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# Effect of Selenium and Vitamin E Dietary Deficiencies on Chick Lymphoid Organ Development[1] (42361)

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*Abstract.* Diets specifically deficient in selenium (Se) and/or vitamin E or adequate in both nutrients were fed to chicks from the time of hatching. Lymphoid organs (bursa, thymus, and in some instances, spleen) were collected from chicks 7–35 days of age. Growth of the chicks fed these diets was monitored over the experimental period as was lymphoid organ growth. The development of the primary lymphoid organs was further assessed by histological techniques and the organ contents of vitamin E ($\alpha$-tocopherol) and Se were determined. Specific deficiencies of either Se or vitamin E were found to significantly impair bursal growth as did a combined deficiency. Thymic growth was impaired only by the combined deficiency diet. Severe histopathological changes in the bursa resulted from the combined deficiency and these were detectable by 10–14 days after hatching. These changes were characterized by a gradual degeneration of the epithelium and an accompanying depletion of lymphocytes. Similar changes, although slower to develop and less severe, were observed in the thymus as a result of the combined deficiency. When both serum and tissue levels of vitamin E and Se were monitored, it was observed that these were rapidly and independently depleted by the specific deficiency diets. These data suggest that the primary lymphoid organs are major targets of Se and vitamin E dietary deficiencies and provide a possible mechanism by which immune function may be impaired. 

Both vitamin E and selenium (Se) have been shown to exert a considerable influence on a variety of immune and growth parameters. The effects of these nutrients on the immune system would seem to be somewhat broad or generalized since there is evidence that dietary deficiencies of either or both produce impairments in T-cell activity (1–3), humoral immunity (4–6), phagocytic activity (7–9), and general lymphocyte mitogenic responsiveness (3, 10). Dietary supplementation with levels of either Se or vitamin E (6, 11) at levels that exceed normal nutritional requirements appear to have general enhancing effects on the immune system.

Both Se and vitamin E function in the maintenance of biological membranes. Vitamin E, in its role as a biological antioxidant, aids in the prevention of lipid hydroperoxide formation through its ability to quench free radicals (12). Se is an essential component of the enzyme, Se-dependent glutathione peroxidase (SeGSHpx), which functions to reduce hydrogen peroxide and lipid hydroperoxides to the corresponding alcohols (13). The combined actions of these nutrients thus serve to protect unsaturated membrane phospholipids and protein sulfhydryl groups from oxidations that may impair cellular function (14). Given their important roles in the cellular antioxidant defense system, it is not surprising that a deficiency condition in either or both nutrients might seriously affect lymphocyte function. What has not been previously examined, however, is what effect(s) deficiencies of these nutrients might have on the primary lymphoid organs and the production of immunocompetent lymphocytes. The studies reported here have thus sought to examine at the organ, cellular, and biochemical levels the impacts of vitamin E and/or Se deficiencies on the development of these primary lymphoid organs.

**Materials and Methods.** *Animals and diets.* All studies used male Cornell K-strain Single Comb White Leghorn chickens (15). Chicks were sexed at hatching and randomly assigned to dietary treatment groups. Each treatment

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group was housed separately in thermostatically controlled battery brooders with raised wire floors. Animals were maintained with a 15-hr day length and were given feed and water *ad libitum*.

A Se-deficient, vitamin E-free semipurified basal diet was used in all studies (16). It contained approximately 0.02 ppm total Se as determined by fluorometric analysis (17). This basal diet was supplemented with 0 or 100 IU vitamin E (as *all-rac*-α-tocopherol)/kg of feed and with 0 or 0.10 ppm Se (as $Na_2SeO_3$) in a $2 \times 2$ complete factorial design. Body weights were monitored weekly.

**Tissue collection and histological processing.** Animals were killed by asphyxiation and the primary lymphoid organs collected. All lymphoid organs were cleaned of adherent nonlymphoid tissue and were weighed. To adjust for differences in body weights between treatment groups, all organ weights were expressed relative to live body weight. Those tissues that were to be processed for histological study were fixed in 10% phosphate-buffered Formalin. These were later embedded in parafin, sectioned at 5–6 μm, mounted, and stained with Harris' hemotoxylin and eosin.

*Determination of lymphoid cell numbers.* Lymphoid tissues were collected and immediately transferred to ice-cold Hank's balanced salt solution (HBSS). The tissues were then dissociated by extensive teasing with mouse-tooth forceps. The resultant suspension was filtered through cheesecloth and the cells washed twice in fresh HBSS. Following this, the cells were suspended in a known volume of HBSS, diluted appropriately, and the number of mononuclear leucocytes was counted using a hemocytometer.

*Analytical methods.* Blood was taken by anterior cardiac puncture. Serum activities of SeGSHpx were assayed by the glutathione reductase coupled assay of Paglia and Valentine (18) as modified by Lawrence and Burke (19). Results are expressed as nanomoles of NADPH oxidized per minute per milligram of protein. Serum vitamin E levels were determined by reverse-phase high-performance liquid–liquid chromatography using a $C_{18}$ column with detection by molecular fluorescence using a modification of the method of Combs and Combs (20). The method was standardized using *all-rac*-α-tocopherol and was capable of detecting α-tocopherol levels down to 0.01 μg/mg of tissue.

Similar procedures were used to determine the Se and vitamin E contents of the primary lymphoid organs. Tissues were rapidly excised and held on ice. These were mixed with 3–4 volumes of cold 0.05 *M* Tris–HCl buffer, pH 7.4, containing 0.154 *M* KCl and homogenized using a motor-driven Teflon–glass homogenizer at 4°C. Soybean trypsin inhibitor (Sigma Chemical Co., St. Louis, Mo.) was added at 1 mg/ml to the homogenizing medium. Homogenates were centrifuged at 12,000*g* for 10 min at 4°C, after which the supernatants were recovered for vitamin E and Se determinations as described above.

*Statistical analyses of data.* Analyses of variance were performed on all quantitative data to determine the level of significance of treatment effects. Significant differences (i.e., $P < 0.05$) were further examined using the protected LSD *t* test (21) to analyze planned comparisons with the control (i.e., vitamin E- and Se-adequate) treatment.

**Results.** The effects of the dietary treatments on the growth of the primary lymphoid organs and spleen are shown in Fig. 1. It is readily apparent that the growth of the bursa, thymus, and spleen were each severely impaired in those animals fed the unsupplemented basal diet. These effects were observed even after adjustments were made for differences in body weights. Of particular interest, however, is the observation that while the combined deficiency impaired bursal growth, a simple deficiency of either Se or vitamin E also produced a severe depression in bursal growth (Fig. 1) after 4 weeks of treatment. This occurred in the absence of any adverse effects on general body growth. Similarly, a simple deficiency of vitamin E significantly depressed splenic growth over this same period. Dietary deficiencies in both vitamin E and Se produced the most severe depression in splenic growth. Finally, simple deficiencies of either vitamin E or Se did not impair thymic growth; however, the combined deficiency markedly depressed thymic weights at 3 and 4 weeks of age.

Several similar experiments were performed with tissue collection occurring over a broad range of ages (data not shown). The results consistently demonstrated that the bursa is

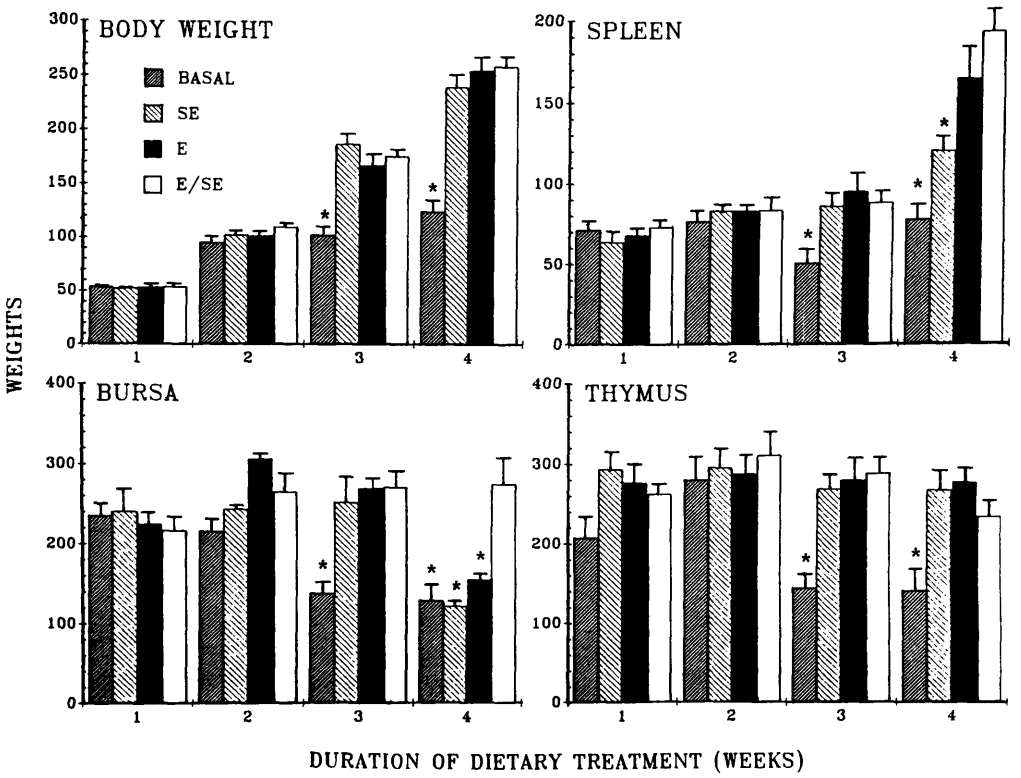


FIG. 1. Effect of dietary treatments on body and lymphoid organ weights. Body weights are expressed in grams and organ weights are given as mg/100 g body weight. Those receiving the E/Se diet were supplemented with both vitamin E and Se as described under Materials and Methods; the E treatment group received vitamin E supplements only, the Se treatment group received Se supplements, and the Basal group received a diet deficient in both vitamin E and Se. There were seven animals per treatment and time period, and each bar represents the mean with the SEM indicated. Significant differences ($P < 0.05$) from the fully supplemented control (i.e., the E/Se treatment) at a given time period are indicated by the asterisk.

particularly susceptible to vitamin E and/or Se deficiencies. Significant depressions in bursal growth and development were produced in some experiments after feeding chicks the basal diet for only 10–17 days after hatching (Table I). During this time, body weights were not significantly affected by the dietary treatment. Significant depressions in splenic weights also were noted to occur routinely at a relatively early age (14–17 days) as a result of a vitamin E deficiency. This resulted whether a vitamin E deficiency was produced simply or in combination with a Se deficiency. Thymic growth, however, characteristically was not impaired by these short treatments and depressed growth occurred only when the diet was deficient in both nutrients for several weeks.

To assess the effect of impaired growth on the ability of these lymphoid organs to generate and maintain a viable lymphocyte population, lymphoid cell suspensions were prepared from both the spleen and bursa and cell counts were performed. As can be seen from Table I, the number of lymphoid cells from either a primary or secondary lymphoid organ source was significantly reduced as a result of dietary treatment by as early as 17 days. In the case of the bursa, this reduction would appear to be directly proportional to the impairment in bursal growth and development, since a transformation of the data to the numbers of lymphoid cells per milligram of bursal tissue resulted in no significant differences between treatment groups (Table I). For the spleen, however, the difference in cell numbers

TABLE I. EFFECT OF DIETARY SE AND VITAMIN E ON LYMPHOID ORGAN WEIGHTS AND CELL NUMBERS

| Dietary treatment[1] | | | | | | | |
|---|---|---|---|---|---|---|---|
| Vit E[4] (IU/Kg) | Se[5] (ppm) | N[2] | Body wt (g) | Spleen wt (mg) | Bursa wt (mg) | Splenocytes[3] ($\times 10^6$) | Bursacytes ($\times 10^6$) |
| 0 | 0 | 46 | 75.3 ± 1.7[6] | 52.5 ± 4.4* | 128.1 ± 6.6* | 16.7 ± 0.4* [0.318][7] | 19.0 ± 0.5* [0.148] |
| 100 | 0.1 | 30 | 78.6 ± 2.3 | 79.6 ± 3.8 | 181.7 ± 7.2 | 32.9 ± 0.2 [0.413] | 26.8 ± 0.2 [0.147] |

[1] All animals were fed the indicated dietary treatments for 17 days.
[2] Number of animals per treatment group.
[3] Cell counts were determined on 5 samples/group.
[4] Provided as all-*rac*-alpha-tocopheryl acetate.
[5] Provided as $Na_2SeO_3$.
[6] Mean ± SEM.
[7] Number in brackets designate the number of mononuclear leucocytes after adjustment for organ weights (leucocytes $\times 10^6$/mg of tissue).
* Indicates a significant difference from the E/Se supplemented group at the $P < 0.01$ level.

was not explained entirely on the basis of the reduced splenic growth. Even after the adjustment for differences in organ weights, the E/Se-adequate group had approximately 30% more lymphoid cells present than did those in the Basal (vitamin E- and Se-deficient) treatment group.

The effects of the dietary treatments on serum levels of vitamin E and SeGSHpx were determined to ascertain whether the appropriate nutritional deficiency had been established. Deficiencies, as indicated by subnormal plasma tocopherol concentrations and plasma SeGSHpx activities, were produced within 1–2 weeks of feeding the vitamin E- and/or Se-deficient diets (Fig. 2). The serum tocopherol and/or SeGSHpx levels continued to decrease through the third week to reach baseline (SeGSHpx) or undetectable ($\alpha$-tocopherol) levels in those animals fed the vitamin E- and/or Se-deficient diets.

The levels of $\alpha$-tocopherol and SeGSHpx in the primary lymphoid organs from animals fed the experimental diets were also examined. It should first be noted that the initial tissue levels of $\alpha$-tocopherol within the thymus were very similar to the levels found in the plasma while the concentrations within the bursa were approximately half this level (Fig. 2). These relationships were maintained in animals fed a vitamin E-supplemented diet. Tissue concentrations of $\alpha$-tocopherol fell rapidly in both the bursa and thymus as well as in the plasma of those animals fed vitamin E deficient diets so that $\alpha$-tocopherol was at near undetectable levels after 3 weeks.

A similar change was observed for SeGSHpx tissue activities over the 3-week experimental period. Initial tissue levels were approximately one-half of those found in the serum at that time; these fell rapidly when the chickens were fed Se-deficient diets. By the end of 3 weeks, tissue SeGSHpx activity had also dropped to almost undetectable levels (Fig. 2). These changes paralleled similar decreases in serum SeGSHpx activity. Animals receiving Se-supplemented diets demonstrated no significant changes in SeGSHpx activity in either serum or primary lymphoid tissues.

In order to evaluate more critically possible changes produced by the dietary treatments on the primary lymphoid organs, tissues from animals fed these diets for 7 to 35 days after hatching were examined using conventional histochemical techniques. As might have been predicted from the effects of these treatments on gross lymphoid organ growth, the bursa was the first lymphoid organ to be negatively affected by dietary deficiencies of these nutrients (Fig. 3). By 10–14 days of age, bursal sections from animals fed the basal diet showed a notable increase in vacuolation of the bursal epithelium surrounding the plicae (individual bursal folds) containing the follicles (Figs. 3A, B). These vacuoles appeared to contain fluid and/or a light-staining fibrillar ma-

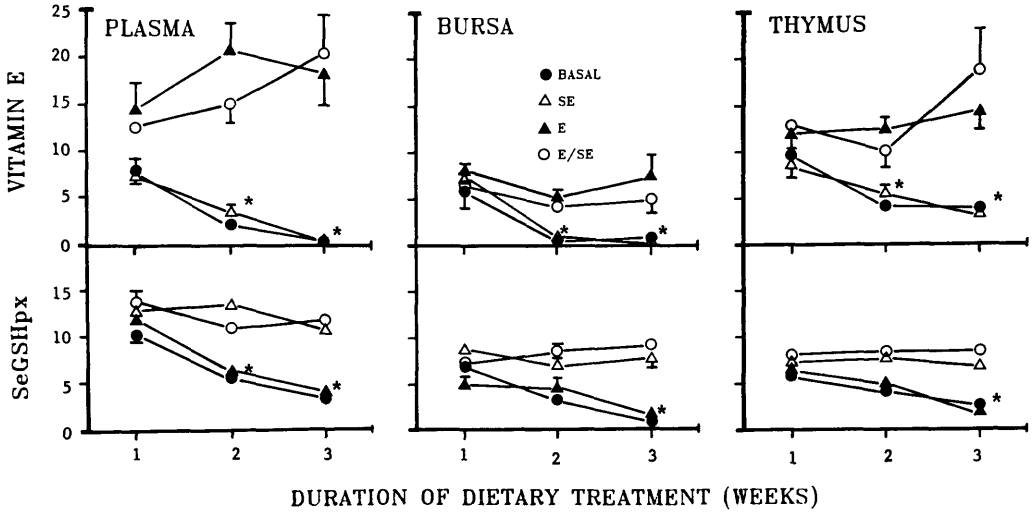


FIG. 2. The effect of the dietary treatments on the plasma and primary lymphoid organ tissue vitamin E ($\alpha$-tocopherol) and SeGSHpx concentrations. Plasma vitamin E concentrations are given as $\mu$g/ml while tissue concentrations are given as $\mu$g/g of tissue. SeGSHpx concentrations are given as nmoles NADPH oxid./min/mg of protein. Those receiving the E/Se diet were supplemented with both vitamin E and Se as described under Materials and Methods; the E treatment group received vitamin E supplements only, the Se treatment groups received Se supplements, and the Basal group received a diet deficient in both vitamin E and Se. Each point represents the mean of five determinations from individual animals fed one of the experimental diets for the period indicated. Vertical bars designate the SEM for each point if the SEM exceeded the area occupied by the data point. Significant differences ($P < 0.05$) from the fully supplemented control (i.e., the E/Se treatment) are indicated by an asterisk.

trix. They later were observed ruptured with a resultant collapse of the overlying epithelial tissue; this presumably resulted in the "notched" or serrated appearance characteristic of many of these sections. By 21–24 days after hatching, a decrease in lymphoid cell numbers within the individual bursal follicles also became apparent (Fig. 3C). In the latter stages of this accelerated bursal atrophy, the epithelial layer surrounding the plicae had disintegrated and the bursa had become a sac-like structure of fibrous tissue with few lymphocytes present (Fig. 3D).

Examination of bursal sections from animals fed diets deficient in either vitamin E or Se did not reveal major histological differences from the E/Se-adequate control, although there was some evidence of increased vacuolation in bursal sections from chicks fed either the Se-deficient or the E-deficient diet. The simple deficiencies never resulted in the severe degeneration of the bursal epithelium that was seen in animals fed the basal diet, however. The bursal sections from chicks with a simple nutrient deficiency usually exhibited only a reduced organ size consistent with the decreased bursal growth already discussed for the singly deficient diets.

Thymic sections were also examined (Fig. 4). No consistent changes in histological appearance from the E/Se-supplemented control (Fig. 4A) were noted during the first 2–3 weeks of development. However, after approximately 21–24 days, some degeneration was noted in both the cortical and medullary areas of thymic sections from animals raised on the basal diet (Fig. 4B). This was characterized by discrete foci lacking both epithelial and lymphoid cells; this thymic degeneration thus resembles the "starry sky" phenomenon described by Weiss (22) as characteristic of thymic atrophy or degeneration. Large cells, apparently serving a phagocytic function, were often found in these areas. Shortly thereafter (i.e., by 24–28 days) definite decreases in lymphocyte numbers were readily detected within the thymus; this was particularly notable in the cortical region (Fig. 4C).

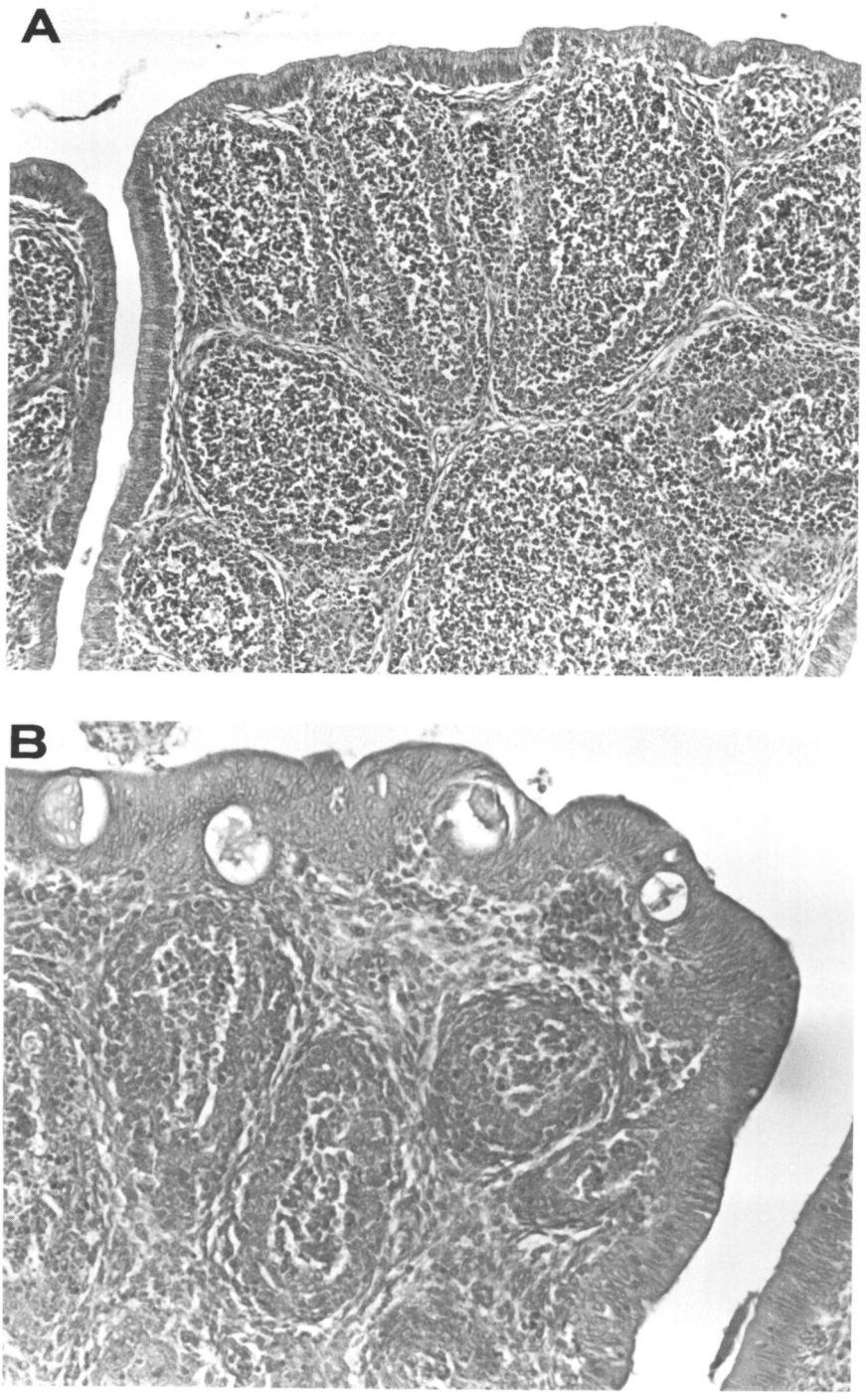


FIG. 3. Photomicrographs of bursal sections from animals fed the experimental diets described under Materials and Methods. All sections were stained using conventional H&E staining technique. Total microscopic magnification for each photomicrograph is indicated in parentheses. (A) Bursal section (30×) from a 14-day-old chick fed the fully supplemented E/Se diet. (B) Bursal section (60×) from a 14-day-old chick fed the Basal diet. (C) Bursal section (30×) from a 24-day-old chick fed the Basal diet. (D) Bursal section (30×) from a 35-day-old chick fed the Basal diet.

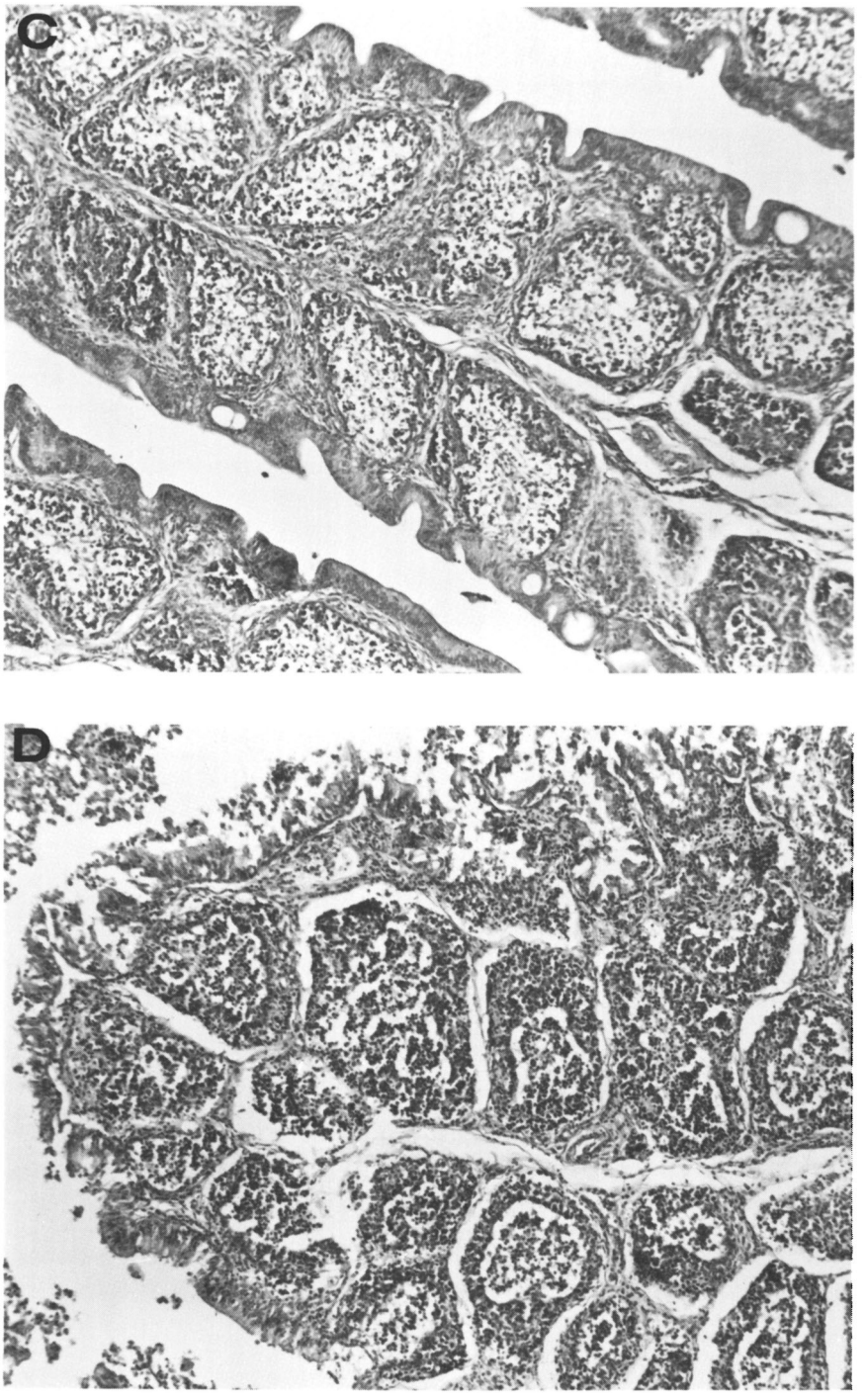


FIG. 3—*Continued.*

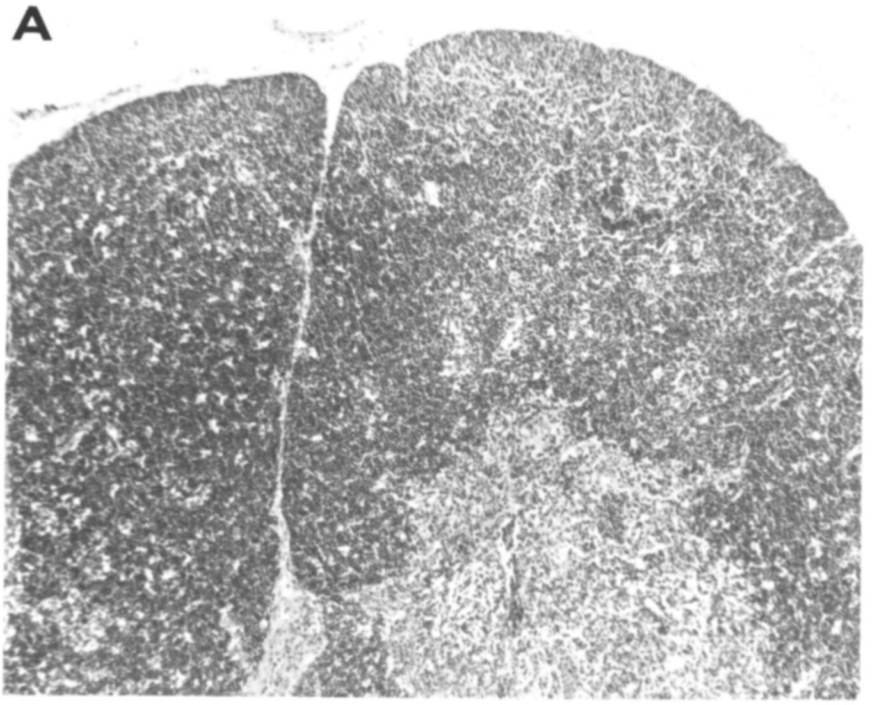


FIG. 4. Photomicrographs of thymic sections from animals fed the experimental diets described under Materials and Methods. All sections were stained using conventional H&E staining technique. Total microscopic magnification for each photomicrograph is indicated in parentheses. (A) Thymic section (30×) from a 28-day-old chick fed the fully supplemented E/Se diet. (B) Thymic section (50×) from a 21-day-old chick fed the Basal diet. (C) Thymic section (30×) from a 28-day-old chick fed the Basal diet.

**Discussion.** Numerous studies have demonstrated that the level of vitamin E available to the animal through the diet (3–5) can influence the animal's ability to make an effective immune response (reviewed by Ref. (23)). Less work has been directed towards defining the role of Se in either immune development or immune function. There is, however, considerable evidence that Se also can influence immune response capabilities (reviewed by Ref. (24)). Because of the well-defined nutritional requirements of the chicken and the clear morphological dichotomy that exists within the avian immune system, the vitamin E- and/or Se-deficient chick model has proven to be an excellent one for the study of the roles of these nutrients on immune development. Earlier work within this model (4) provided evidence that nutritional deficiencies of either vitamin E or Se during early development can retard the development of T-dependent humoral immune function while a combined vitamin E and Se deficiency prolonged the immune impairment.

The present work sought to examine the following questions: (a) what is the direct effect of vitamin E and/or Se deficiencies on the development of the chick primary lymphoid organs, and (b) how do the kinetics of nutrient depletion during dietary deficiencies compare between the primary lymphoid organs and the serum? The first question was initially examined at the gross level by monitoring the effects of either simple or combined vitamin E and Se deficiencies on general body and lymphoid organ growth. A severe and prolonged deficiency of both vitamin E and Se can produce a marked depression in feed consumption (16). Severe protein–calorie malnutrition, in turn, is known to adversely affect immune function (25). However, recent studies by Eskew *et al.*, (3) found that rats pair-fed a diet adequate in

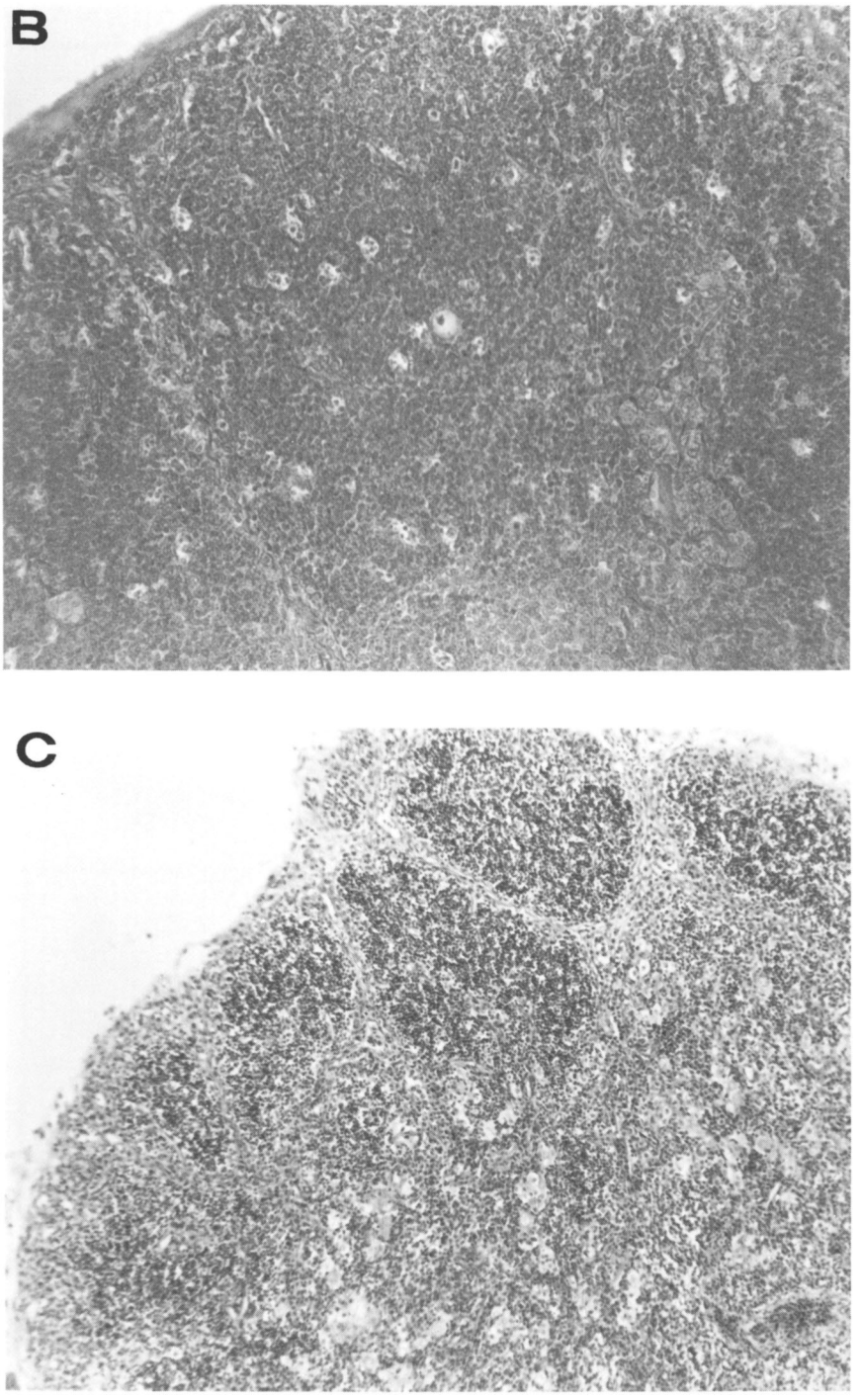


FIG. 4—*Continued.*

vitamin E and Se exhibited elevated immune function when compared to their E/Se-deficient counterparts but were not significantly different from the *ad libitum*-fed controls. General body growth was adversely affected by either the combined deficiency or the pair-feeding treatments, but this effect was not observed until after 3 weeks. Diets deficient only in vitamin E or Se did not depress growth rates during the 5-week experiment but did produce several differences in immune parameters between the simple deficiency groups and E/Se-supplemented controls.

These results in the rat (3) are very consistent with the data reported here with the chick. No depression in the growth rates of E/Se-deficient chicks was observed until 2–3 weeks after hatching. The diets deficient in only one of these nutrients produced no depression in body growth. Simple vitamin E or Se deficiencies did, however, result in a significant depression of lymphoid organ growth, particularly in the bursa. Given the function of the bursa in early B-cell ontogeny (26), treatments that affect the growth and development of this organ might also be expected to influence humoral immunity. This supposition is strongly supported by previous work within this model system (4).

Splenic growth was depressed in animals fed vitamin E-deficient diets, but was not affected by the dietary level of Se. Depression in spleen weights was also seen in the absence of any overall depression of body growth. A first interpretation of these data was that this was largely related to the hemolytic anemia induced in chronically vitamin E-deficient chicks (27). However, the mononuclear leucocyte counts revealed that the vitamin E deficiency also produced an actual depletion of leucocytic cells in the spleen. This observation is further supported by differential counts performed in our laboratory (9) which consistently showed depressed numbers of circulating lymphocytes in chicks fed the Basal diet. The fact that a simple Se deficiency did not produce a change in chick splenic growth is consistent with recently reported experiments examining the effects of Se deficiencies on immune function in mice (28).

These same studies (28) also found that thymic growth was not affected by Se deficiency in either the first or the second generations. This is consistent with the present data where thymus growth appeared to be unaffected by simple vitamin E or Se deficiencies. Only the combined vitamin E- and Se-deficiencies over several weeks resulted in any impairment of thymic growth.

While the growth of the primary and secondary lymphoid organs may provide some indication of the effects of the dietary treatments, a more critical evaluation was facilitated by a histological examination of these organs. The fact that bursal epithelial degeneration appeared to precede later lymphoid depletion is of interest. Since this epithelium of the primary lymphoid organs is known to provide an inductive microenvironment for B-lymphocyte development (26), degeneration of this epithelium would be expected to negatively effect the development and function of these lymphocytes. Several studies (26, 29) have demonstrated that the "follicle-associated epithelium" or FAE of the bursa is particularly important in the development of the bursal follicles and their associated lymphocytes. Clearly, Se and/or vitamin E deficiencies can result in a functional impairment of humoral immunity (4). The histological analysis results strongly support the hypothesis that this impairment may involve the ontogeny of functional B lymphocytes.

The specific cell types within the thymus which are first affected by vitamin E and/or Se deficiencies are less obvious due to the nature of thymic morphology and to the dense populations of lymphocytes within the loose thymic epithelial network. The first visible indication of histopathological change within the thymus is the appearance of foci containing neither epithelial cells nor lymphocytes. The subsequent severe depletion of lymphocytes is similar to the changes occurring within the bursa. Thus, the observed functional immune impairment(s) may also be due to a decreased production of thymus-dependent lymphocytes.

The data presented here provide information regarding the normal levels of SeGSHpx and vitamin E within the primary lymphoid organ tissues and evidence that a dietary deficiency of Se or vitamin E results in a rapid depletion of the reserves of these nutrients.

Whitacre (30) examined the levels of SeGSHpx in a variety of tissues including liver, spleen, kidney, heart, pancreas, and duodenum. Only the duodenum and pancreas had SeGSHpx activities as low as were found in the bursa and thymus. Previous work has demonstrated that histopathological changes due to a severe uncomplicated dietary Se deficiency occurs only in the acinar pancreas (31). SeGSHpx activities found within the primary lymphoid organs that are comparable to those within the pancreas combined with the obvious inability of the lymphoid organs to store SeGSHpx may contribute to the observed sensitivity to vitamin E and/or Se dietary deficiencies. It is also apparent that vitamin E can be as rapidly depleted from these tissues as was SeGSHpx.

Finally, it should be noted that all of the changes in the primary lymphoid organs and in immune function resulting from dietary Se and/or vitamin E deficiencies are highly correlated. The observed decreases in humoral immune activity (4), depressed lymphoid organ growth and development, histopathological changes, and changes in the tissue levels, occur concomitantly. The temporal nature of these factors suggests an underlying basic interrelationship between the immune response parameters and the deleterious effects of dietary vitamin E- and Se-deficiencies on primary lymphoid organ development.

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