0163-4984/98/6201-2-0007 $10.50

Changes in Blood Chemistry, Hematology, and Histology Caused by a Selenium/Vitamin E Deficiency and Recovery in Chicks

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Received February 3, 1997; Accepted May 1, 1997

ABSTRACT

Exudative diathesis, a condition caused by a selenium (Se)/vitamin E deficiency, was studied in chicks. Trios of chicks that showed clinical signs of exudative diathesis were matched for severity. One was injected subcutaneously with 0.5 mL distilled water, and the other two received 15 μg of Se in 0.5 mL distilled water. A chick fed a diet with supplemental Se also received 0.5 mL distilled water. Blood was collected from three chicks 2 d after injection, and from the other chick, 6 d after injection. After blood was collected, pectoral muscle and bone marrow were collected. Deficient chicks showed varying degrees of necrosis in pectoral muscle, whereas recovering chicks had extensive fibrosis in pectoral muscle. An analysis of blood showed differences in CO_2, glucose, Se, glutathione peroxidase, alanine aminotransferase, aspartate aminotransferase, and creatine kinase. Heterophils and monocytes were increased in deficient chicks; lymphocytes, basophils, and hemoglobin decreased. After 6 d of recovery, all of the changes noted above were correcting toward normal. Eosinophils, in contrast, were unaffected by a deficiency, but increased in recovering chicks. It is hypothesized that cytokines associated with the inflammatory response accentuate the clinical signs of exudative diathesis.

Index Entries: Chicks; selenium; inflammatory response; necrosis; exudative diathesis.

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INTRODUCTION

Exudative diathesis in chicks was described by Dam and Glavind *(1)*. Transparent fluid accumulation was noted in subcutaneous connective tissue, generally around the breast and abdomen. In addition to the fluid, mild hemorrhage and accumulation of leukocytes were reported. The changes resembled a sterile inflammation. Capillary walls were interpreted as abnormally permeable in the inflamed tissue, allowing the escape of plasma, but usually not erythrocytes. Vitamin E was subsequently found to protect against exudative diathesis *(2)*.

The discovery of selenium (Se) as an essential nutrient identified a second nutrient capable of preventing exudative diathesis *(3,4)*. Se status in the chick affects the activity of the enzyme glutathione peroxidase *(5)*. It has been proposed that Se, through glutathione peroxidase, prevents peroxidation of unsaturated lipids of the capillary endothelial cell plasma membrane *(6)*. Vitamin E was proposed to protect against exudative diathesis by preventing oxidation within the lipid portion of the membrane. Damage to the capillary endothelial cells was thought to allow escape of fluids that characterize exudative diathesis.

The main objective of this research was to describe a Se/vitamin E deficiency through changes in blood chemistry, hematology, and histology. Recovery following Se supplementation is also reported using the same criteria. Emphasis is placed on the inflammatory response and its possible role in promoting exudative diathesis.

MATERIALS AND METHODS

Commercial broiler breeders were fed a diet with no supplemental Se or vitamin E for 1 mo prior to collection of eggs for this study. Chicks that hatched from these eggs showed no signs of a deficiency. Twenty-six chicks were fed a low-Se diet (Table 1) with no added Se, vitamin E, or synthetic antioxidant. Ten chicks were fed the same diet that had 0.1 mg selenite Se added/kg.

Several chicks first showed signs compatible with exudative diathesis at 10 d of age. Signs observed were lethargy and dark discoloration of the skin in the breast area. A trio of deficient chicks was matched for severity. One was injected subcutaneously with 0.5 mL of distilled water. The other two were injected subcutaneously with 15 µg of selenite Se in 0.5 mL of distilled water. A chick that was fed the Se-supplemented diet was also injected subcutaneously with 0.5 mL of distilled water. Two days later, blood was collected from the heart of three of the chicks. One milliliter was withdrawn for determination of Se content *(7)*. An aliquot of the blood from each chick was delivered to a tube with EDTA (1.5 mg/mL blood). This blood was used for manual counting of leukocytes and hemoglobin determination. The remainder of the blood was delivered to a tube with heparin

Table 1
The Low Selenium Basal Diet

Ingredient	% of Diet
Corn	11.48
Soybean Meal (48%)	15.00
Torula Yeast	20.00
Glucose	22.50
Corn Starch	22.50
Dicalcium Phosphate	0.87
Limestone	1.56
Iodized Salt	0.40
Animal Fat	3.00
Soybean Oil	2.00
D,L-Methionine	0.32
L-Arginine	0.12
Trace Mineral[1]	0.05
Vitamin Mix[2]	0.20

[1]The following was supplied in mg/kg of diet: Mn, 50; Fe, 50; Cu, 5.0; and Zn, 50.

[2]Supplied per kg of diet: retinyl palmitate, 1500 I.U.; cholecalciferol, 800 I.C.U.; riboflavin, 3.6 mg; pantothenic acid, 10 mg; vitamin B12, 0.1 mg; choline chloride, 650 mg; vitamin K, 0.5 mg; niacin, 17.0 mg.

(30 U/mL blood). Plasma from the blood was used to determine blood chemistry values** and glutathione peroxidase activity *(8)*. After blood collection, the chicks were euthanized with carbon dioxide. A section of the superficial pectoral muscle and left femur were collected from the chicks and placed in 10% neutral-buffered formalin. The remaining chick from the original four recovered for 6 d after the Se injection. An identical procedure was then followed with that chick. The process was repeated seven times over a 17-d period. When a matched trio of Se-deficient chicks was identified, procedures were initiated. A total of eight trios were identified for this experiment. On day 20, one trio of deficient chicks was injected subcutaneously with 10 mg of *d,l*-α-tocopherol dissolved in 0.5 mL corn oil.

Analysis of variance (ANOVA) was used to determine statistical significance of blood chemistry and leukocyte data. The results from aspartate aminotransferase and creatine kinase were transformed to natural logarithms before analysis. When significant treatment effects were found, means were statistically separated using least significant difference.

For histological examination, femurs were decalcified in a solution containing equal parts of 20% sodium citrate and 50% formic acid. Pectoral muscle and decalcified femurs were embedded in paraffin and then stained with hematoxylin and eosin. Myeloid to erythroid (M:E) cellular ratios were estimated from femur sections.

**Boehringer Mannheim/Hitachi 911, Indianapolis, IN.

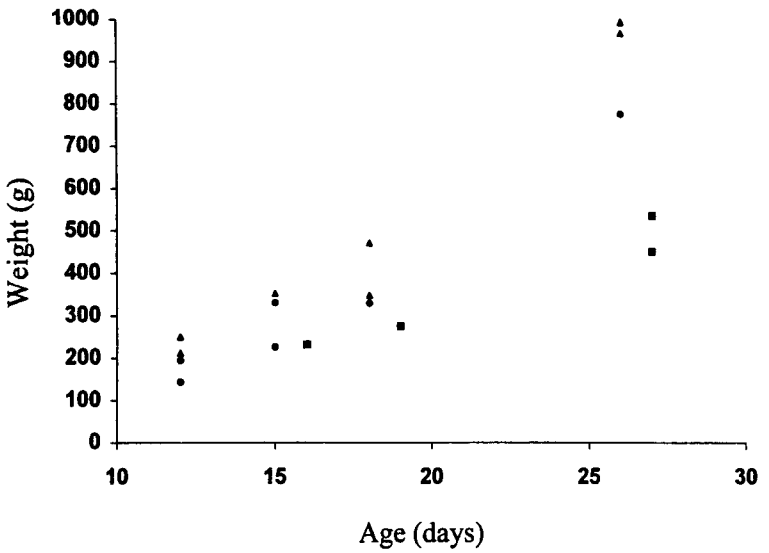

Fig. 1. Age at which each chick was sampled and its weight at that time. A = Se adequate, D = Se deficient, C = Se corrected for 6 d. ● D, ■ C, ▲ A.

RESULTS

The low-Se diet contained 0.068 mg Se/kg. Conditions used for this experiment caused exudative diathesis in several chicks at 10 d of age. Most of the chicks fed the low-Se diet developed exudative diathesis during the following 17 d. The age at which each chick was sampled and the weight of the chick at that time are shown in Fig. 1.

Data are not presented from those chicks whose deficiency was corrected for 2 d; the results were similar to those from deficient chicks. An exception is the histological results from pectoral muscle, which is described later.

Several blood chemistry parameters were affected by the Se status of the chick (Table 2). Carbon dioxide, glucose, selenium, and glutathione peroxidase activity decreased as a result of the deficiency. Plasma sodium increased in chicks whose Se deficiency had been corrected for 6 d. Activity of alanine aminotransferase, aspartate aminotransferase, and creatine kinase were increased by the deficiency.

The hemoglobin content of red blood cells was altered ($P = 0.06$) by Se status. Results were: Se adequate, 8.06 ± .44 g/dL; Se deficient, 6.77 ± 0.48 g/dL; and Se corrected, 8.72 ± 0.53 g/dL. Leukocyte numbers were also affected (Table 3). Total leukocyte count in deficient chicks was more than double the number present in Se-adequate chicks (Table 3). The increase was owing to more than a fivefold increase in heterophils. In addition, monocytes approximately tripled. Lymphocytes and basophils

Table 2
Blood Chemistry as Affected by Selenium Status

Criterion	Unit	Selenium Status		
		Adequate	Deficient	Corrected[1]
		(n=7)	(n = 6)	(n = 5)
Carbon dioxide	mEq/L	23.14 ± 1.10[a]	19.80 ± 1.20[ab]	18.20 ± 1.31[b]
Glucose	mg/dL	292.20 ± 11.76[a]	215.00 ± 12.71[b]	278.57 ± 13.92[a]
Sodium	mEq/L	150.86 ± 1.31[b]	149.60 ± 1.41[b]	157.00 ± 1.55[a]
Selenium	µg/mL	.08 ± .01[a]	.01 ± .01[c]	.04 ± .01[b]
Glutathione peroxidase	EU/mL	.37 ± .03[a]	.12 ± .03[c]	.23 ± .04[b]
Alanine aminotransferase	iu/L	.14 ± .39[b]	2.00 ± .42[a]	0 ± .46[b]
Aspartate aminotransferase	iu/L	179 ± 62[b]	699 ± 63[a]	372 ± 64[b]
Creatine kinase	iu/L	1556 ± 1480[c]	28001 ± 1550[a]	8433 ± 1620[b]

[a,b,c]Means in a row with different letters are significantly different (P < .05).
[1]Samples were collected 6 days after injection with 15 µg Se.

Table 3
Numbers of Leukocytes (× 10^9/L) as Affected by Selenium Status

Cell	Selenium Status		
	Adequate	Deficient	Corrected[1]
	(n=7)	(n = 6)	(n = 5)
Leukocytes	25.19 ± 5.01[b]	53.74 ± 5.41[a]	29.72 ± 5.93[b]
Lymphocytes	13.86 ± 1.58[a]	6.20 ± 1.66[b]	9.36 ± 1.87[ab]
Heterophils	7.19 ± 3.39[b]	40.05 ± 3.66[a]	13.04 ± 4.01[b]
Eosinophils	.64 ± .92[b]	.60 ± .99[b]	4.62 ± 1.09[a]
Basophils	1.56 ± .25[a]	.12 ± .27[b]	.88 ± .30[ab]
Monocytes	1.94 ± 1.01[b]	6.77 ± 1.10[a]	1.82 ± 1.20[b]

[a,b]Means in a row with different letters are significantly different (P < .05).
[1]Samples were collected 6 days after injection with 15 µg Se.

were less numerous from deficient chicks. When the Se deficient chick was supplemented for 6 d, leukocyte numbers were generally between those of the adequate and deficient chick; however, eosinophils increased during healing. Based on a percentage distribution, lymphocytes were more than half of the cells in Se-adequate chicks, but heterophils were approximately three-fourths of all cells in deficient chicks (Table 4).

Histologic studies assessed the tissue damage. Pectoral muscle of Se-adequate chicks was normal (Fig. 2). Deficient chicks had coagulative necrosis of myocytes ranging from rare foci to severe diffuse (Fig. 3), accompanied by scattered hemorrhage. Many areas with severe necrosis had accompanying multifocal pyogranulomatous to granulomatous inflammation and mild to moderate fibrosis. Two days after Se injection, pectoral muscle had lesions similar to those of Se-deficient chicks, except

Table 4
Selenium Effect on the Distribution (%) of Leukocytes

Cell	Selenium Status		
	Adequate	Deficient	Corrected[1]
	(n=7)	(n = 6)	(n = 5)
Lymphocytes	55.02 ± 3.60[a]	11.54 ± 3.88[c]	31.49 ± 4.26[b]
Heterophils	28.54 ± 4.22[c]	74.55 ± 4.56[a]	43.88 ± 4.99[b]
Eosinophils	2.54 ± 1.58[b]	1.12 ± 1.71[b]	15.55 ± 1.87[a]
Basophils	6.19 ± 1.06[a]	.22 ± 1.15[b]	2.96 ± 1.25[b]
Monocytes	7.70 ± 2.15[ab]	12.60 ± 2.32[a]	6.12 ± 2.54[b]

[abc]Means in a row with different letters are significantly different ($P < .05$).
[1]Samples were collected 6 days after injection with 15 µg Se.

Fig. 2. Normal skeletal myocytes and capillaries in pectoral muscle sample from Se/vitamin E-adequate chick. Hematoxylin-and-eosin stain (×300).

the fibrosis was more extensive and many of the necrotic myocytes had mineralization of sarcoplasm in Se-injected chicks. Early regeneration was present as evidenced by mild nuclear hypertrophy, nuclear proliferation and rowing, and increased basophilia of cytoplasm. Six days after Se injection, myocyte necrosis and heterophilic inflammation were uncommon, but cellular regeneration and fibrosis were common (Fig. 4). Myocytes were variable in cell diameter and nuclear size. Cytoplasm was basophilic and reduced in volume.

Bone marrow of chicks with adequate Se had an M:E cellular ratio of 1 (Fig. 5). Se-deficient chicks had an M:E ratio of 0.45 because of increased immature erythroid cellular elements (Fig. 6). Lymphoid follicles showed mild lymphocyte depletion and apoptosis. The M:E ratios were 0.75 and 1 on days 2 and 6, respectively, after Se injection.

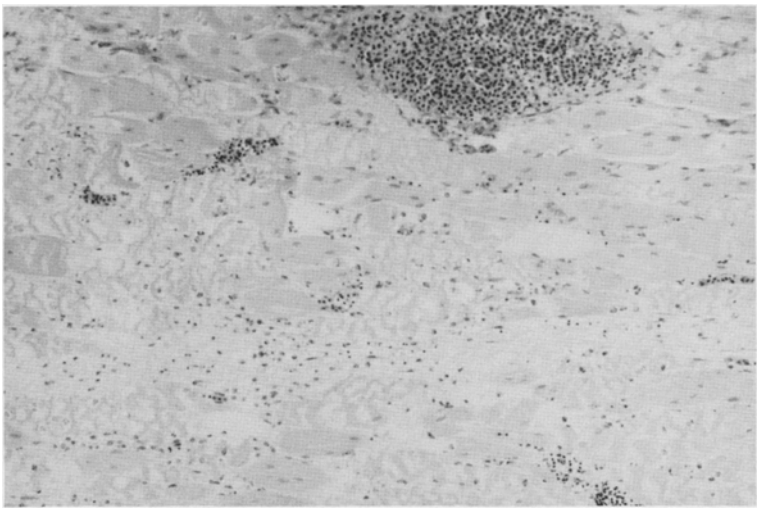

Fig. 3. Diffuse necrosis evident as loss of striations, fragmentation, and dissolution of skeletal myocytes in pectoral muscle of a vitamin E/Se-deficient chick. Hematoxylin-and-eosin stain (×210).

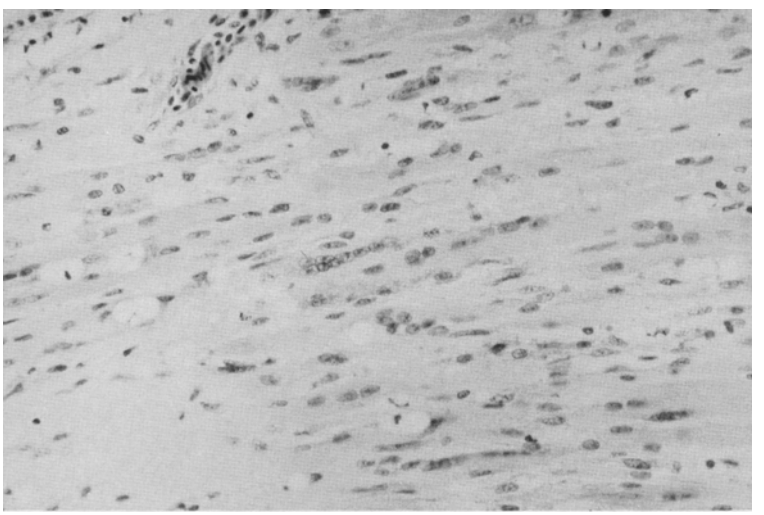

Fig. 4. Vacuolation and necrosis of myocytes with regeneration evident as myocyte nuclear hypertrophy and rowing in pectoral muscle of a deficient chick 6 d after Se injection. Hematoxylin-and-eosin stain (×330).

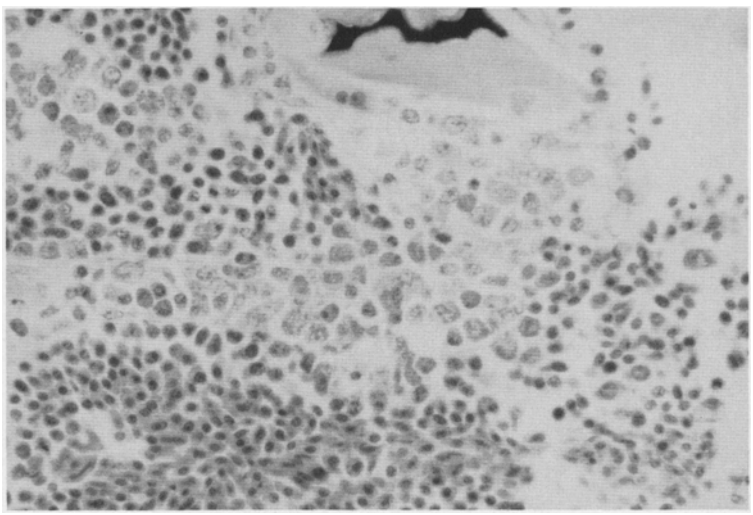

Fig. 5. Normal ratio of myeloid and erythroid cellular elements in femoral bone marrow of vitamin E/Se-adequate chick. Hematoxylin-and-eosin stain (×480).

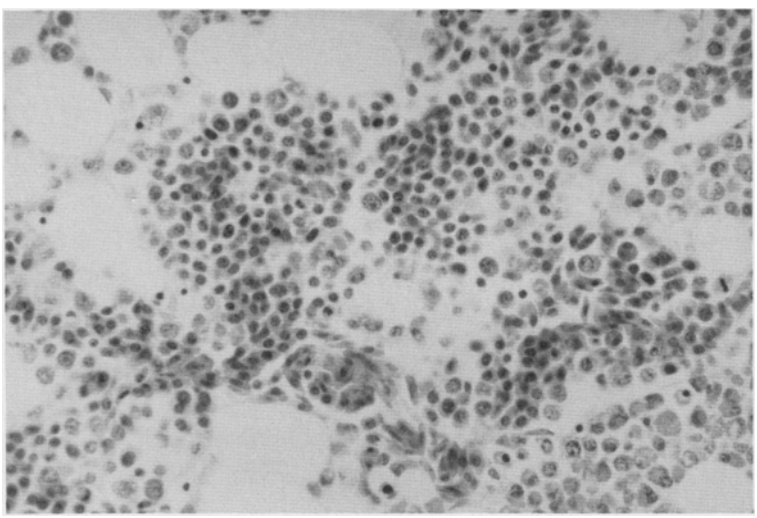

Fig. 6. Decreased ratio of myeloid-to-erythroid cellular elements from erythroid hyperplasia in femoral bone marrow of vitamin E/Se-deficient chick. Hematoxylin-and-eosin stain (×480).

Deficient chicks treated with tocopherol showed no recovery. They ceased eating and drinking, and were no longer able to move 2 d after injection, at which time they were euthanized. In contrast, chicks injected with Se showed increased movement, eating, and drinking within several hours.

DISCUSSION

In avian species, deficiency of vitamin E or Se has profound pathophysiologic effects on multiple cell types, especially skeletal, but also smooth and cardiac, muscle *(9,10)*. During the acute stages of lesion development *(9,11)*, a deficiency produces loss of striations, hyalinization, necrosis, and segmentation of skeletal muscle. The subacute stage is indicated by histocytic inflammation, fibroplasia, and proliferation of myocyte nuclei. The chronic stage is dominated by fibroplasia, myocyte atrophy, and regenerative processes. In the current study, deficiency of Se produced degeneration and necrosis of skeletal myocytes and an associated increase of several enzymes in the blood (Table 2) accompanying the destruction of myocytes. Tissue necrosis can trigger an inflammatory response *(12)*. Doubling of leukocytes in the hemogram (Table 3) is likely driven by chemotactic cytokines produced in the inflammatory response. Numbers of heterophils and monocytes increased several times; however, the demand for different leukocytes varied, because lymphocytes and basophils decreased.

The inflammatory response associated with an Se/vitamin E deficiency in a chick may be responsible for exudative diathesis. Transparent fluid accumulation and slight hemorrhaging *(1)* might result from cytokines produced by leukocytes. Cytokines released at the site of injury facilitate adherence of immune system cells to vascular endothelial cells *(12)*. TNF-α, IL-1, and IL-6 act locally on endothelial cells to increase vascular permeability, possibly leading to the accumulation of transparent fluid. These cytokines also may have contributed to the hemorrhage and coagulative necrosis that is evident (Fig. 3), eventually resulting in anemia. This hypothesis explains exudative diathesis as a pathological response to cytokines generated by the inflammatory response, instead of as a generalized deterioration of the vascular system owing to a lack of glutathione peroxidase *(6)*. Alternatively, direct damage to capillary endothelial cell plasma membranes by lipid peroxidation may have been responsible for increased vascular permeability and development of exudative diathesis *(5)*.

Correction of the deficiency was associated with rapid repair of the damaged myocytes and replacement by fibrous tissue. After 6 d, the total leukocyte counts approached that of the Se-adequate chicks. Numbers of each cell were correcting toward that found in the Se-adequate chicks, except for eosinophils, which were several times as prevalent in the corrected chicks as in the adequate and deficient chicks.

Se deficiency produced an increased M:E ratio, presumably from an increased erythropoiesis. Such a change could be a response to loss of erythrocytes from hemorrhage or hemolysis, or from defective erythropoietic maturation. Se deficiency has been reported to cause erythrocyte hemolysis and/or anemia in the rat, chick, dog, monkey, and humans *(13)*. In the present study, anemia, indicated by decreased

hemoglobin concentration, could have exerted a demand for increased erythrocyte production, and thus a compensatory increase in bone marrow erythropoiesis.

ACKNOWLEDGMENTS

The authors thank Mauria Watts and Frank Cihla, Department of Animal Sciences, for the selenium and glutathione peroxidase assays. Salaries and research support were provided by state and federal funds appropriated to the Ohio Agricultural Research and Development Center, The Ohio State University. Article Number: 7-97.

REFERENCES

1. H. Dam and J. Glavind, Alimentary exudative diathesis, *Nature* **142,** 1077–1078 (1938).
2. H. Dam and J. Glavind, Alimentary exudative diathesis, a consequence of E-avitaminosis. *Nature* **143,** 810–811 (1939).
3. E. L. R. Stokstad, E. L. Patterson, and R. Milstrey, Factors which prevent exudative diathesis in chicks on Torula yeast diets [Abstract], *Poultry Sci.* **36,** 1160 (1957).
4. M. L. Scott, J. G. Bieri, G. M. Briggs, and K. Schwarz, Prevention of exudative diathesis by Factor 3 in chicks on vitamin E-deficient Torula yeast diets [Abstract], *Poultry Sci.* **36,** 1155 (1957).
5. T. Noguchi, A. H. Cantor, and M. L. Scott, Mode of action of selenium and vitamin E in prevention of exudative diathesis in chicks, *J. Nutr.* **103,** 1502–1511 (1973).
6. M. L. Scott, M. C. Nesheim, and R. J. Young, *Nutrition of the Chicken,* 3rd ed., M. L. Scott and Associates, Ithaca, pp. 350–353 (1982).
7. T. S. Koh and T. H. Benson, Critical re-appraisal of fluorometric method for determination of selenium in biological materials, *J. Assoc. Off. Anal. Chem.* **66,** 918–926 (1983).
8. R. P. Burk and R. A. Lawrence, Glutathione-peroxidase activity in selenium deficient rat liver, *Biochem. Biophys. Res. Commun.* **71,** 952–958 (1976).
9. C. Riddell, Muscular system, in *Avian Histopathology,* American Association of Avian Pathologists, Kennett Square, PA, pp. 27–30 (1987).
10. C. Riddell, Cardiovascular system, in *Avian Histopathology,* American Association of Avian Pathologists, Kennett Square, PA, pp. 31–36 (1987).
11. R. E. Austic and M. L. Scott, Nutritional diseases, in *Diseases of Poultry,* 9th ed., B. W. Calnek, H. J. Barnes, C. W. Beard, W. M. Reid, and H. W. Yoder, Jr. eds., Iowa State University Press, Ames, IA, pp. 45–71 (1991).
12. J. Kuby, *Immunology,* 2nd ed., W.H. Freeman, New York, pp. 7–15, **313–316** (1994).
13. L. R. McDowell, Selenium, in *Minerals in Animal and Human Nutrition,* Academic, New York, pp. 294–332 (1992).