The Effect of Vitamin A Deficiency and Newcastle Disease on Lymphoid Cell Systems in Chickens (37487)

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The very high mortality from measles in malnourished children in Africa is well known. There is much evidence that the cellular immune response is depressed in malnourished children. For example, Harland found that children with kwashiorkor had impaired response to tuberculin (1); a report from Smythe's group stated that children with protein-calorie malnutrition (PCM) had thymic atrophy and depressed cell-mediated immune responses (2); Sellmeyer *et al.* observed very low lymphocyte-transformation values in children with either PCM or measles (3); and Watts reported reduced thymus weights in malnourished children (4). Thus the lethal effect of measles may be linked with sudden dysfunction of the cellular immune system already impaired by malnutrition. A similar effect is described in an experimental model in the present report. We had previously found that 3-wk-old chicks raised on a diet deprived of vitamin A and other nutrients developed atrophy of lymphoid tissues, and when infected with Newcastle disease virus (NDV) gave higher yields of virus than normally fed chicks. The present paper shows that while pure vitamin A deficiency produces only moderate loss of lymphocytes from thymus and bursa of 3-wk-old chicks, infection of A-deprived chicks with NDV causes rapid subtotal or total loss of lymphocytes from thymus and bursa in addition to rapid loss of body weight. Neither A-deprivation alone nor NDV alone caused these effects.

Materials and Methods. Three groups of chicks from a commercial source were placed on one of three separate diets from the time of hatching to the termination of the experiment; each group consisted of 18 chicks. The following diets were used: (a) "Growena" normal growing mash; (b) a specially formulated vitamin A-deprived chick diet (General Biochemicals, Catalogue No. 170740), supplemented daily with 6.4 mg of vitamin A palmitate/kg of diet; and (c) the same vitamin A-deprived diet as mentioned in (a) but without the supplemental palmitate.

At age 21 days, 12 chicks/diet group were intranasally inoculated with 1 drop/nostril undiluted allantoic fluid stock of NDV-B strain, titers $10^{7.5}$–$10^{9.0}$; 6 chicks/diet group were not inoculated and were used as controls. Two NDV-infected and 1 control chick/diet group were killed on Days 1, 2, 3, 5, 7, and 9 after NDV inoculation; thymi and bursae were removed, processed histologically and 5 μm sections were then stained with hematoxylin–eosin. Three separate consecutive experiments of similar design showed essentially the same results. Except when otherwise designated, the results of the third experiment are described in this report.

Results. 1. The results are summarized in Table I. The thymus and bursa of Growena-fed uninoculated control chicks showed no abnormal histology (Figs. 1, 5). In NDV-infected chicks on this diet, bursae showed some regression in both specimens on Day 7 post-NDV; 1 of 2, on Day 9, had a patch of polymorphonuclear eosinophils in the subepithelium of a plicate tip. The thymus of NDV-infected chicks was normal until degrees of partial loss of cortical cells were observed on Days 5, 7, and 9, with mild polymorphonuclear cell invasion mostly at the cortex–medulla junction areas; Hassall's corpuscles showed some increase on Days 7 and 9 post-NDV.

2. Control chicks on A-deprived, palmitate-

TABLE I. Effects of Diet Alone (c) and of Diet Plus NDV Infection in Chicks.[a]

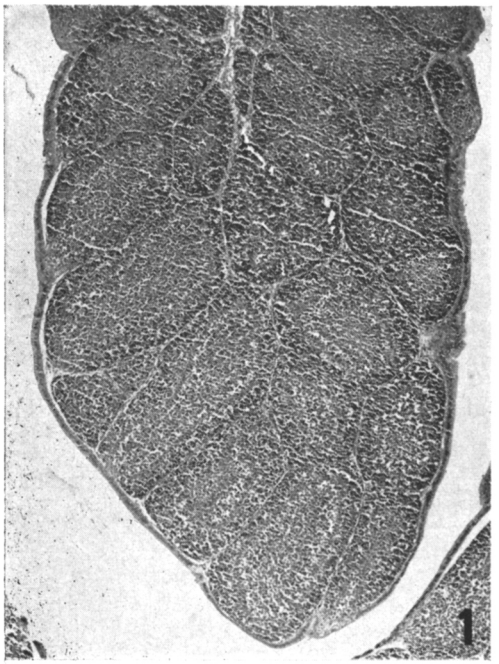
After NDV (days)	Growena		A-deprived with supplement		A-deprived throughout	
	Bursa	Thymus	Bursa	Thymus	Bursa	Thymus
1	—	—	—	—	++	++
1	—	—	—	—	++	+
1_c	—	—	±	—	+	—
2	—	—	+	—	+±	+
2	—	—	—	—	++	++
2_c	—	—	±	—	—	—
3	—	—	—	—	++++	++++
3	—	—	—	—	+++	++
3_c	—	—	—	—	+	—
5	—	+	±	—	+++	+++
5	—	++	+	++	+++	++
5_c	—	—	+++	—	+	±
7	±	++	±	+	+++	++
7	±	+	±	+	++++	++++
7_c	—	±	+	—	++	++
9	±	++	—	+±		
9	—	++	±	++	++++	++++
9_c	—	—	—	—	+++	+

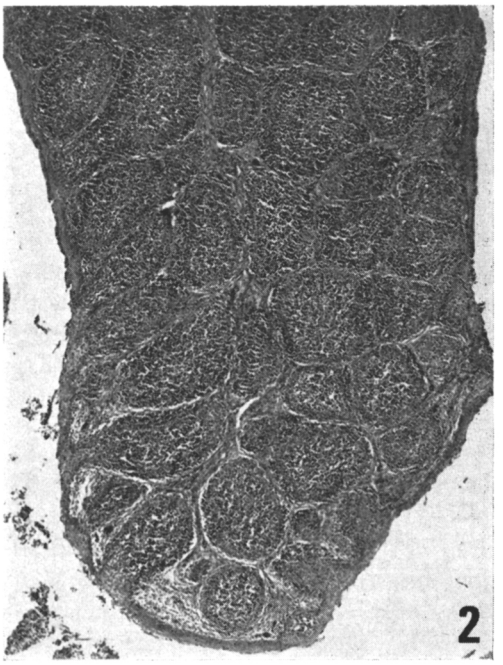
[a] On a normal mash diet, "Growena"; a specially formulated A-deprived diet supplemented daily with 6.4 mg/kg of food of vitamin A palmitate; and a specially formulated A-deprived diet. ± indicates a slight histologic effect; + indicates a definite effect on integrity of cell populations; ++ indicates a significant effect; +++ indicates a severe effect; ++++ indicates atrophy or total loss of cortical lymphocytes.

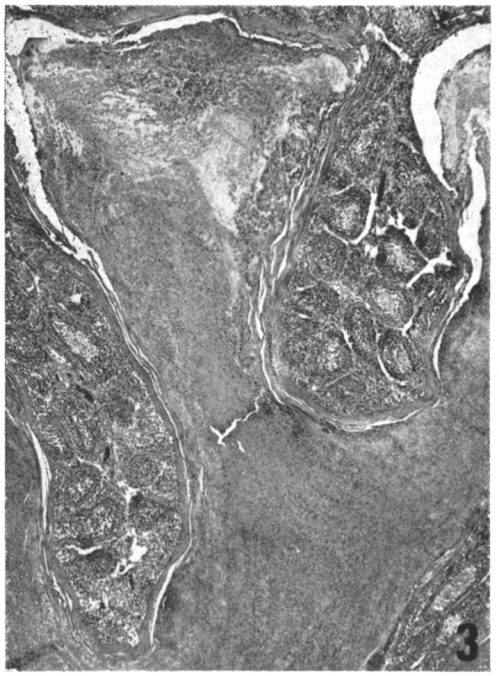
supplemented diet, showed mild abnormalities in the bursae (except for one extremely atrophic bursa on Day 5, for which we have no ready explanation) with degrees of minor involution, cysting, epithelial ruffling; no abnormalities in the thymus of the control chicks were observed (Fig. 2). The bursae in 6 of 12 NDV-infected chicks showed mild epithelial derangement (metaplasia?) and polymorphonuclear eosinophil invasion at such sites; mild sloughing of the epithelium was found in 1 of 2 chicks accompanied by polymorphonuclear cell invasion. The thymus in NDV-infected chicks was not affected until Day 5, when 1 of 2 chicks showed a loss of cortex of about 10% and a lack of normal incursions of the surface into the gland body. Days 7 and 9 showed the same percentage of cortex loss in addition to incursions of polymorphonuclear eosinophils at the cortex–medulla junction and, on Day 9, a distinct increase in thymic corpuscles.

3. In NDV-infected chicks on the A-deprived diet, the bursae of the uninfected control chicks showed varying degrees of reduced overall size; beginning Day 3 (*i.e.*, Day 24 of A deprivation), generally increasing metaplasia and, on Days 7 and 9, epithelial metaplasia and keratinization (Fig. 3), sloughing, and polymorphonuclear eosinophil invasion were observed. There was a large plasma cell population in the subepithelium adjacent to the heavily keratinized area on Day 9. Loss of follicle cortex, and epithelization of medulla were not noticed until Day 9. The thymi of A-deprived, uninfected control chicks were normal until Day 5 when there was a significant invasion of polymorphonuclear cells of one cortex-medulla area, and, on Day 7, patchy focal loss of cortex, with curious concentric denuded epithelial reticulum in the areas of loss (Fig. 6).

NDV-infected bursae showed great increase in interfollicular fibrous epithelial metaplasia and keratinization; there were polymorpho-







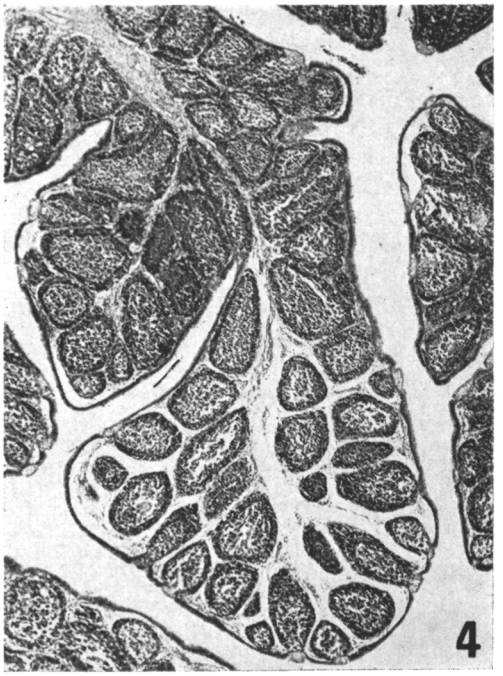
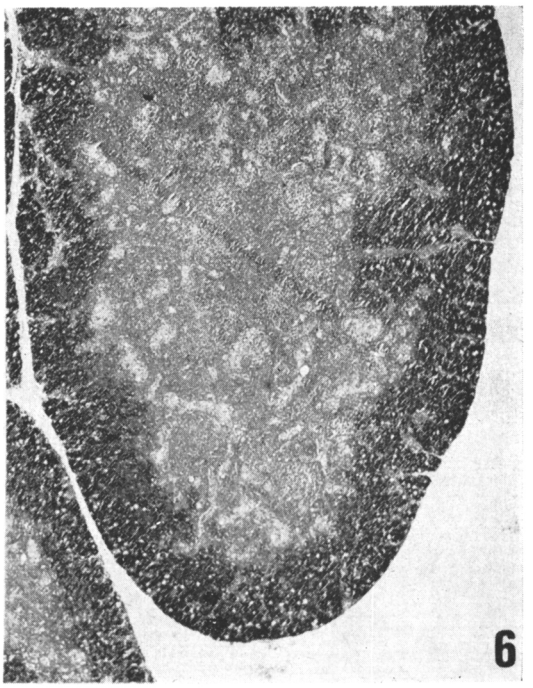


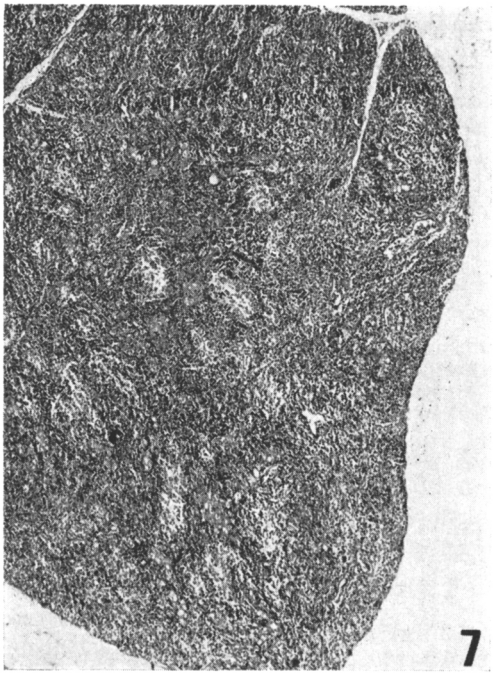
FIG. 1. Tip of plica, normal bursa, 30-day chick, no NDV. 45×.

FIG. 2. Bursa of 31-day chick depleted of vitamin A since hatching; some fibrosis, moderate metaplasia and keratinization; no NDV. 45×.

FIG. 3. Bursa of 31-day chick depleted of vitamin A since hatching, infected 9 days with NDV; extreme atrophy, heavy keratinization, great exudate (2 plicae). 45×.

FIG. 4. Bursa of 31-day chick starved for 6 days, no NDV. 45×.





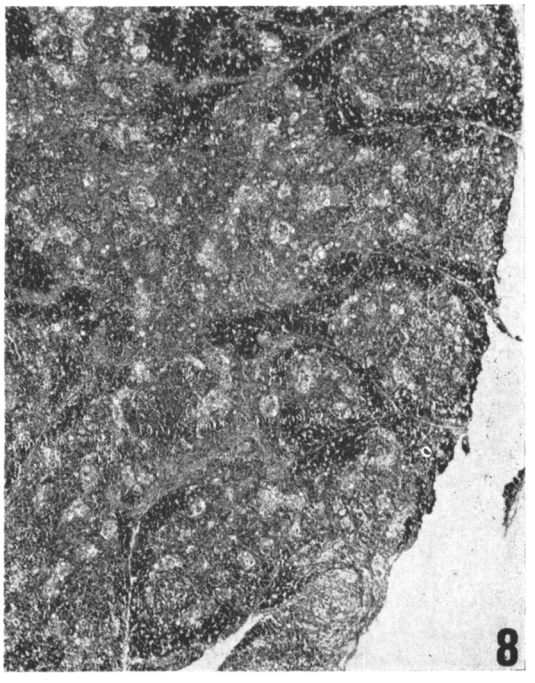


FIG. 5. Normal thymus, 30-day chick, no NDV. 40×.

FIG. 6. Thymus of 31-day chick depleted of vitamin A since hatching; moderate "starry sky" cortex, some loss of cortical width; no NDV. 40×.

FIG. 7. Thymus of 31-day chick depleted of vitamin A since hatching, infected 9 days with NDV; total loss of cortex, epithelized matrix and cortex. 40×.

FIG. 8. Thymus of 31-day chick starved 6 days, no NDV. 40×

nuclear eosinophils at such sites and in adjacent blood vessels, some loss of cortex, cell debris in medulla of follicle, areas of highly secretory epithelial cells, and much exudate on Days 1 and 2 after NDV inoculation. On Day 3, advanced atrophy (which was extreme in one specimen), great loss of cortex, interruption of the vascular ring, and many epithelial mucous cysts became evident. On Day 5, both bursae were small and fibrous; many degenerating cells were present in the medulla; heavy metaplasia, keratinization, polymorphonuclear cell invasion and numbers of plasma cells were observed below the basement membrane. On Day 7, the infected chicks were different from the uninfected controls in that about 25% of the epithelium was keratinized, heavy polymorphonuclear cell invasion took place at such sites, and polymorphonuclear eosinophils were replacing cortex cells in some areas. By Day 9, the epithelium of nearly all primary plicae were keratinized; there were many accumulations of polymorphonuclear cells in cortical areas; the organ was generally atrophic, degenerate, but surprisingly there were only few dead cell remnants in the highly epithelized medullae.

NDV-infected thymus cortices on Day 1 were significantly (at least one third) decreased in one specimen, less so in the other one; thymic corpuscles of the NDV-infected specimen increased in size and number compared to those of the control specimen; polymorphonuclear cells were not seen in the border zone, yet many destroyed cells showed in cleared areas of the cortex. Day 2 was similar to Day 1; Day 3 showed 1 of 2 thymi completely devoid of cortex, the entire thymus appearing as a reticular epithelial mesh in which pale-staining cells were held. Polymorphonuclear eosinophils were commonly observed on the periphery of the former cortex, and thymic corpuscles were often enormous, some heavily staining with alcian blue (AB) and/or periodic acid Schiff stain (PAS).

On Day 7, one specimen showed little change over those observed on Day 5, but the thymus of the other chick was essentially devoid of thymocytes; in addition, almost half of the total thymic tissue consisted of thymic corpuscles, tags of reticulum, degenerating cells, thick-walled vessels, and vacuoles containing intact and degranulated polymorphonuclear cells. Again there were numbers of polymorphonuclear eosinophils in the former cortex. The single thymus specimen on Day 9 was large; when observed histologically, it completely lacked cortex, the entire tissue consisting of pale sheet-like cells, huge numbers of Hassall's corpuscles, and great numbers of polymorphonuclear cells, some larger than the usual granulocytes, and it had rod-shaped granules (Fig. 7).

In a related experiment, progressive body weights in chicks on these diets were of particular significance. Up to and including the day of virus inoculation, all 4 groups showed progressively increasing parallel rates of weight gain. Beginning Day 1 after NDV infection, the vitamin A-deficient group alone began to lose weight[1] (Chart 1). By Days 5 to 11, the weights in this group were an average of 60–70% of the infected groups raised either on normal mash or on the supplemented A-deficient diet, and of those raised on the A-deprived diet but without NDV inoculation.

In an additional experiment, 12 chicks were maintained for 20 days after hatching on the special diet which lacked vitamin A, but was supplemented daily with vitamin A palmitate. All food, but no water, was withheld beginning Day 20 of life. Six of these chicks were inoculated with NDV on the following day, while 6 remained uninoculated. Either 1 or 2 chicks of each group were killed on Days 2, 4, 5, and 6. The bursae of both groups showed progressive involution and degrees of fibrosis; the thymi increasingly had a starry appearance and narrowed cortices (Fig. 8). On Days 5 and 6, the main difference between the thymi of uninfected and infected specimens was a subtotal loss of cortex in the one and total loss in the other; there was a great numerical increase in thymic corpuscles in both. Granulocytes were not a feature in either.

[1] Another experiment showed similar results with both groups.

CHART I

WEIGHT INCREASE OF CHICKS ON TWO DIFFERENT DIETS
BEFORE AND AFTER INOCULATION OF NEWCASTLE DISEASE VIRUS IO[9]

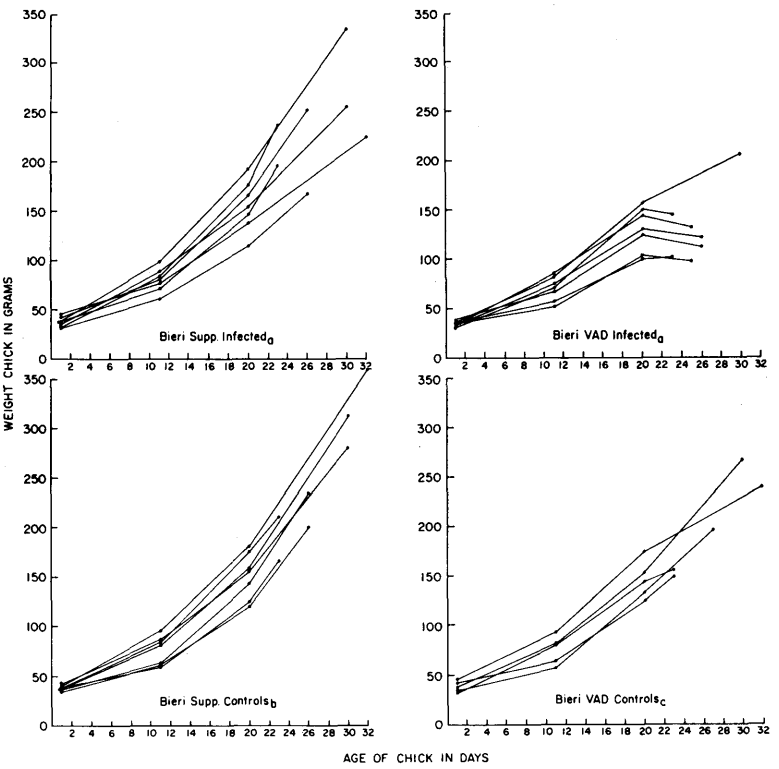


CHART 1. [a] Chicks in both infected groups were inoculated on day 21. [b] Bieri Supp. = the same diet as "Bieri VAD" to which a supplement of A-vitamin palmitate is added daily. [c] Bieri VAD = full nutritional diet for chickens from which vitamin A has been deleted.

Discussion. It has been noted clinically for many years, that acute infection can precipitate overt signs of malnutrition in children, and that malnutrition and infection can interact to intensify the effects of both. The role of the lymphocyte cell system in these interactions has recently been implicated as a major factor (1–4). The experiments here reported indicate that in a growing animal lack of a single dietary factor may allow relatively normal growth *until* coupled with an acute infectious disease. The fast developing synergistic effect in the avitaminosis-A/NDV model apparently includes rapid destruction of both "T" and "B" cells.

The direct effect of hypovitaminosis A on epithelial cells, particularly corneal cells, cells of respiratory mucous membranes, and ducts of oculonasal glands (5) and taste buds (6) are characteristic of this deficiency in humans or experimental animals. Yet aspects of this familiar pattern are unexplained. For example, in A-deficient areas of the world many people become blind from corneal opacity in only one eye, suggesting that a secondary factor such as irritation or subclinical infection has precipitated corneal keratinization on the one side. In our previous experiments on nutritionally deficient chickens, it was clear that keratinized cells replaced mucociliated cells of nasal mucosae only in areas desquamated by NDV infection; areas adjoining such desquamated foci did not slough and did not become keratinized (5). So A deficiency plus virus infection acted synergistically to produce focal keratinization, just as A deficiency plus

irritation or infection may cause unilateral keratinization of the cornea.

Other secondary factors may trigger other synergistic interactions between malnutrition and infection. The very high lethality of measles reported in malnourished children in certain areas of Africa has rarely been reported in equally malnourished children in India. This suggests that localized dietary or genetic factors might intensify, or modify, effects of PCM on the immune system of very young children. Lack of vitamin A is not a likely candidate in this case, since A deficiency is common in India and rare in Africa; but some other (pyridoxine?) deficiency may be involved focally in Africa, and/or a more generalized genetic factor may afford degrees of protection in Indian children.

Complete starvation produced rapid loss of lymphocytes from both "executive" lymphoid organs, and infection seemed to accelerate the process principally in the thymus (7). This experiment was not designed to simulate marasmus, but to establish in this model whether lack of food would specifically affect the cortical areas in these two organs, as Jolly's (8) experiments on the pigeon bursa had suggested.

The chicken is an effective model in which such interactions may be explored, because of clear differentiation of the organs in which lymphocytes are processed to function in cell-mediated (thymus) and immunoglobulin-producing (bursa) immune responses; specific diets can be designed and certain genetic strains are available if indicated.

Summary. A diet without vitamin A but otherwise nutritionally complete was given to chicks from time of hatching. Control diets with adequate vitamin A were given to two other groups. At age 21 days, Newcastle disease virus (NDV) was nasally inoculated into all 3 groups; controls of each group remained uninoculated. Between Days 1 and 3 after NDV inoculation the A-depleted chicks showed significant loss of lymphocytes from the cortex of both the thymus and the bursa of Fabricius, while those on control diets did not show any loss. On Days 5 to 9, there were minimal to moderate effects on populations of lymphocytes in these organs of the group of chicks given adequate vitamin A, but consistently much more severe effects became apparent in the A-depleted group in which 3 of 7 (42%) of the cortices were essentially devoid of lymphocytes. Granulocytes were prominent in areas of thymic and bursal cortex depleted lymphocytes. Noninfected A-deprived chicks and chicks on a normal diet showed relatively modest depletion of cortical lymphocytes from both organs beginning 5 days after NDV infection.

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