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Influence of Dietary Selenium and Vitamin E on the Humoral Immune Response of the Chick[1] (41051)

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Abstract. Nutritional deficiencies in vitamin E and/or selenium (Se) caused impaired immune function, as measured by the humoral response to ovine erythrocytes by young chicks, but only at low antigen doses. In the 2-week-old chick, both vitamin E and Se were required for optimum immune function; however, at 3 weeks of age, either vitamin E or Se was sufficient for optimum immune function. At this developmental stage, Se appeared to be capable of replacing vitamin E with regard to the immune system.

Titrations of dietary vitamin E in the presence of adequate Se and the reciprocal experimental regime did not result in immune enhancement. However, the range of concentrations of each factor employed were within nutritional and nonpharmacological levels. In contrast, high dietary Se produced significant immune suppression in male but not female chicks. These data suggest that Se may be an important component of immune function, and its effect is influenced by antigen concentration, sex, and the ontogenic state.

Many factors are known to affect the ability of an animal to mount an effective immune response. One aspect which has received increasing attention is the nutritional status of the animal receiving an antigenic challenge (1). One nutrient whose effect on immune responsiveness has been extensively examined is vitamin E (2). At pharmacological dosages, vitamin E has been demonstrated to have an immunostimulatory effect on the humoral response (3, 4), to increase delayed hypersensitivity reactions (5), and to affect mitogenic responsiveness (6). Dietary deficiencies of vitamin E have been shown to lead to depressed antibody synthesis (7).

Selenium (Se) is an essential trace element, the function of which is closely related to that of vitamin E (8, 9). Selenium is an integral component of the enzyme glutathione peroxidase (SeGSHpx) (10), which is involved in the reduction of hydrogen or fatty acid peroxides thus protecting the cell from uncontrolled lipid and/or protein oxidation. The possible role that Se might play in influencing immune responsiveness has also been examined. Enhanced antibody formation in mice (11–13) and in rabbits (14) has been reported when Se supplements were given in the presence of vitamin E.

The primary purpose of the experiments described in this paper was to further assess the effects of Se on the humoral immune response. These effects were evaluated in terms of the variables of antigen dosage, the sex and developmental status of the responding organism, and the interaction of vitamin E with Se on the antibody response.

Materials and Methods. *Animals and diets.* The animals used for these studies were Single Comb White Leghorn chickens of the Cornell K strain (15) and Cornell Special C strain (16). At hatching, chicks were randomly assigned by sex to each dietary treatment. Each sex and treatment group was housed separately in thermostatically controlled battery brooders with raised wire floors. Chicks were raised with a 15-hr day length. Feed and water were provided *ad libitum*.

Chicks were fed the low Se (determined by fluorometric analysis (17) to contain less than 0.02 ppm total Se), vitamin E-free semipurified diet of Combs (18). The basal diet was supplemented as indicated with

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0.10 ppm of Se (as Na_2SeO_3) and/or 100 IU/kg of vitamin E (as *all-rac*-α-tocopheryl acetate). Those diets to which either vitamin E or Se were added are termed E or Se diets, respectively. When both nutrients were added to the basal diet, the diet is designated as E/Se.

Biochemical procedures. Blood was obtained by anterior cardiac puncture. The vitamin E activity of serum was estimated on the basis of total fat-soluble reducing activity determined in xylene extracts of sera by a ferric reduction method using bathophenanthroline as the indicator and *all-rac*-α-tocopherol as the standard (19). Results were expressed as milligrams of *all-rac*-α-tocopherol equivalents per decaliters. The Se-dependent glutathione peroxidase (SeGSHpx) was assayed by the glutathione reductase coupled assay of Paglia and Valentine (20) as modified by Lawrence and Burke (21). Results were expressed as nanomoles of NADPH oxidized per minute per milliliter of serum.

Determination of antibody. The antigen used to assess immune responsiveness in these studies was the T-cell-dependent antigen, ovine erythrocytes (SRBC). Injections were given intracardially to the young chicks in a 0.2-ml volume of SRBC suspended in 0.1 *M* phosphate buffer, pH 7.4, containing 0.85% saline. The dosage of SRBCs was a variable in several of the experiments, but all cells used either for immunization or antibody titration were obtained aseptically from the same donor sheep. The concentration of cells per milliliter was standardized by spectrophotometric determinations of hemoglobin content following complete hemolysis (22). Concentrations were also checked by microscopic cell counts and an adjusted 1.0% suspension of SRBC had a concentration of 2.8×10^8 cells/ml.

Chicks were bled by anterior cardiac puncture 7 days following immunization. Serum samples were processed and titered individually by the microtiter hemagglutination method (23). Antibody production was expressed as $\log_2$ titers of the reciprocal of the highest dilution producing visible agglutination of the test antigen.

Experimental treatments. Experiment 1: Day-old chicks were divided into two dietary groups, one receiving the basal diet and one receiving the E/Se supplemented diet. Those chicks that were to be placed on the basal diet were produced from hens maintained on the low Se, low vitamin E Diet B of Combs and Scott (24). This diet is estimated to contain 13.6 IU vitamin E/kg and 0.038 ppm Se. Chicks to be raised on the E/Se diet in this experiment and all chicks for subsequent experiments were obtained from hens fed a practical-type corn-soy breeder diet which was supplemented with vitamin E. Animals were immunized 2 weeks after hatching with a 20% (5.60×10^9 cells/ml) SRBC preparation. Hemagglutinating antibody titers were determined 7 days after immunization in all experiments. *Experiment 2:* Day-old chicks were again divided into two dietary groups and fed either the basal or the E/Se diet. Immunization was at 2 weeks after hatching with 5% (2.8×10^8 cells), 1.0% (5.6×10^7 cells), or 0.1% (5.6×10^6 cells) suspensions of SRBC. *Experiment 3:* Day-old chicks were divided into four dietary groups and each group fed one of the four experimental diets already described. Animals were immunized 2 weeks after hatching with either a 1.0% or 0.5% suspension of SRBCs. *Experiment 4:* Day-old chicks were divided and fed the four experimental diets for 3 weeks prior to immunization. A single dosage of 1.0% SRBC was administered to all birds. *Experiment 5:* All chicks were fed the E/Se diet after hatching and maintained on this diet for 2 weeks. They were then divided into four dietary groups and fed one of the four experimental diets for an additional 2 weeks. At this time it was determined by measurements of serum reducing substances and SeGSHpx levels that appropriate deficiency conditions had been established depending on the dietary treatment. Animals were then immunized with either 1.0% or 0.5% suspensions of SRBC. *Experiment 6:* Day-old chicks were divided into six dietary groups and each group fed a diet containing 100 IU/kg vitamin E and a Se supplement ranging from 0 to 1.0 ppm. Four weeks after hatching all animals were immunized with 1.0% SRBCs. *Experiment 7:*

TABLE I. EFFECTS OF VITAMIN E/Se STATUS ON THE HUMORAL RESPONSE OF 2-WEEK-OLD CHICKS RECEIVING 20% SRBC IMMUNIZING DOSAGE

Dietary treatment	Anti-SRBC antibody (log_2 titers)	Body weight (g)	SeGSHpx[a] (nmole NADPH/min/ml)	Total reducing substances[b] (mg/dl)
Basal	6.41 ± 0.36 (55)[c]	98.2 ± 2.24	18 ± 6	0.076 ± 0.043
E/Se supplemented[d]	6.45 ± 0.24 (27)	109.9 ± 2.93[e]	136 ± 9[e]	1.855 ± 0.98[e]

[a] Mean ± SE of eight determinations.
[b] Equivalents *all-rac*-α-tocopherol, mean ± SE of eight determinations.
[c] Mean ± SE with number of animals per treatment shown parenthetically.
[d] Basal diet supplemented with 100 IU/kg vitamin E and 0.1 ppm Se.
[e] Significantly different from data within the column at $P < 0.05$.

This experiment was the reciprocal of the preceding experiment. Chicks were divided into six dietary groups and each group fed a diet containing 0.1 ppm Se and a vitamin E supplement ranging from 0 to 160 IU/kg. Immunization was the same as in the preceding experiment.

Statistical treatment of data. All samples were coded and randomized for titration and the data were organized into the respective diet and sex groups only after the antibody titers had been read and recorded. Analysis of variance was performed on all data to determine whether any sex, diet, or antigen dosage effects existed. In Experiments 1–5, no significant ($P > 0.05$) effects of sex were determined; therefore, the data for males and females within a dietary or dosage treatment group were pooled.

Results. The first experiments were designed to determine whether the presence or absence of nutritionally adequate but nonpharmacologic levels of vitamin E and/or Se would influence the ability of young chicks to mount an effective antibody response. Results (Table I) show that 2-week-old chicks fed the basal diet were depressed in body weight and serum SeGSHpx activities. These chicks were severely depressed in serum vitamin E levels due to prior maternal depletion. The anti-SRBC antibody titers of those chicks fed the basal diet and immunized with a 20% SRBC preparation were not depressed when compared to the antibody production of those chicks fed the E/Se diet. This experimental design was repeated twice more, once again using the K strain and once using Cornell special C strain chicks (a partially inbred strain which is homozygous with respect to the major histocompatibility complex (B^{13}/B^{13})). Essentially identical results for all parameters examined were obtained.

To examine the effect of lower antigen concentrations within this system, chicks of the same age and fed identical diets were immunized with 5, 1.0, or 0.1% SRBC suspensions. The data obtained (Table II) demonstrate that little difference exists between the two dietary treatments in their ability to influence antibody formation to

TABLE II. EFFECTS OF BASAL AND E/Se DIETS ON THE HUMORAL RESPONSE OF 2-WEEK-OLD CHICKS IMMUNIZED WITH VARYING SRBC DOSAGE LEVELS

	Anti-SRBC antibody (log_2 titers)		
Dietary treatment	Immunizing dose (0.1% SRBC)	Immunizing dose (1.0% SRBC)	Immunizing dose (5.0% SRBC)
Basal	1.38 ± 0.35 (40)[a]	4.24 ± 0.53 (28)	4.56 ± 0.56 (39)
E/Se supplemented[b]	1.50 ± 0.39 (28)	6.54 ± 0.52[c] (28)	5.43 ± 0.54 (28)

[a] Mean ± SE with number of animals per treatment shown parenthetically.
[b] Basal diet supplemented with 100 IU/kg vitamin E and 0.1 ppm Se.
[c] Significantly different from data within the column at $P < 0.01$.

TABLE III. EFFECTS OF VITAMIN E AND Se ON THE HUMORAL RESPONSE AND NUTRITIONAL STATUS OF 2-WEEK-OLD CHICKS

Dietary treatment		Anti-SRBC antibody		SeGSHpx[a]	
Vit. E (IU/kg)	Se (ppm)	Immunizing dose 0.5% SRBC (log_2 titer)	Immunizing dose 1.0% SRBC (log_2 titer)	(nmole NADPH/ min/ml)	Total reducing substances[b] (mg/dl)
0	0	2.31 ± 0.35 (26)[c]	2.96 ± 0.46 (25)	9 ± 3	0.633 ± 0.112
100	0	2.04 ± 0.38 (28)	3.94 ± 0.48 (28)	5 ± 2	1.980 ± 0.355[d]
0	0.1	2.29 ± 0.45 (28)	3.46 ± 0.54 (26)	106 ± 5[d]	0.636 ± 0.128
100	0.1	4.25 ± 0.54[d] (28)	5.46 ± 0.45[d] (26)	128 ± 7[d]	2.032 ± 0.270[d]

[a] Mean ± SE of six determinations.
[b] Equivalents *all-rac*-α-tocopherol, mean ± SE of six determinations.
[c] Mean ± SE with number of animals per dosage group shown parenthetically.
[d] Significantly different from data within the column at $P < 0.01$.

SRBC at the highest (5.0%) antigen dosage. However, the lack of vitamin E and Se had significant ($P < 0.01$) detrimental effects on antibody titers when the antigen level approached a limiting dosage (e.g., the 1.0% SRBC dosage). The overall low responsiveness to the 0.1% SRBC dosage indicates that this antigen dosage was insufficient to elicit a significant production of antibody and thus to allow discrimination of dietary effects on the immune system.

Having established that dietary levels of vitamin E and Se could significantly influence antibody production and that the antigen concentration was an important factor in producing this result, the individual effects of vitamin E and Se were examined. The results of these experiments (Table III) show that deficiencies in either vitamin E or Se had effects on the humoral response comparable to the combined deficiency. All deficiency diets resulted in antibody titers significantly ($P < 0.01$) less than those produced by vitamin E and Se adequate chicks.

In contrast to these results were the findings of an experiment in which chicks were maintained on the same four diets for 3 weeks prior to immunization. These data (Table IV) demonstrate that supplementation with vitamin E and/or Se produced significantly ($P < 0.05$) higher titers than were found in chicks fed the basal diet.

The suggestion that adequate dietary levels of vitamin E and Se are critical in the ontogeny of the immune system and the development of an effective humoral response was further examined in the fifth experiment. As can be seen (Table V), vitamin E and/or Se deficiencies produced following 2 weeks of normal vitamin E–Se nutriture had no significant effects ($P > 0.05$) on the antibody response. However, chicks fed the E/Se supplemented diet throughout the experiment appeared to have slightly higher antibody titers at both immunization dosages. This suggests the possibility of dietary effects below the level of sensitivity of the present experimental design.

The final set of experiments were designed to partition and to titrate the individual effects of vitamin E and Se on antibody production. In the first of these, the dietary level of vitamin E was held constant (100 IU/kg) and the amount of selenium present was varied from 0 to 0.5 ppm. Re-

TABLE IV. EFFECT OF DIETARY VITAMIN E AND Se ON THE HUMORAL RESPONSE OF 3-WEEK-OLD CHICKS IMMUNIZED WITH 1.0% SRBC

Dietary treatments		
Vit. E (IU/kg)	Se (ppm)	Anti-SRBC antibody (log_2 titer)
0	0	2.79 ± 0.54[a] (19)[b]
100	0	5.16 ± 0.30 (49)
0	0.1	4.29 ± 0.33 (48)
100	0.1	4.58 ± 0.31 (46)

[a] Significantly different from data within the column at $P < 0.05$.
[b] Mean ± SE with number of animals per dosage group shown parenthetically.

TABLE V. EFFECTS OF VITAMIN E, Se, AND ANTIGENIC DOSAGE ON THE HUMORAL RESPONSE OF CHICKS PREVIOUSLY FED A DIET ADEQUATE IN BOTH VITAMIN E AND Se

Dietary treatment		Anti-SRBC antibody		SeGSHpx[a]	
Vit. E (IU/kg)	Se (ppm)	Immunizing dose 0.5% SRBC (log_2 titer)	Immunizing dose 1.0% SRBC (log_2 titer)	(nmole NADPH/ min/ml)	Total reducing substances[b] (mg/dl)
0	0	5.71 ± 0.33 (28)[c]	5.22 ± 0.49 (23)	10 ± 7	0.124 ± 0.006
100	0	5.88 ± 0.30 (26)	5.64 ± 0.42 (25)	5 ± 3	1.676 ± 0.130[d]
0	0.1	5.58 ± 0.38 (24)	5.29 ± 0.53 (21)	125 ± 17[d]	0.271 ± 0.065
100	0.1	6.09 ± 0.27 (22)	6.35 ± 0.32 (23)	130 ± 10[d]	2.424 ± 0.164[d]

[a] Mean ± SE of six determinations.
[b] Equivalents *all-rac*-α-tocopherol, mean ± SE of six determinations.
[c] Mean ± SE with number of animals per dosage group shown parenthetically.
[d] Significantly different from data within column at $P < 0.05$.

sults (Fig. 1A) revealed that the antibody response of both males and females fed diets differing in Se content were essentially the same ($P > 0.05$) through the 0.2 ppm Se diet. However, at the highest dietary level of Se (0.5 ppm) antibody production of the males was significantly ($P < 0.001$) depressed. To determine whether this phenomenon was repeatable and to determine the effect of even higher levels of Se, the experiment was again performed with dietary levels of Se extending to 1.0 ppm. Similar results were obtained in a second experiment (Fig. 1B). Only the antibody titers of the males were significantly depressed at 0.5 ppm Se ($P < 0.05$). An even greater decline in antibody production was observed in the males fed 1.0 ppm Se ($P < 0.001$).

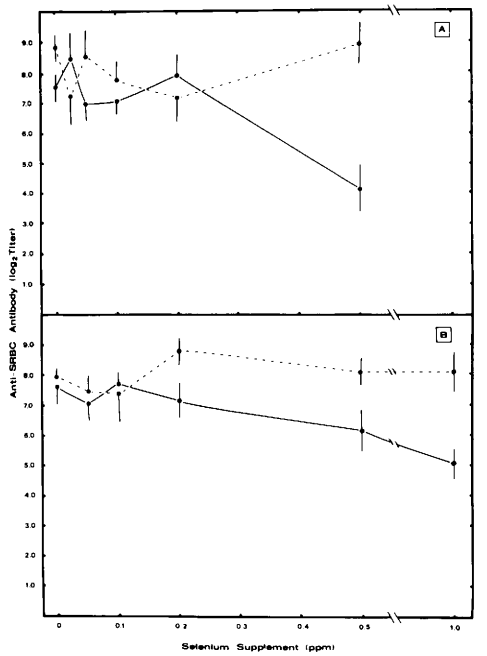

FIG. 1. The influence of dietary Se on the antibody response to a 0.2-ml immunizing dosage of a 1.0% SRBC suspension by 4-week-old vitamin E adequate male (●——●) and female (●– – –●) chicks. The results of two different experiments are shown in panels A and B. Each point represents the mean of 15–20 animals; vertical bars designate the SEM.

Chicks fed Se supplemented (0.1 ppm) diets with levels of vitamin E ranging from 0 to 160 IU/kg did not show any significant differences ($P > 0.05$) due either to diet or sex (Fig. 2). A repetition of this experimental design verified these results.

Discussion. Numerous studies have investigated the effects of nutritional status on immune function. Vitamins A, B_{12}, C, E, niacin, and pyridoxine have all been shown to affect immune function (1, 25). In addition, several amino acids and the minerals iron, zinc, and selenium are known to influence immunity (1, 25). Vitamin E and Se supplements in excess of dietary requirements have been implicated in enhanced disease resistance (26–28). While vitamin E and Se are known to interact in the function of the cellular antioxidant defense system (8), the mechanism of their influence(s) on the immune system is not at all clear. In addition, the variable parameters of sex, ontogenic state, and antigen

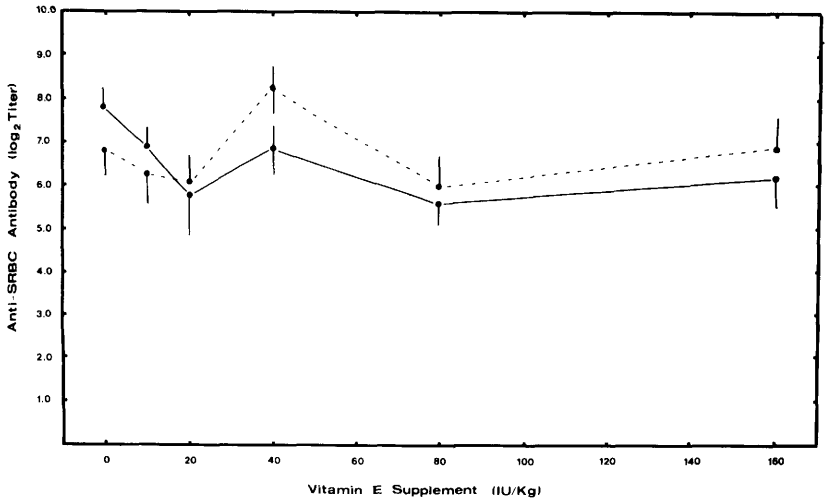

FIG. 2. The influence of dietary vitamin E on the antibody response to a 0.2-ml immunizing dosage of a 1.0% SRBC suspension by 4-week-old Se adequate male (●——●) and female (●- - -●) chicks. Each point represents the mean of 15–20 animals; vertical bars designate the SEM.

dosage have not been examined in previous studies of vitamin E and Se. The present findings, therefore, demonstrate the importance of these variables in assessing the influence of these and perhaps other nutrients on the immune system.

The serum vitamin E levels reported for the depleted chicks in the first experiment were considerably below those reported in subsequent experiments. This was due to the use of chicks from vitamin E and Se depleted hens in the early experiments. It was determined, however, that this severe deficiency in chicks placed on the basal diet was undesirable for further experimentation. This was primarily due to the decreased hatchability and viability of the chicks from depleted hens. The later experiments in which newly hatched chicks were maintained on the basal diet for several weeks would not have been possible using embryonically depleted chicks. The fact that significant reductions in antibody production could be obtained in chicks made deficient in vitamin E and/or Se after hatching, however, suggests that there are postembryonic aspects of the developing immune system that are sensitive to such deficiencies.

The results show that antigen dosage level may be a critical factor in visualizing the effect of changes in nutritional status on the immune system. When deficiencies in both vitamin E and Se were produced, immune impairment was observed only at low antigen dosages (29). Preliminary studies in this laboratory (Marsh, unpublished data) have indicated that maximal antibody formation was obtained using approximately a 10% SRBC immunizing dosage in K-strain chickens. Higher antigen concentrations appeared to be in antigen excess and gave suppressed antibody titers. Therefore, the present study employed antigen levels selected to yield submaximal antibody production while still sufficient to stimulate a humoral response in more than 99% of all immunized animals on adequate diets.

The insensitivity of the antibody response to dietary manipulations at high antigen concentrations was not an isolated phenomenon since it was obtained in multiple experiments using birds from two different genetic strains. Previous investigations (30) reported no suppression in immunity of young vitamin E-deficient chicks or in depleted hens; however, these results may have been due to the high level of antigen administered (0.2 ml 20% SRBCs). Using this antigen concentration, we also failed to detect any effect of nutritional deficiency on immune function.

Immune impairment at low antigen concentrations could have particular practical

implications in disease resistance. The vitamin E and/or Se deficient chick might be unable to respond to a chronic low level infection thus requiring a higher threshold level of progressive infection before immunostimulation occurs. The fact that decreased immune responsiveness was observed in young chicks (2–4 weeks) is of further practical significance as this age is a critical period of susceptibility to viral oncogenesis (31). This is also the period during which maternal antibody declines to undetectable levels and yet the chick has not reached maximal immunoresponsiveness.

Results from this study indicate that the 2-week-old chick requires both vitamin E and Se for optimal immune function. This finding is in agreement with the observations of Spallholtz *et al.* (11, 12), who found that supplements of both vitamin E and Se to mice resulted in higher antibody titers than in those of animals deficient in one of these nutrients. The above-mentioned studies (11, 12) and the present results with the 2-week-old chick suggest that vitamin E is absolutely required for optimum immune function. This suggestion does not extend to the results from older chicks. Studies with the 3-week-old chick demonstrated that Se alone could facilitate optimum immune function and thus have a sparing effect on the requirement for vitamin E in the antibody response of the more mature chick. This observation is further verified by the vitamin E titration studies in the 4-week-old chick receiving Se but no vitamin E supplements. Therefore, it seems possible that Se may be able to duplicate some of the effects on the immune system previously attributed to vitamin E.

The contrasting results obtained with 2- and 3-week-old chicks may indicate changing nutritional requirements during the ontogeny of the immune system. These data suggest that during early development of the immune system, both vitamin E and Se are required while in later development, either factor may be sufficient. This hypothesis is further supported by the findings that chicks fed adequate diets during the first 2 weeks of development and subsequently depleted in vitamin E and/or Se showed no significant immune depression (Table V). The inability of other investigators to observe immune suppression in adult chickens depleted in vitamin E but not Se also support this hypothesis (30). A more comprehensive investigation of nutritional effects on immune system development is currently in progress.

While the exact mechanism of interaction for vitamin E and/or Se with the immune system is unknown, vitamin E has been reported to influence T-helper-cell activity (4), phagocytic activity (27), mitogenic responsiveness (6), and the level of prostaglandin synthesis in immune organs (32, 33). The effect of vitamin E on any one of these parameters would be expected to influence antibody formation. The effect of Se on these and other parameters has been studied less extensively. However, Serfass and Ganther (34) found that Se-deficient rats have reduced killing activity by polymorphonuclear neutrophils and Spallholz *et al.* (11, 12) found that supplemental Se in mice results in enhanced humoral responsiveness.

In the results reported here, no immune enhancement was observed due to either vitamin E or Se supplementation. However, immune enhancement has only been reported for nutritional dosages of vitamin E approaching pharmacological levels (3, 7, 30). The vitamin E and Se supplements used in the present experiments were well below such levels and, therefore, were apparently not sufficient to promote immune enhancement.

A surprising result of this investigation was the finding that dietary Se, in slight excess of the level required by the chick for protection from deficiency diseases, produced significant depressions in antibody titers in male but not female chicks. Se toxicity, as manifest by decreased growth and mortality does occur in the chick. Hill (35) has shown that 40 ppm Se added as Na_2SeO_3 to a corn-soya based diet produced more than 20% mortality of chicks within 2 weeks. However, Se (as Na_2SeO_3) is less toxic to chicks fed the basal diet used in the present experiments: 50 ppm Se produced only 13% mortality of chicks within 4 weeks (El-Begearmi and Combs, unpub-

lished observations). The fact that the level of Se resulting in impaired immune function is one to two orders of magnitude below that known to be toxic suggests that the immune system may be one of the first systems affected by Se at levels insufficient to produce clinical toxicity. Levels of Se shown by these studies to decrease antibody production in male chicks naturally occur in grains from high Se regions (36) of the world; therefore, this may be a matter of practical concern.

Both the chick and the rat show differential sensitivity to Se between the sexes. The impairments in growth and in hepatic oxidative drug metabolism in Se deficiency are greater in male than in female rats (37). The chick has recently been found to show the same responses to Se deficiency (38). Furthermore, the male chick has been found to be more sensitive to the toxic effects of high levels of Se than is the female (38). Thus, it is apparent that both nutritional and supranutritional levels of Se may be metabolized differently by males and females. The nature of the differential immune sensitivity of the males to Se is currently under investigation.

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