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Impaired Antibody Production in Chicks Fed Diets Low in Vitamin A, Pantothenic Acid or Riboflavin.* (28418)

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The deleterious effects of specific dietary deficiencies upon antibody production to a variety of antigenic stimuli have been noted (1-3). In a recent investigation(4), diets deficient in pyridoxine and pantothenic acid markedly impaired antibody production to influenza virus in rats, whereas thiamine deficiency failed to exert an effect. Axelrod *et al.*(5) showed that pyridoxine deficiency in guinea pigs depressed circulating antibody formation to diphtheria toxoid in both the primary and secondary phases. Stoerk and Eisen(7) and Stoerk *et al.*(8) found that male albino rats immunized in a state of pyridoxine deficiency developed serum antibody levels far below those of inanition controls (paired weight) and full fed controls.

Most of these studies have been conducted with animals severely deficient in a particular nutrient. There is need for study with animals only partially deprived of a specific nutrient, since the deficiency state in such studies would bear a closer relationship to the degree of nutritional deficiency usually encountered in man.

This study was designed to determine the effect of a partial, single deficiency of Vit. A, pantothenic acid and riboflavin on antibody production to *Salmonella pullorum* antigen in chicks. The authors are unaware of similar work in the chick.

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Methods. Two hundred day-old White Rock male chicks were randomly distributed into 5 groups of 40 each, weighed, wing-banded, and maintained to 4 weeks in thermostatically controlled battery brooders with wire floors. They were allowed feed and water *ad libitum*. Chicks were individually weighed at weekly intervals. Feed consumption was recorded.

The chicks in 3 of these groups were fed a diet suboptimal in riboflavin, pantothenic acid, or Vit. A, respectively, while those in the other 2 groups received complete diets, containing different levels of pyridoxine. A basal diet suboptimal in riboflavin, pantothenic acid, and Vit. A was prepared and these vitamins were added to give the levels in control A diet except for the vitamin studied. The basal diet shown in Table I was calculated to contain per kilogram, 975 I. U. Vit. A, 4.6 mg pantothenic acid, 2.1 mg riboflavin and 5 mg pyridoxine. These levels are 45, 50 and 72% of the chick's requirements listed by the National Research Council(6) for Vit. A, pantothenic acid and riboflavin, respectively. The complete diets (control A and B) supplied 9960 I. U. Vit. A, 24.6 mg pantothenic acid and 9.7 mg riboflavin per kilogram. All diets contain 13 mg of pyridoxine per kilogram, except for control B diet which contains 5 mg per kilogram. This latter level is 175% of N. R. C. requirement. The vitamin levels used in con-

TABLE I. Composition of Basal Diet.

Ingredient	%
Soybean oil meal (50% protein)	30.00
Isolated soybean protein*	10.00
Cerelose (glucose monohydrate)	48.43
Corn oil	8.00
DL-methionine	.25
Glycine	.30
Ethoxyquin†	.02
Choline chloride	.25
Vitamin mix‡	.50
Trace mineral mix§	.20
Calcium carbonate	.50
Sodium chloride	.50
Defl. rock phosphate (18% P; 32% Ca)	.75
Potassium chloride	.20
Magnesium sulfate, heptahydrate	.10

* C-1 assay protein obtained from Archer Daniel Midland Co., Cincinnati, Ohio.

† 1-2-dihydro, 6-ethoxy, 2-2-4-trimethylquinoline.

‡ Supplied per kg of diet: Vit. A, 975 I.U.; Vit. D_3, 1500 I.C.U.; alpha tocopherol acetate, 53 mg; menadione sodium bisulfate, 6.6 mg; Vit. C, 44 mg; cobalamin, 0.05 mg; folacin, 6.6 mg; d-biotin, 0.66 mg; niacin, 176 mg; and thiamine hydrochloride, 18 mg.

§ Supplied in parts per million of diet: manganese, 90; iron, 37; copper, 3; iodine, 1.8; and zinc, 30.

trol A diet, though higher than the N. R. C. requirements, are similar to those normally found in purified or semi-purified chick diets. Since the results obtained with the diets containing different levels of pyridoxine were similar, both were treated as controls.

At the end of 3rd week, preinoculation agglutination tests were conducted in order to ascertain the presence of reactors. Only 5 birds exhibited slightly suspicious reactions and were discarded.

At 4 weeks of age, 20 chickens of representative body weights were selected from each group for treatment with pullorum antigen and transferred to growing batteries. Also, 10 chicks were kept from the group which received control A diet to serve as uninoculated controls. Each chick in the other groups was injected intravenously on the 28th, 31st and 34th day of age with 0.25, 0.5, and 1 ml of concentrated pullorum antigen,† respectively. During this period the chicks continued to receive their respective diets.

Two weeks after the last injection (7th wk), 5 ml of blood were drawn by heart puncture from each chick. Serum was separated after standing at 37°C for 2 hours, and agglutinin responses were determined(9). The individual serum was diluted 2-fold from 1 in 10 serially to 1 in 1280, with 1% sterilized saline in 10 × 100 mm tubes. Undiluted serum was held at 10°F no longer than a week prior to testing. The pullorum antigen used in the agglutination tests was diluted with an alkalinized, buffered saline solution,‡ to yield a density of approximately 50 × McFarland nephelometer, Tube 1. The antigen-serum mixtures were shaken for 2 minutes, kept at 37°C for 12 hours and read. The end point was taken as the greatest dilution (before addition of antigen suspension) which showed a non-uniform clumped sediment extending essentially over the bottom of the tube. One week later, blood was collected again and the test repeated.

Results. The agglutinin responses shown by control and vitamin deficient chickens are presented in Table II. The sera of chicks which had been fed a diet partially deficient in either Vit. A, pantothenic acid or riboflavin exhibited a lower ($P<.01$) average agglutinin response than that of controls. A larger percentage of partially deficient chicks showed lower titers than did controls, even though some chicks exhibited normal or higher titers within each group. The control groups of chicks showed the highest agglutinin response among all the groups challenged with pullorum antigen. Agglutinin titers were slightly lower for each group with sera obtained 3 weeks as compared with 2 weeks after the last injection of pullorum antigen. However, the relative differences in titers due to diet variables were maintained.

Table III presents data concerning body weight, feed/gain ratio and the weights of bursa of Fabricius, thymus, spleen and adrenal glands of chicks fed adequate or vitamin-low diets. There was greatest reduction of growth in riboflavin deficiency, followed by pantothenic acid. Chicks fed vitamin A-low diet maintained normal growth in com-

† Pullorum tube antigen concentrate obtained from Vineland Poultry Laboratory, Vineland, N. J.

‡ Contains 0.25% Phenol., 0.85% sodium chloride, buffered to maintain pH 8.2 to 8.5, (from Vineland Poultry Laboratory, Vineland, N. J.).

TABLE II. Agglutinin Response of Chicks Fed Adequate or Vitamin-Low Diets to 8 Weeks.

Treatment	No. of chicks	Mean agglutination titer 7th wk	8th wk	Avg	Relative % titer*
Uninoculated control	10	22	8	15	12
Control A	19	130	118	124	100
" B	18	155	121	138	109
Vitamin A-low	20	70†	56†	63†	51
Pantothenic acid-low	20	58†	38†	48†	39
Riboflavin-low	19	51†	35†	43†	35

* A value of 100 assigned as the avg value for control group A.
† Differs from control A ($P < .01$).

TABLE III. Body Weight Gain, Feed/Gain Ratio, and Weights of Bursa, Thymus, Spleen and Adrenal Glands of Chicks Fed Adequate or Vitamin-Low Diets to 8 Weeks.

Diets	Avg 8-wk body wt, g	Feed/Gain ratio	mg/100 g body wt* Bursa	Thymus	Spleen	Adrenal
Control A	1507	2.10	87	644	264	16
" B	1503	2.10	90	512	212	14
Vitamin A-low	1481	2.11	58†	739	215	14
Pantothenic acid-low	1372†	2.14	69	601	239	16
Riboflavin-low	1115‡	2.13	69	723	234	17

* Averages for 6 chicks per group.
† Differs from control A ($P < .05$).
‡ Differs from control A ($P < .01$).

parison to controls. Despite this, the partial deficiency of Vit. A. interfered significantly with optimum agglutinin response (Table II and Fig. 1).

The weights of the bursa, as a function of body weight, averaged significantly less in chicks fed the vitamin A-low diet as compared to the controls (Table III). Chicks fed either pantothenic acid- or riboflavin-low diets also exhibited lower bursa weights; however, these were not significantly different from those of controls. No significant effect of the deficiencies studied were observed on thymus, spleen and adrenal weights. Feed consumption per unit gain was similar for all groups (Table III).

Discussion. Underdahl and Young(10) did not find diets deficient in Vit. A, D, or E to affect appreciably production of swine influenza antibodies. Axelrod(3) stated that the effects of Vit. A, D, and E on antibody production are inconsistent and these factors do not appear to exert a major role in acquired immunity. However, other workers have found that Vit. A deficiency reduces antibody production in deficient animals(11, 12,13). Recently, Harmon *et al.*(14) found that pigs receiving adequate Vit. A showed greater response in antibody production against *S. pullorum* antigen than was observed in vitamin A-deficient pigs. A significant reduction in agglutinin response of chicks fed diets partially deficient in either Vit. A, pantothenic acid or riboflavin, was observed in this study.

In addition to work cited above concerning the adverse effects of pyridoxine and

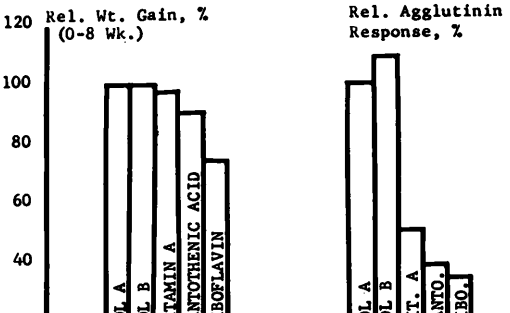

FIG. 1. Relative weight gain and relative agglutinin response of chicks fed adequate and vitamin-low diets.

pantothenic acid deficiencies on antibody production in rats(4) and guinea pigs(5), a combined deficiency of pyridoxine and pantothenic acid(15) but not a single deficiency of pyridoxine(16), markedly impairs antibody production in man.

In this study, chickens fed riboflavin-low diet exhibited the greatest reduction in agglutinin response, whereas those fed the vitamin A-low diet showed the least among the 3 groups which received vitamin-low diets. These partially deficient diets supplied 45, 50 and 72% of the requirement listed by the N. R. C.(6) for Vit. A, pantothenic acid and riboflavin, respectively. Using this reference for adequacy, it would appear that riboflavin was most critical and Vit. A least critical for antibody production in the chicks. However, the riboflavin deficiency was more severe than the deficiency of either Vit. A or pantothenic acid as measured by body weight gains. No other visible manifestations of these deficiencies were noted.

The average 8-week body weights of chicks fed diets containing suboptimal levels of Vit. A or pantothenic acid were 98% and 91% of that of controls, respectively, but their mean agglutinin response was only 51 and 39% of that of controls, respectively. These observations strongly indicate that the dietary need for these nutrients for optimum antibody production is appreciably greater than the levels required for rapid growth.

The possible role of inanition which usually accompanies acute nutritional deficiency was given consideration as a factor in the impairment of antibody production. Accumulated evidence(4,7,8,17,18) argues strongly against any significant role of inanition *per se*. In the present study, feed/gain ratio was similar for all groups, and normal or near normal body weights were obtained except for the riboflavin-deficient group. Hence, the specific vitamin deficiency, and not inanition, appears to have been responsible for the reduction in circulating antibodies.

The deleterious effects of vitamin deficiencies upon antibody production may be related to degeneration or lack of proper development of lymphoid tissues. Lesions of lymphoid cells occur in pyridoxine-deficient animals(3,7). Beaver(19) has shown that lymphoid tissues become hypoplastic in vitamin A-deficient, germ-free rats. The role of bursa on antibody production is well established(20,21,22). Sadler and Glick(20), observed bursa size to influence antibody production in chicks. Burnet(23) also indicated that the bursa, but not the thymus, is involved in formation of antibodies in chicks. The lower bursa size, observed when the vitamin-low diets were fed in this study, is consistent with those observations. However, the bursa may not be the only organ involved in antibody formation, since the vitamin A-low chicks having the lowest bursa weights exhibited agglutinin responses slightly higher than others fed either pantothenic acid- or riboflavin-low diets.

Summary. Chicks fed diets partially deficient in either Vit. A, pantothenic acid or riboflavin showed significantly lower agglutinin response to *S. pullorum* antigen as compared with controls. The weight of bursa, in relation to body weight, was less in chicks fed the vitamin-low diets, being significantly lower than that of controls only for the chicks fed the vitamin A-low diet. No significant differences were observed in thymus, spleen or adrenal weights. The results indicated that the dietary need for these nutrients is appreciably greater for optimum antibody production than the levels required for rapid growth.

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Time Factor in Reversibility of Myocardial Metabolic Changes in Hemorrhagic Shock.* (28419)

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Myocardial failure may play an important role in the terminal cardiovascular collapse that occurs in hemorrhagic shock(1). This could be partly due to the lowered coronary perfusion pressure, which limits coronary flow response. It has also been shown that there is an abnormal pattern of myocardial metabolism in shock(2,3). Although previous studies have shown that transfusion may partly reverse this abnormal metabolic position of the heart(3,4), no attempts have been made to identify the relation of this reversibility to the duration of experimental shock. The present studies were designed with this problem in mind, and the response of the heart to transfusion was tested in dogs subjected to shock of varying durations.

Methods. The studies were carried out on 18 normal mongrel dogs. They were given morphine sulfate (3 mg/kg subcutaneously) followed by a 50/50 mixture of Nembutal, and dial-urethane† (0.25 cc/kg intravenously). Intravenous heparin was given (5 mg/kg) as well as an intramuscular injection of 100,000 units of penicillin and 0.25 g of streptomycin. A catheter was inserted through the jugular vein and maneuvered into the coronary sinus with fluoroscopic visualization, and a polyethylene tube was inserted into the thoracic aorta by way of a femoral artery. Blood samples were drawn simultaneously from each catheter, and analyzed for oxygen, carbon dioxide, lactate, and pyruvate by methods previously described(2).

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† The dial-urethane was generously supplied by Ciba Pharmaceutical Co., Summit, N. J.