Experiment I were changed from a commercial diet to the experimental, semipurified diet and after they were fed this diet for 5 days, the PCV values for all the ducks significantly decreased ($P < .05$) irrespective of the presence or absence of vitamin E and/or selenium in the diet (Table 4). Furthermore, the PCV values remained relatively constant unless the ducks were stressed with malaria, in which case the influence of malarial infection decreased the PCV values further.

Muscle Creatine

The average muscle creatine value for the control, supplemented animals in the first experiment was 4.23 mg./gm., compared to 2.39 mg./gm. for the control, unsupplemented animals (Table 5). This difference in average values proved to be statistically significant ($P < .05$).

Plasma Alpha Tocopherol, Serum Glutamic-Oxalacetic Transaminase (SGOT), Total Erythrocyte Counts, and Dialuric Acid Hemolysis Prior to Injection of *Plasmodium* Organisms

After 5 days during Experiment I the average plasma alpha tocopherol values for the ducks in supplemented Groups 1 and 3 were .46 and .62 mg./100 ml., which were higher than the plasma values of .39 and .34 mg./100 ml. for the ducks in nonsupplemented Groups 2 and 4, respectively (Table 6). In Experiment II after 14 days, the average plasma alpha tocopherol value for each group, except Group 6, was decreased compared to the values determined at the onset of the study. The decrease seemed to be inversely related to the amount of dietary vitamin E, since average plasma tocopherol values ranged from .22 and .06 mg./100 ml. for vitamin E deficient, Groups 1 and 2 to .43 and .41 mg./100 ml., respectively, for high vitamin E supplemented, Groups 5 and 6 (Table 7).

Table 4. Experiment I: Summary of average packed cell volume (% PCV)

Group	Treatment	Time (days) 0	5	9	14
1	Basal + 50 E + .2 Se*	33	26[P]	28[P]	27[P]
2	Basal	34	28[P]	28[P]	29[P]
3	Basal + 50 E + .2 Se + Malaria**	37	31[P]	24[P]	5[P]
4	Basal + Malaria	35	30[P]	21[P]	---

*Vitamin E units I.U./kg. of feed; selenium .2 ppm.

**Malaria infection was initiated on experimental Day 7.

P > .05.

Table 5. Experiment I: Muscle creatine (mg./gm.) values

Bird No.	(Control, Basal + E + Se*)	Bird No.	Group 2 (Control, Basal)
1	4.02	6	2.15
2	4.84	7	2.21
3	4.17	8	2.89
4	4.07	9	2.29
5	4.03	10	2.43
Average	4.23 ± .34	Average	2.39 ± .30[P]

*50 I.U./kg. vitamin E + .2 ppm selenium.

P > .05.

Table 6. Experiment I: Summary of parameters by groups

Parameter	Time (da.)	Group Diets* 1 50 E + .2 Se	2 Basal	3** 50 E + .2 Se	4*** Basal
Plasma alpha tocopherol (mg./100 ml.)	0	.70	---	.61	1.01
	5	.46	.39	.62	.34$_P$
	9	.35	.43	.67	.13^P
	14	---	---	.27	.39
SGOT (Trans Ac units)	0	44	38	36	52
	5	36	30$_P$	28	33$_P$
	9	36	218^P	45$_P$	180^P
	14	19	380^P	480^P	2400+^{P}
Dialuric acid hemolysis (%)	9	5.3	27.4^P_P	9.1	51.0^P_P
	14	2.6	29.5^P	20.0	33.0^P
Total erythrocytes (mill./cu.mm.	0	1.33	1.50	1.57	1.56
	5	1.65	1.65	1.71	1.78
	9	1.58	1.42	1.37$_P$	1.19$_P$
	14	1.59	1.75	0.33^P	0.27^P
Parasit. erythrocytes (% per 500 cells)	9	0.0	0.0	3.9	4.5
	14	0.0	0.0	46.0	61.0

*Vitamin E units I.U./kg. of feed; selenium ppm.

**Malaria infection initiated on experimental Day 7.

P > .05.

Table 7. Experiment II: Summary of parameters by groups

Parameter	Time (da.)	Group Diets* (Basal +) 1**	2** .2 Se	3** 25 E .5 Se	4** 50 E .05 Se	5** 100 E	6** 100 E .2 Se
Plasma alpha tocopherol (mg./ 100 ml.)	0	.57	.40	.45	.47	.51	.35
	14	.22	.06[P]	.28[P]	.36	.43	.41
	17	.09[P]	.11[P]	.35	.64	.79	.63
SGOT (Trans Ac units)	0	18	15	19	25	17	37
	14	88	45	74	44	28	43
	17	830[P]	108[P]	114[P]	116[P]	112[P]	157[P]
Dialuric acid hemolysis (%)	0	1.1	0.6	0.8	1.0	0.7	1.3
	14	28.4[P]	13.8[P]	0.3	0.3	0.3	0.4
	17	10.0	11.1	12.6	10.8	8.4	4.8[P]
Total erythrocytes (mill./cu.mm.)	0	1.37	1.63	1.41	1.41	1.39	1.16
	14	1.18	1.31	1.46	1.02	1.41	1.33
	17	1.62	1.75	1.50	1.54	1.30	1.58
Parasit. erythro-cytes (% per 500 cells)	14	0.0	0.0	0.0	0.0	0.0	0.0
	17	28.5	28.5	28.5	24.3	25.0	19.8

*Vitamin E units I.U./kg. of feed; selenium ppm.

**Malaria infection was initiated at experimental Day 14.

P > .05.

The SGOT values for all 4 groups during the initial 5 days of Experiment I were not significantly different (Table 6). However, by experimental Day 9 the average SGOT value for Group 2 (basal-fed ducks) was 218 Trans Ac units, which was significantly higher ($P < .05$) compared to the average SGOT value of 36 for Group 1 (vitamin E-selenium, supplemented ducks). In contrast, during the first 14 days of Experiment II the average SGOT values did not appreciably increase for any of the 6 groups, although average SGOT values of 88 and 74 Trans Ac units for Groups 1 and 3, respectively, were elevated compared to the other groups (Table 7).

There was no significant change in the total erythrocyte counts for the ducks in either the first or second experiment prior to the time when the *Plasmodium* parasite was injected, although ducks in Groups 1 and 4 of Experiment II were somewhat anemic (Table 7). Furthermore, the total erythrocytes for the control ducks (Groups 1 and 2, Experiment I) never exceeded the more acceptable normal figure of $2\text{-}3 \times 10^6$ erythrocytes/cu.mm. for comparable aged ducks fed commercial diets (Table 6).

By means of the *in vitro* dialuric acid method the tendency toward hemolysis was evident after 9 days of Experiment I. There was a significant difference ($P < .05$) between the average of 27.4% for Group 2, the vitamin E deficient ducks, compared to an average of 5.3% for Group 1, the vitamin E supplemented ducks (Table 6). Similarly, the average dialuric acid hemolysis value for Group 1 (basal-fed ducks) of Experiment II was 28.4%. In addition, the average hemolysis value for Group 2 (basal-fed, high selenium supplemented ducks) was 13.8% in contrast to low values for Groups 3 through 6 which were supplemented with varying levels of vitamin E and selenium (Table 7).

Plasma Alpha Tocopherol, SGOT, Total Erythrocyte Counts, Dialuric Acid Hemolysis, and Parasitized Erythrocytes after Injection of *Plasmodium* Organisms

In Experiment I after 6 days of infection the average plasma alpha tocopherol value for the infected, supplemented ducks (Group 3) was .27 mg./100 ml. This was lower than values noted earlier in the experiment for the same group (Table 6). The average plasma tocopherol value for the infected, basal-fed ducks (Group 4) was .39 mg./100 ml., which was higher than previous values; however, only 1 duck in the group was still alive at the time of determination. Unfortunately, no values for either control group were available during this same time period. In contrast to Experiment I, the average plasma tocopherol values determined in Experiment II after 3 days of malarial infection actually increased in all groups except Group 1 (basal-fed ducks) when compared to their plasma alpha tocopherol values just prior to infection (Table 7). In the case of Group 1 the average plasma tocopherol value for these ducks decreased from .22 mg./100 ml. before infection to .09 mg./100 ml. 3 days after infection. Furthermore, the combined average of plasma alpha tocopherol values for Groups 5 and 6 (both high vitamin E supplemented ducks) was significantly elevated ($P < .05$) compared to the corresponding plasma value just prior to the infectious phase of the experiment (Figure 2).

When the SGOT values were determined after 6 days of malarial infection during Experiment I, the average SGOT values were 480 and 2400 Trans Ac units, respectively, for Group 3 (supplemented ducks) and Group 4 (basal-fed ducks), which was significantly elevated ($P < .05$) above previous values for each group prior to infection (Table 6 and Figure 3). Similarly, after 3 days of malarial infection during Experiment II the average SGOT value for Group 1 (basal-fed ducks) was 830 Trans Ac units, which was significantly higher ($P < .05$) compared to SGOT values for Groups 2 through 6 (Table 7).

In both experiments the average total erythrocyte count for all the infected groups was relatively the same during early stages of acute malarial infection but significantly decreased ($P < .05$) by the terminal stages of the infection as best demonstrated by total erythrocyte counts of .33 and .27 million/cu.mm. for ducks in Groups 3 and 4 of the first experiment (Table 6). During this same time period it appeared that the average total erythrocyte count for ducks in both control Groups 1 and 2 of the same study did not decrease.

After 2 days postinfection (experimental Day 9) of Experiment I the tendency toward dialuric acid hemolysis was greatly enhanced for the ducks in Group 4. At the terminal aspects of the infection (experimental Day 14) there was apparently less differentiation between hemolysis values of Groups 3 and 4 (Table 6). In Experiment II at the end of the 3-day postmalarial infection period (experimental Day 17), the tendency for hemolysis was enhanced for all groups. Only the average hemolysis value for Group 6 (high vitamin E-selenium, supplemented ducks) was noticeably different, being less elevated than the corresponding average hemolysis values for Groups 1 through 5 (Table 7).

Based on the evidence from Experiment II there apparently was no significant difference for average parasitized erythrocytes for duck Groups 1 through 6, although Group 6, the high supplemented vitamin E-selenium ducks, had a slightly lower average count than all the other groups (Table 7).

The Influence of Vitamin E and Selenium on Survival Time and Mortality Rates of Infected Ducks

During the course of Experiment I all of the infected ducks of Gropus 3 and 4 died, whereas only 1 of 5 and 3 of 5 ducks of Groups 1 and 2, respectively, died (Table 8). In addition, there was a definite

Table 8. Survival time and mortality rate of ducks in Experiments I and II

Experiment	Group	Treatment	Days Survived (average)	Mortality Rate
I	1	Basal + 50 E + .2 Se	25.0[E]	1/5
	2	Basal	21.4[E]	3/5
	3	Basal + 50 E + .2 Se + Malaria*	14.0	5/5
	4	Basal + Malaria*	13.2	5/5
II	1	Basal + Malaria**	18.0	5/5
	2	Basal + .2 Se + Malaria	21.5	5/5
	3	Basal + 25 E + .1 Se + Malaria	20.5	5/5
	4	Basal + 50 E + .05 Se + Malaria	20.3	5/5
	5	Basal + 100 E + Malaria	21.0	5/5
	6	Basal + 100 E + .2 Se + Malaria	21.0	5/5

*Malaria infection was initiated on experimental Day 7.

**Malaria infection was initiated on experimental Day 14.
Vitamin E units I.U./kg. of feed; selenium ppm.

E = All living ducks euthanatized after 28 experimental days.

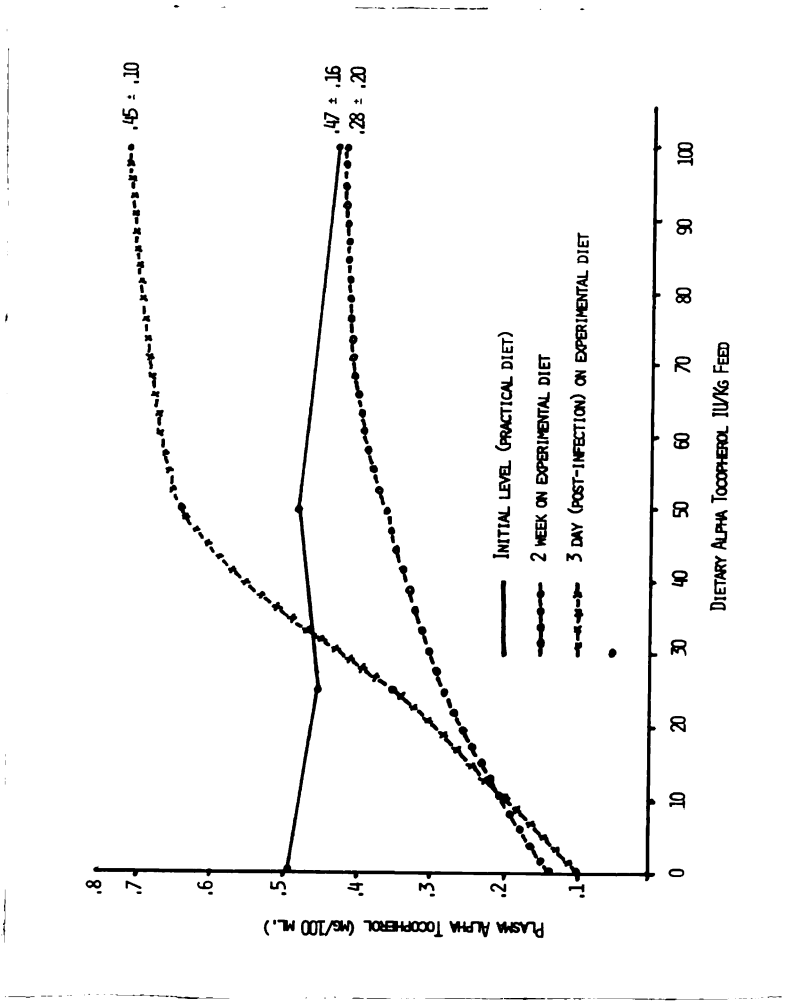


Figure 2. Relation of plasma alpha tocopherol levels to dietary alpha tocopherol levels in Experiment II.

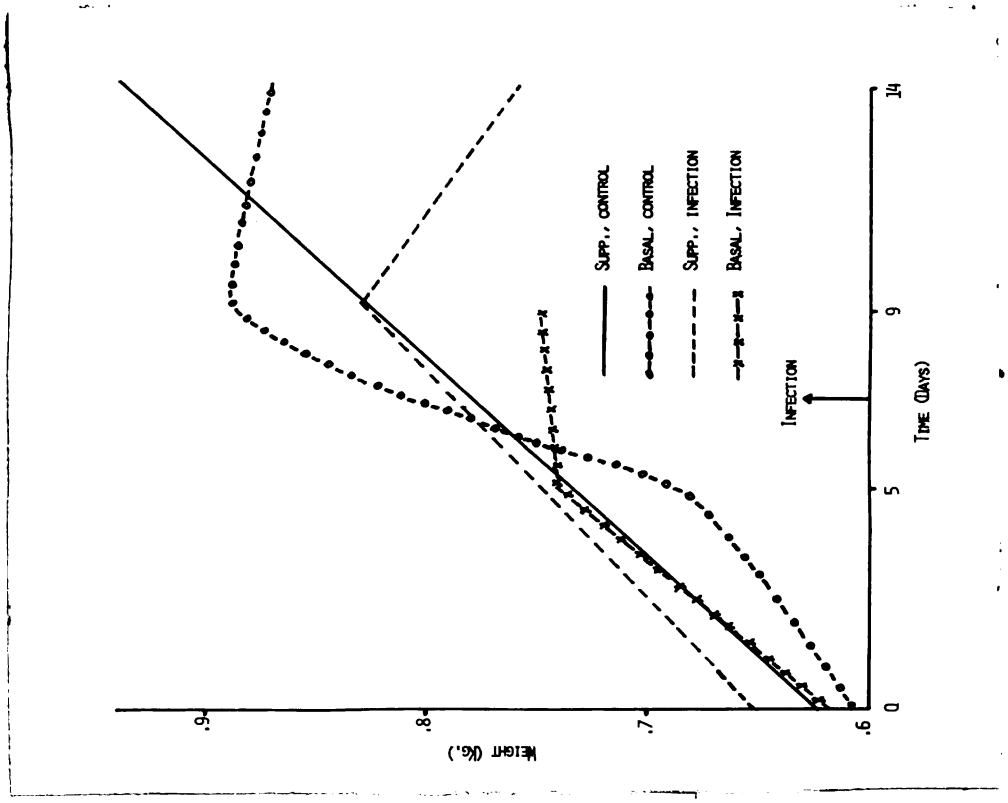


Figure 1. Average body weights by groups in Experiment I.

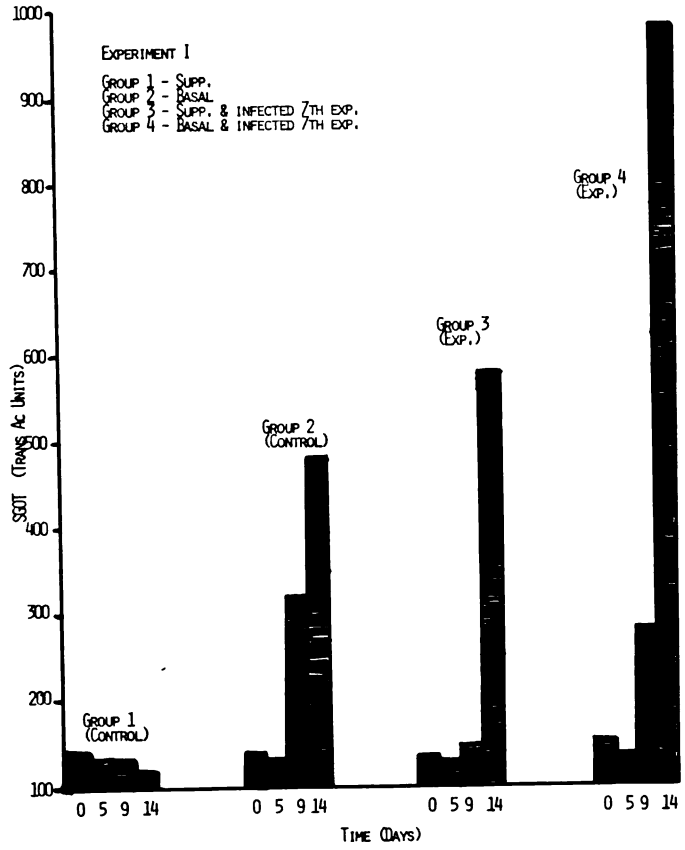


Figure 3. Changes in SGOT levels in Experiment I.

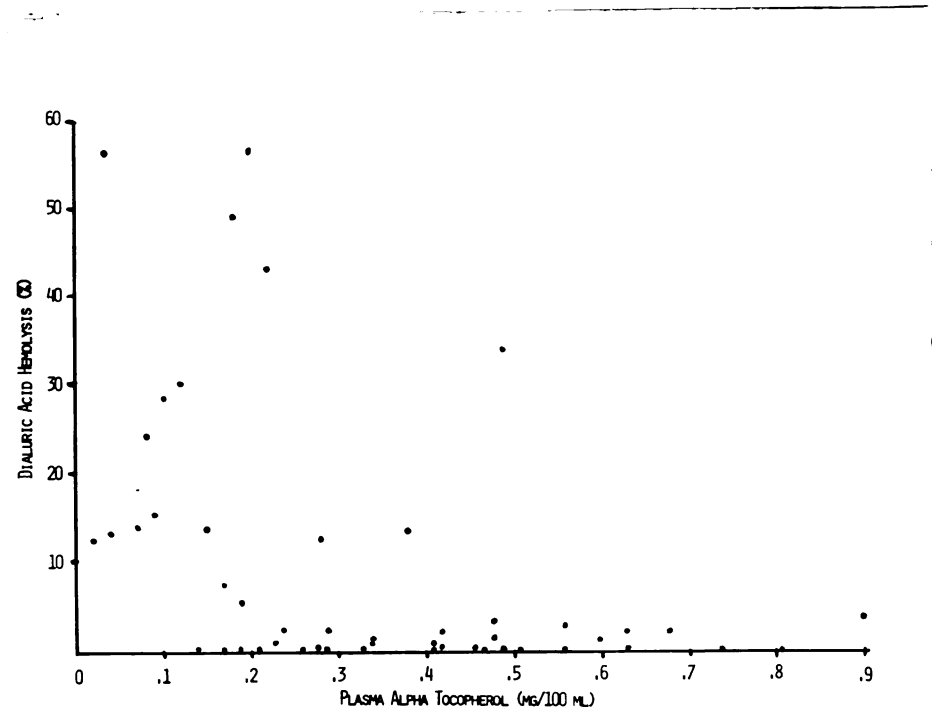


Figure 4. Correlation of dialuric acid to plasma alpha tocopherol (Experiment II). [Based on 57 determinations.]

difference in survival times between Groups 1 and 2 and a slight difference between Groups 3 and 4. The results of the second study seem to confirm the findings that basal-fed, malarious ducks (Group 1) were likely to die earlier than infected ducks fed supplemented vitamin E and/or selenium (Table 8).

Pathology

Most prominent gross lesions of noninfected, vitamin E deficient ducks were pale skeletal muscles and subserosal, focal, white opaque areas of the gizzard musculature. In contrast, typical gross malarial lesions were hydropericardium, splenomegaly, hepatic congestion and anemia. Microscopically, Zenker's necrosis of skeletal muscle and necrosis and hyaline degeneration of intestinal and gizzard smooth muscle were most characteristic of vitamin E-selenium deficiency lesions. Most representative of histological malarial lesions were phagocytosis of malarial pigment and parasitized erythrocytes occurring in necrotic splenic tissue, swelling and necrosis of liver parenchymal cells, and nephrosis. A summary of the most prominent histological lesions for Experiments I and II are given (Table 9). The following describes in more detail the general gross and histological appearance of the most significant lesions.

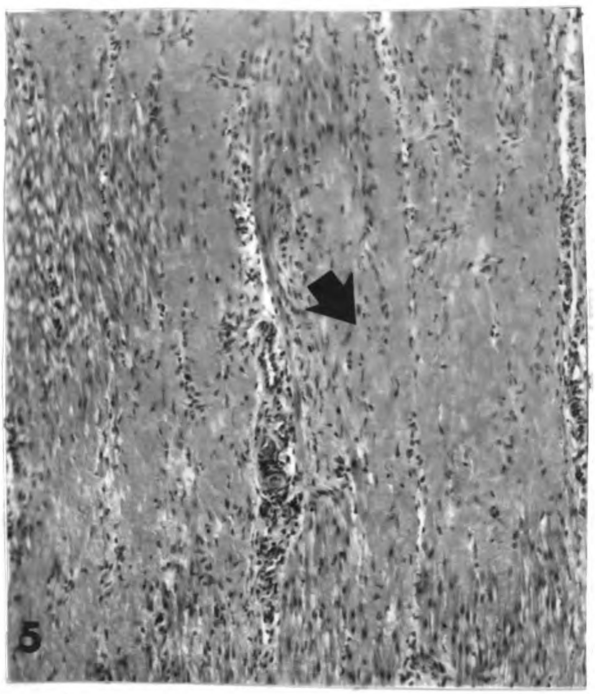
Smooth Muscle. Although no lesions of the intestinal tract were observed grossly, microscopic focal areas of smooth muscle necrosis were evident in the small intestine of several vitamin E-selenium deficient ducks (Figures 5 and 6). Gizzards of several ducks on vitamin E-selenium devoid diets had focal, grayish areas beneath the serosal surface. On histological examination these areas consisted of fragmenting and hyalinizing smooth muscle fibers, some of which were being replaced

Table 9. Summary of prominent histopathological lesions in Experiments I and II

Group	Basal Diet +*	Nutritionally Related				Malaria Related		
		Skeletal Myopathy	Gizzard Myopathy	Intest. Myopathy	Myocarditis	Pancreatitis	Cardiac Degen.	Nephrosis
Experiment I								
1	50 E + .2 Se	0/5	0/5	0/5	0/5	1/5	0/5	0/5
2	Basal only	5/5	3/4	2/5	1/5	0/4	0/5	0/4
3	50 E + .2 Se + Malaria	0/5	0/5	0/5	0/5	0/1	5/5	2/4
4	Malaria	4/5	1/5	1/5	0/3	1/3	3/3	4/5
Experiment II								
1	Malaria	4/4	2/4	1/4	1/4	1/1	3/4	2/3
2	.2 Se + Malaria	1/4	0/4	0/4	1/3	---	2/3	2/2
3	25 E + .1 Se + Malaria	0/4	0/4	0/4	0/4	4/4	4/4	3/4
4	50 E + .05 Se + Malaria	0/4**	0/4	0/4	0/4	2/2	4/4	2/3
5	100 E + Malaria	0/4	0/4	0/4	0/4	1/1	3/4	2/3
6	100 E + .2 Se + Malaria	0/4	0/4	0/4	0/4	1/1	2/4	3/4

*Vitamin E units I.U./kg. of feed; selenium ppm.

**One duck had a focal area of mineralization rather than diffuse myopathy.



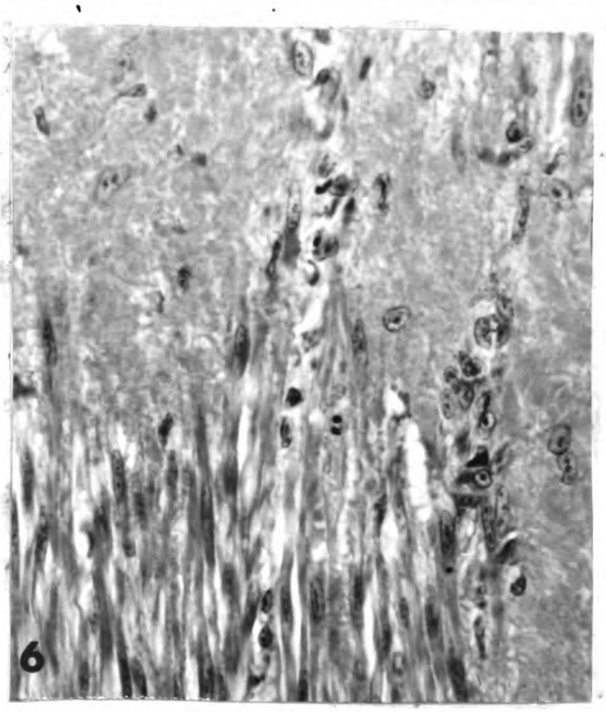


Figure 5. Focal areas of necrosis (arrow) and adjacent areas of normal tunica muscularis of the duodenum of a vitamin E-selenium deficient duck. H & E stain; x 136.

Figure 6. Higher magnification of Figure 5. H & E stain; x 540.

with fibrous connective tissue (Figures 7, 8 and 9). Gizzard smooth muscle from one of the malarious, vitamin E-selenium deficient ducks had focal areas of mineralization (Figure 10). Ducks fed .2 ppm selenium but no vitamin E did not have these smooth muscle lesions. Furthermore, malarial infection in adequately supplemented ducks did not cause smooth muscle myopathy lesions.

Cardiac Muscle. In general, hydropericardium and flaccid dilated hearts were grossly evident in malarious ducks, irrespective of their dietary vitamin E-selenium levels. Microscopically, many of the hearts had evidence of mild fatty degeneration with occasional limited heterophilic cellular infiltration. However, in 1 noninfected, vitamin E deficient and in 2 *Plasmodium spartani*-infected, vitamin E deficient ducks there was microscopic evidence of severe focal cardiac inflammation characterized by necrosis of cardiac muscle fibers and cellular infiltration of heterophils and, in 1 case, macrophages and plasma cells (Figures 11, 12, 13 and 14). These lesions were confined to the epicardium of 1 heart and the myocardium of the other 2 hearts.

Skeletal Muscle. Skeletal muscles, particularly the pectoral and quadriceps muscles, of avitaminosis E ducks had a pale mucoid appearance. Microscopically, the most consistent changes were swelling and fragmentation of the muscle fibers with subsequent sarcolemmal nuclei proliferation and occasional heterophilic cellular infiltration (Figures 15 and 16). In the second experiment 1 of 4 deficient ducks with malaria also had focal areas of mineralization in its degenerating quadriceps muscles (Figure 17).

Pancreas. In the first experiment none of the pancreatic tissue had detectable gross lesions. Microscopically, 1 duck out of 5 control

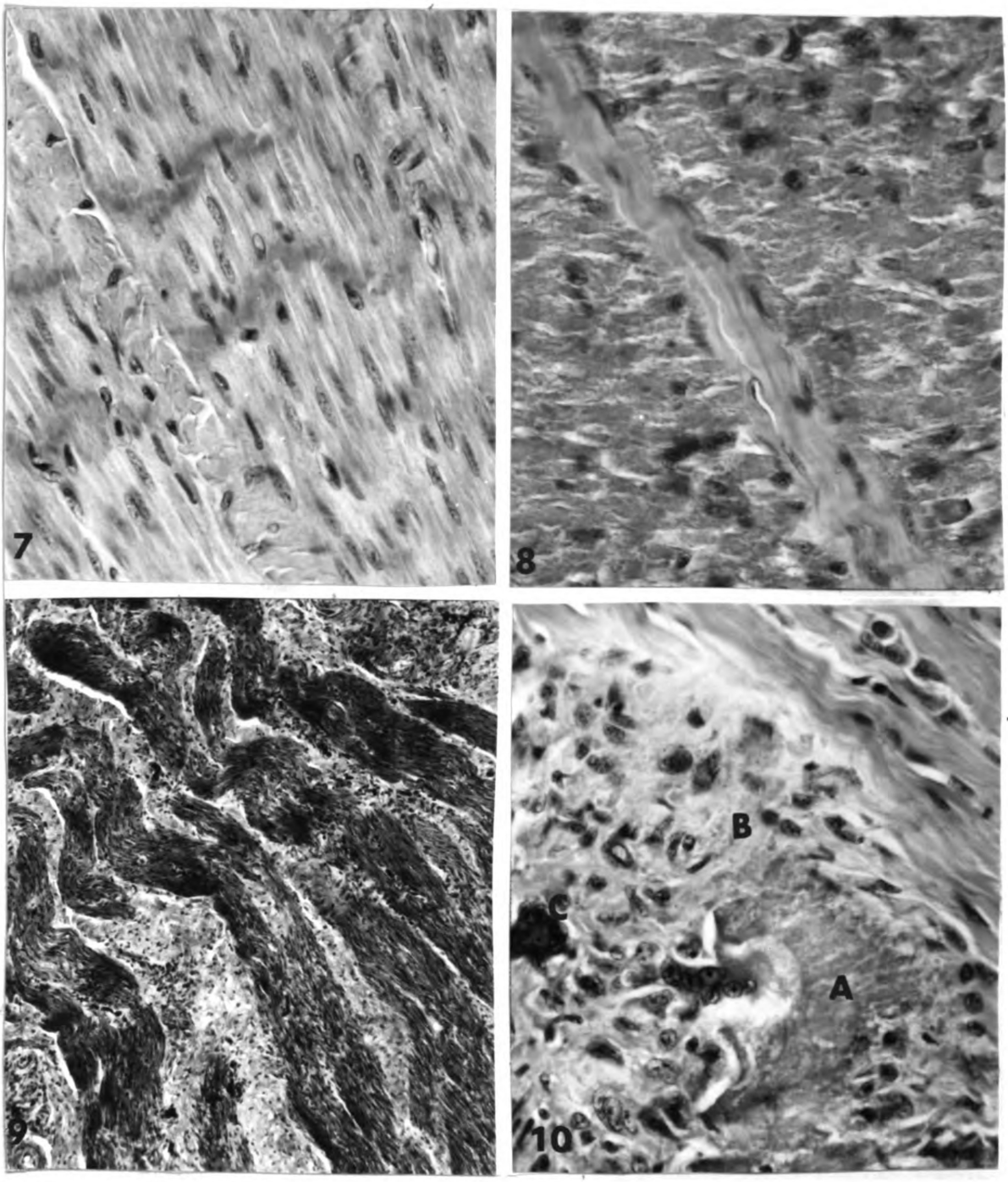


Figure 7. Gizzard smooth muscle from a vitamin E-selenium fed duck. H & E stain; x 540.

Figure 8. Hyaline degeneration, fragmentation, and necrosis of gizzard smooth muscle of a vitamin E-selenium deficient duck. H & E stain; x 540.

Figure 9. Fibrosis of gizzard smooth muscle of a vitamin E-selenium deficient duck. Gomori's trichrome stain; x 136.

Figure 10. Mineralization (A), fibrosis (B), and giant cell formation (C) of the gizzard of a vitamin E-selenium deficient, malarious duck. H & E stain; x 540.

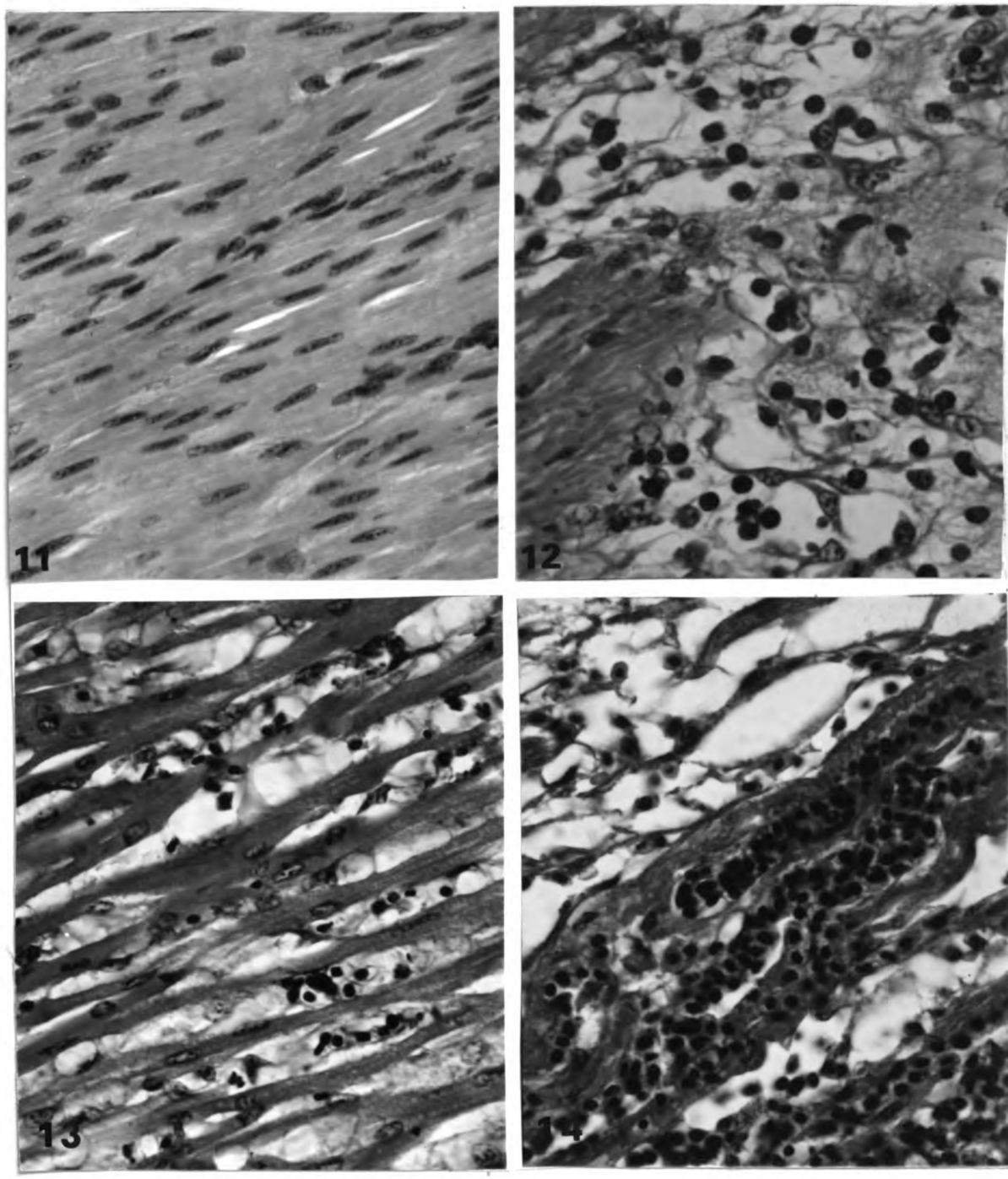


Figure 11. Cardiac muscle from a vitamin E-selenium fed, noninfected duck. H & E stain; x 540.

Figure 12. Cardiac muscle fiber necrosis with heterophilic cellular infiltration from a vitamin E-selenium deficient duck. H & E stain; x 540.

Figure 13. Cardiac muscle degeneration characterized by fatty metamorphosis from a vitamin E-selenium fed, malarious duck. H & E stain; x 540.

Figure 14. Myocarditis characterized by heterophilic cellular infiltration and petechial hemorrhage in a vitamin E-selenium deficient, malarious duck. H & E stain; x 540.

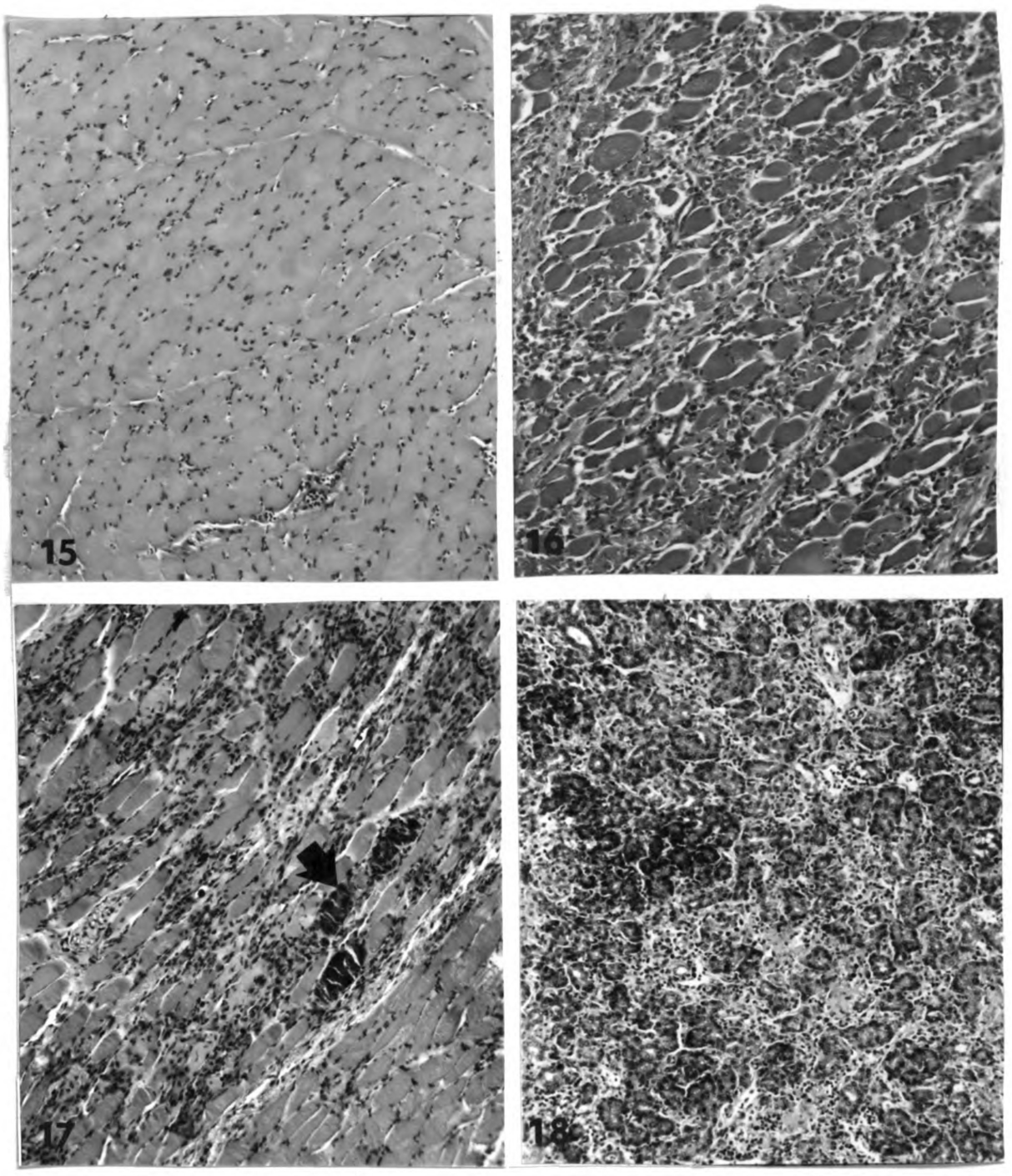


Figure 15. Skeletal muscle of a vitamin E-selenium supplemented duck. H & E stain; x 136.

Figure 16. Fragmentation, swelling, and sarcolemmal nuclei proliferation in the skeletal muscle of a vitamin E-selenium deficient duck. H & E stain; x 136.

Figure 17. Mineralization (arrow), sarcolemmal proliferation, and fragmentation in the skeletal muscle of a vitamin E-selenium deficient, malarious duck. H & E stain; x 136.

Figure 18. Necrotizing pancreatitis with hyaline degeneration and heterophilic infiltration in the pancreas of a malarious duck. H & E stain; x 136.

supplemented ducks (Group 1) had a focal area of necrosis involving the pancreas, whereas 1 out of 3 vitamin E-selenium deficient, *Plasmodium lophurae*-infected ducks (Group 4) had widespread necrotizing pancreatitis with minimal heterophilic cellular infiltration (Figure 18). In contrast every pancreas collected at random from 11 *Plasmodium spartani*-infected ducks of the second trial had widespread necrotizing pancreatitis characterized by hyaline droplet formation, necrosis of the acinar cells and cellular infiltration, mainly heterophils with some lymphocytes.

Kidneys. No evidence of any significant renal injury or inflammation was observed in noninfected, vitamin E-selenium supplemented ducks or in noninfected basal-fed ducks. Grossly, kidneys of all malaria-infected ducks appeared congested and, upon incision, exuded blood. The microscopic renal lesions of ducks infected with *Plasmodium lophurae* (Experiment I) consisted of tubular necrosis, congested glomeruli, and minimal cast formation. In contrast, renal lesions of ducks infected with *Plasmodium spartani* (Experiment II) were best characterized by: tubular degeneration consisting of cloudy swelling, hyaline droplet formation and fatty metamorphosis; hyaline, granular and fatty tubular casts; tubular necrosis; and glomerular congestion.

Cerebellum. No gross or histological cerebellar lesions were evident for any duck in Experiments I and II.

Cerebrum. There were no apparent cerebral lesions in uninfected ducks, either supplemented or deficient for vitamin E and selenium. Congestion of capillaries and minimal neuronal degeneration were typical findings in *Plasmodium*-infected ducks. "Malarial granulomas" or glial nodules seen in human malarious brains, were not evident in the cerebral tissue of infected ducks.

Liver. No significant gross or histological lesions were found in the hepatic tissue of noninfected supplemented or basal-fed ducks. In contrast, the livers of the malarious ducks were congested, darkened and, at times, enlarged. Microscopically, the liver parenchymal cells were undergoing degeneration and eventual necrosis which created a centrilobular pattern in less severely affected livers. Quite striking was the presence of abundant dark brown granular pigment undergoing phagocytosis. Since this pigment was negative to Prussian blue iron stain and proved to be birefringent, it apparently was malarial pigment rather than hemosiderin. Moreover, the sinusoids and central veins were congested and some degree of cellular infiltration, principally lymphocytes with some heterophils, was evident by being most concentrated in the vicinity of the portal vessels.

Spleen. The only significant pathology found in splenic tissue was from malarious ducks, irrespective of their dietary vitamin E and selenium levels. Grossly, splenomegaly and congestion were quite pronounced. Microscopically, the cells of both the red and white pulp were undergoing degeneration and subsequent necrosis and malarial pigment and parasitized erythrocytes were undergoing phagocytosis by primarily cell types of the reticuloendothelial system.

DISCUSSION

This research was conducted because of the need for general information on the interrelationship of infection and nutrition and there are no detailed reports of similar work. The general results suggest the duck was a good experimental model to study the interrelationship of vitamin E-selenium deficiency and malarial infection, since it was susceptible to both. Furthermore, the ducks were economical to purchase, readily consumed the experimental diet, and were of a size to manifest clinical signs and to obtain tissues in large enough amounts for analysis.

Apparently average daily gains of ducks in both experiments are not only affected by malaria but by vitamin E and selenium as well. It was evident in Experiment I that the slowest gaining ducks were those both infected and vitamin E-selenium deficient, while the fastest growing ducks were those noninfected and supplemented. In addition, the results of Experiment I suggest that during malarial infection, supplemented ducks were less retarded in their growth rates than infected, vitamin E-selenium deficient ducks.

During the course of Experiment I, the packed cell volume (PCV) values changed significantly. After consuming the semipurified diet for 5 days the average group PCV values were significantly depressed by as much as 20%. By experimental Day 9 the PCV values did not become further depressed for both the supplemented and basal-fed, noninfected ducks (Groups 1 and 2), while the PCV values continued to decline for both infected Groups 3 and 4. Since the PCV values decreased initially for

all 4 groups by experimental Day 5, it was apparent that the red blood cells were being influenced by some other factors than vitamin E, selenium or malarial infection. Workers such as Stowe and Whitehair (1962) and Hayes, Nielsen and Rousseau (1970) have postulated that the high amounts of polyunsaturated fatty acids such as in cod liver oil-based diets may have *in vivo* hemolytic properties when absorbed.

The results of the muscle creatine procedure indicated that the severity of the nutritionally-induced skeletal muscle myopathy in this research was comparable to the nutritional myopathy of ducklings described previously by Pappenheimer and Goettsch. The only difference was that in this research the results were more uniform.

The only significant depression in average plasma alpha tocopherol values during Experiment I occurred on experimental Day 9 when the plasma value decreased to .13 mg./100 ml. for the ducks in basal-fed Group 4, which had been infected for 2 days. During the same time period none of the other plasma alpha tocopherol values became significantly depressed, not even for Group 2, the noninfected basal-fed ducks. In contrast, all the plasma alpha tocopherol values except for the high vitamin E-selenium supplemented ducks (Group 6) were depressed during the first 14 noninfectious days of Experiment II. Moreover, during the early course of malarial infection (3 days postinfection), plasma alpha tocopherol values increased in all groups except for Groups 1 and 2, in which plasma alpha tocopherol values remained significantly lower.

The possible transient decrease for plasma alpha tocopherol values during the second experiment may be due to the dietary antagonism of polyunsaturated fatty acids (Green *et al.*, 1967; Hayes and Rousseau, 1970). Conversely, Trager (1943), reporting on biotin deficient, malarial ducks, noted that plasma biotin levels actually increased during the

early course of the infection; such also appeared to be true for the average plasma tocopherol values during the infectious phase of Experiment II. Furthermore, it seemed apparent that in order for this effect to occur the animals should not be depleted of vitamin E. The possible explanation for this phenomenon might be twofold: (1) intestinal absorption of vitamin E may have been actually enhanced during early malarial infection; and (2) the possibility there is a release of vitamin E from vitamin E rich storage depots such as the liver that is injured by the infectious process or adipose tissue that may be mobilized in an energy deficit state.

Increases in serum glutamic-oxalacetic transaminase (SGOT) values were evident for vitamin E-selenium deficiencies by 9 to 14 experimental days of Experiments I and II. These increases are attributed to muscle myopathy. Furthermore, it would appear that SGOT changes due to malaria occurred as early as 2 to 3 days postinfection. In addition, the most significantly elevated SGOT values were for malarious, vitamin E-selenium deficient ducks in Groups 4 and 1 of Experiments I and II, respectively. SGOT values were not as elevated for infected ducks that had vitamin E and selenium supplementation. Apparently, minimal levels of 25 I.U./kg. of vitamin E and/or .2 ppm selenium were adequate.

The most apparent sensitive biochemical parameter for vitamin E deficiency in the ducks was dialuric acid hemolysis in which a significantly elevated value of 27.4% was reached for the vitamin E-selenium deficient Group 2 of Experiment I as early as Day 9. This was also the most significantly elevated parameter during the 14 noninfectious days of Experiment II for Group 1, the vitamin E-selenium deficient ducks. Since the hemolysis value of 13.8% for Group 2 (basal, high selenium-fed ducks) compared to a hemolysis value of 28.4% for Group 1 (basal-fed

ducks) during the noninfectious phase of Experiment II, it would appear that selenium might partially protect vitamin E-deficient erythrocytes against dialuric acid hemolysis. In addition, hemolysis appeared to be minimal during this same time period when plasma tocopherol values exceeded .25 mg./100 ml. (Figure 4). Furthermore, although the dialuric acid hemolysis method was less reliable in the infectious phase of Experiments I and II, it would appear that hemolysis values were less elevated for high vitamin E-selenium supplemented ducks (Group 3, Experiment I and Group 6, Experiment II).

Total erythrocyte counts even for noninfected, vitamin E-selenium fed ducks were less than the normally accepted range of 2 to 3 x 10^6/ cu.mm. for growing ducks. The susceptibility to dialuric acid hemolysis, depressed packed cell volumes, and lower total erythrocyte counts may reflect a slight hemolytic effect of polyunsaturated fatty acids in the diet. During Experiment I anemia was evident in malarious ducks, irrespective of dietary vitamin E and selenium levels. Moreover, parasitized erythrocyte counts proved to be of little value for assessing a malarious, avitaminosis E state, even though comparable counts in malarious, high vitamin E-selenium ducks appeared to be slightly lower.

At the conclusion of Experiments I and II the longest average survival times were for the control, vitamin E-selenium supplemented ducks. Conversely, the shortest average survival time appeared to be for those ducks infected and deficient in both vitamin E and selenium.

Prominent gross and microscopic lesions from basal-fed ducks were intestinal, gizzard, and skeletal myopathy. Furthermore, the author is unaware of previous reports of this type of intestinal and gizzard myopathy in the duck. In Experiment II, .2 ppm of selenium prevented gizzard and intestinal myopathy for ducks of Group 2 (basal, selenium-fed),

while either vitamin E or selenium protected against the development of skeletal myopathy. Since focal areas of mineralization with skeletal and gizzard myopathy were seen in 1 of 4 vitamin E deficient, malarious ducks (Group 1, Experiment II) and severe myocarditis was seen in 2 of 7 avitaminosis E, infected ducks (Groups 1 and 2, Experiment II), it would appear that the vitamin E-selenium deficiency lesions might be made more severe by malarial infection. In malarious, supplemented animals prominent pathology included primarily splenomegaly, nephrosis, and anemia. In addition, severe pancreatitis was seen at necropsy during Experiment II in all 11 *Plasmodium spartani*-infected ducks from which pancreatic tissue was taken. Although pancreatic lesions were not prominent for the *Plasmodium lophurae* infection of Experiment I, they nonetheless were suspected of being malaria-related. Furthermore, to the best of the author's knowledge, no necrotizing pancreatic lesions related to avian malaria have been previously reported.

In conclusion, the manifestations of vitamin E-selenium deficiency and malarial infection were readily reproducible using the duck as the model animal. While the results of this study were influenced by acute malarial infection, information evaluating the interaction of vitamin E-selenium deficiency with chronic or relapsing malarial infection would be most interesting. Finally, there may have been 2 problems associated with this research. First of all, the semipurified diet was powdery and thus prone to harden if it got wet and tended to promote some feather picking. Secondly, there apparently was a viral contaminant of the *Plasmodium lophurae* and *Plasmodium spartani* stock which, because it mimics malarial pathology, has been referred to as duck infectious anemia (DIA) virus.

SUMMARY

The ducks fed the vitamin E-selenium deficient diets developed clinical signs as early as 9 days on the diet. The most evident clinical signs were a general muscular weakness in vitamin E-selenium deficient ducks and an anemia in the infected ducks. Prominent lesions in this research included: skeletal myopathy (vitamin E deficiency), smooth muscle myopathy (selenium deficiency), and nephrosis, splenomegaly, and anemia (malaria). Because of focal areas of mineralization with skeletal and gizzard myopathy and severe myocarditis found in tissues of several infected, vitamin E deficient ducks, it would appear that vitamin E deficiency lesions may be made more severe by malaria. The plasma alpha tocopherol values decreased significantly ($P < .05$) while the serum glutamic-oxalacetic transaminase (SGOT) values were elevated in vitamin E deficient, malarious ducks. In vitamin E-selenium deficient duck erythrocytes, dialuric acid hemolysis proved to be sensitive and reliable only for the assessment of vitamin E status and not malaria. Furthermore, it appeared that selenium in the presence of vitamin E deficient erythrocytes provided partial protection against *in vitro* dialuric acid hemolysis.

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