Acute Newcastle Viral Infection of the Upper Respiratory Tract of the Chicken

II. The Effect of Diets Deficient in Vitamin A on the Pathogenesis of the Infection

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Keratotic and squamous changes characteristic of vitamin A deficiency were minimal even in chicks which were malnourished and growth stunted and had no vitamin A in their diet. However, when these chicks were infected with Newcastle disease virus (NDV), keratotic changes appeared, most markedly in areas regenerating after infection. In chicks raised on full nutrient diets lacking only vitamin A, keratotic changes appeared in several areas of nasal mucosa but were absent from the mucosa of the inner (under) surface of the maxillary turbinate. Following NDV infection, such changes did appear in the inner lining epithelia. It is suggested that depletion of vitamin A causes regenerating epithelial cells to keratinize. Other effects of combined lack of vitamin A plus NDV infection were exhaustion of lymphoid cells from cranial bone marrow and exhaustion of lymphoid cell systems locally from the nose and paranasal glands. (Am J Pathol 78:417–426, 1975)

THE UNIQUE RELATIONSHIP between vitamin A deficiency and squamous metaplasia of respiratory epithelia is well established. From the early studies of Wolbach and Howe [1] through the recent reports of Hayes,[2] this striking lesion has been a hallmark of the deficiency. The association of infection with vitamin A deficiency is also recognized, but it has not been known whether viral or bacterial infections precede or follow the keratinizing lesions. It has been suggested that keratinization might assist in the spread of bacterial infection.[3]

In a brief report on the histology of the nasal mucous membranes of chicks which had been infected with Newcastle disease virus (NDV) after being raised on a diet deficient in vitamin A, we had noted that the inner surface of the maxillary turbinate scroll was the first area to be desquamated and that it consistently showed the earliest signs of keratinizing metaplasia.[4] Since the original vitamin-A-deficient diet had

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been formulated to produce a deficiency in rats, it was severely deficient in other nutritional requirements for growing chicks. It was then necessary to compare the effects of a nutritionally complete chick diet deficient *only* in vitamin A with the effects of this rat diet which also severely limited growth, on the pathogenesis of NDV. Heavy keratinization of mucous membrane epithelium was produced in infected, vitamin-A-deficient chicks but not in normally nourished chicks. In addition destruction of lymphocytes in the area of infection was a striking effect of the combination of NDV infection and absence of vitamin A.

Materials and Methods

Commercially obtained White Leghorn chickens were maintained on two special diets. The vitamin A test USP (rat diet) was made by the Nutritional Biochemicals Corporation, Cleveland, Ohio. The vitamin-A-deficient diet for chickens was supplied by General Biochemicals (formula courtesy of Dr. J. G. Bieri [5]). To supplement the deficient chicken diet, vitamin A palmitate was added in the amounts indicated in the individual protocols.

The viruses used in these studies were two strains of Newcastle disease virus, the mesogenic B strain, and the vaccine strain.[6,7]

Histologic technics for these studies have been previously described.[8]

Results

There were four experiments in which the multiply deficient diet was administered for different periods of time and two in which it was combined in different ways with a normal mash diet before inoculation with NDV. Each experiment included four sets of chicks: a) normally fed, uninoculated; b) normally fed, NDV-inoculated; c) deprived, uninoculated; and d) deprived, NDV-inoculated. The results of these early experiments have been summarized in Table 1, which shows that only with the continued uninterrupted deficient diet was the keratinization apparent, and this only in chicks which had received the intranasal inoculation with NDV, either the mesogenic B strain or the less virulent vaccine (lentogenic) strain. No keratinization occurred in deficient chicks that did not receive NDV in any of the series.

Figures 1 and 2 show the effects of 1 and 2 days of infection on chicks which had been on the deficient diet for 21 days before infection. Beginning destruction of the cells with very limited inflammatory response is apparent.

Figure 3 shows beginning keratinization as it occurred 10 days after infection with the B strain, and Figure 4 as it occurred 13 days after infection with the vaccine strain. Figure 5 shows that complete recovery and mucosal regeneration had occurred after 7 days of normal diet.

Table 1—Effect of Different Times on Multiply Deficient Diet on Keratinization of Middle Turbinates Following NDV Infection

Virus	No. of chicks with deficiency plus virus	Time on deficient diet before NDV	Time studied after NDV (dys)	Results
B	3	12 days starting at 10 days of age	6–9	Metaplasia at 8 days
B	4	21 days after hatching	1–5	No metaplasia or keratinization
B	6	21 days starting at 11 days of age	1–7	Flattened epithelium at 3 days, keratinization at 5 days
Vaccine	6	12 days starting at 10 days of age	6–13	Keratinization at 13 days, eliminated with 2 days normal diet
B	5	22 days of 80% deprived 20% normal mash	6–13	No keratinization
B	6	Alternating deficient and normal 59 days	3–13	One of 7 with massive keratinization of inner mucosal surface

Vitamin A Deprivation Alone Plus NDV Infection

There were three experiments to determine whether the nasal mucous membranes destroyed by NDV infection in chicks depleted of vitamin A would be replaced by keratinizing epithelia during regeneration. In all experiments, chicks were deprived of vitamin A from the time of hatching, and all of those inoculated with NDV were given the virus on the 22nd day of life. Effects on the thymus and bursa have been published elsewhere.[9] The last experiment compared the pathogenesis of NDV in chicks on a normal mash diet (Group I), with those deprived of vitamin A since hatching (Group II) and those (Group III) on the same diet as chicks in Group II but with vitamin A palmitate supplement (6.7 mg/kg food). Since chicks in Groups I and III did not differ, the comparisons will be made throughout between normally fed chicks (Group I) and vitamin-A-deprived chicks (Group II).

Days 1 and 2 of Infection

Normally Fed Chicks. There was inflammatory cell infiltration and some destruction and desquamation of maxillary and olfactory turbinate epithelia in 2 of 4 chicks on Day 1, destruction of lateral nasal gland ducts in 1, and milder infections in the other 2 chicks. There was no significant effect on plasma cell populations in the Harderian glands.

Vitamin-A-Deprived Chicks. There was submucosal inflammatory cell infiltration, epithelial sloughing and exudate in maxillary turbinates

and nasal fossa floor in 2 of 4 chicks; great distension of all intact acini; mild (one to two layers) to heavy (six to seven layers) laying down of squamous epithelium on tips of turbinates, nasal floor, lower septum and lower lateral walls in 1 of 4 chicks; plasma cells were absent from lateral nasal gland ducts and from intralobular spaces of most Harderian glands. Two chicks showed little evidence of having been infected.

Days 3 and 5 of Infection

Normally Fed Chicks. Heavy to very heavy infiltration of maxillary turbinate submucosae by inflammatory cells, essentially complete thin nonciliated pavement epithelial cover, and modest plasma cell populations in all glands of Harder were seen.

Vitamin-A-Deprived Chicks. While degrees of effect varied among the four specimens, in seven of the eight maxillary turbinates, mucociliated epithelium covered the extremely delicate, almost cell-free submucosae of the inner surface of the turbinate (Figure 6), in striking contrast with the heavy submucosal infiltration in the normally fed, infected birds. There were patches of keratinization in some of these (Figures 7 and 8). There was extensive keratinization of the mucosal surface of the nasal fossa floor in 1 chick, foci of keratinization of the maxillary sinus membrane in several, and bilateral metaplasia of one pair of lateral nasal gland ducts with cystic degeneration of parts of the gland body. There were no visible plasma cells in any of the four pairs of glands of Harder or in the lateral nasal gland ducts.

Days 7 and 9 of Infection

Normally Fed Chicks. There was progressive repair (Figure 9) of all surface epithelia of all turbinates, including partial (7 days) and complete (9 days) ciliated cell cover. The normal pattern of acinar structure was restored. Populations of plasma cells were abundant in the Harderian glands.

Vitamin-A-Deprived Chicks. Infection was apparently very mild in 1 chick, but there was bilateral keratinization of the under surface and tips (free edges) of the maxillary turbinates of all other chicks. In the 9-day turbinates there was complete loss of basophilia in the cartilages underlying the affected tip area.

Changes in Uninfected, Vitamin-A-Deprived Controls

Several of the vitamin-A-deprived controls (after initiation of the experiment) showed some changes probably due to prolonged vitamin-A-deficiency: extreme reduction in the numbers of both ciliated and

mucous acinar cells in the surface membranes and lack of plasma cells in the Harderian and lateral nasal gland tissues. Other controls showed no signs of vitamin A deprivation. Only 1 of the vitamin-A-deprived controls, a Day 9 chick, had very heavy keratinization of the nasal floor, lower septum, lower lateral walls, lateral nasal gland ducts, and termini of the lacrimal ducts. However, in none of the uninfected vitamin-A-deprived chicks in any of the experiments was there keratinization of the central portion of the maxillary turbinate mucous membranes or of the membranes of the sinus or its ostium, both of which were routinely affected after infection.

Marrow Cells and Harderian Gland Plasma Cells

In histologic sections cut through the long axis of the Harderian gland, the frontal bone appears as an inverted triangle with a few slender trabeculae enclosing a spacious marrow. Within the marrow of most of the normally fed and vitamin-A-supplemented chicks there were several rows of large round highly basophilic cells concentrated along the bony framework and sometimes aggregated in patterned masses within the reticulum of the marrow. In equivalent sections in vitamin-A-deprived chicks these cells were relatively scant or lacking. All of the 53 chicks in this comparison were infected with NDV.

Histologic sections through this area in each of the 53 chicks in the experiment were scrambled and numbered, and cell populations in the marrow quantitatively "graded" 0 to 111. When reassigned to their respective groups, the grades were as shown in Table 2. A similar procedure was followed in evaluating the relative population of plasma cells in the Harderian glands in the same sections. While these scores are only qualitative, they suggest that lack of available vitamin A depresses the normal populations of lymphocyte-line cells found in these two anatomic sites, especially during the course of infection.

Discussion

One of the most common interactions between malnutrition and infection in children is that between vitamin A deficiency and respiratory infection. Vitamin A deficiency is known to be common in the Middle East,[10] Indonesia,[10] India[11] and Bangladesh.[12] Respiratory virus infections are extremely common in early childhood in India.[13–15] For this reason the effects of a single virus infection superimposed on vitamin A deficiency alone and on multiple nutritional deficiency were studied in chicks. Both types of diet, *when combined with NDV infection,* produced immune cell depletion and keratinization of epithelial tissues.

Table 2—The Effect of Vitamin A Depletion and Newcastle Disease Virus Infection on the Cells of the Bone Marrow

Days after NDV	Chick	Group I (Growena mash) Cells in marrow	Group I (Growena mash) Plasma cells in Harderian gland	Group II (Vitamin A deficient) Cells in marrow	Group II (Vitamin A deficient) Plasma cells in Harderian gland	Group III (Vitamin A deficient, vitamin A palmitate) Cells in marrow	Group III (Vitamin A deficient, vitamin A palmitate) Plasma cells in Harderian gland
	1	+	++	±	0	+	++
1	2	+	++	+	0	±	+
	c			±	+	+	+++
	1	+	+	0	+	+	+
2	2	+	++	0	0	+	+
	c	+	++	±	0	+	++
	1	+	+	0	±	++	++
3	2	+	+++	0	0	+	+
	c	+	+++	±	0	+	+++
	1	+	+++			+++	+++
5	2	+	++	0	0	++	+
	c	+	++	+	++	±	+
	1	++	+	±	0	+++	+++
7	2	++	++	0	0	++	+++
	c	+	+++	±	++	+	+++
	1					+++	++
9	2	+++	+++	±	+	+++	++
	c	+	++	±	+	+	+

± = very few cells in central marrow, but aggregations along junctures of bony plates; c = uninoculated chicks.

The severely depleted diet caused growth stunting and more rapid mortality.

The most striking lesion of vitamin A deficiency in humans is rupture of the keratinized cornea concomitant with severe infection. This is often unilateral. We have emphasized that focal keratinization of chick nasal mucosae occurred *only* in areas where NDV infection destroyed the epithelium. We suggest that unilateral corneal keratinization and focal mucosal keratinization both represent synergistic effects of avitaminosis A (a conditioning effect) and virus infection (a destructive effect). The vitamin-A-deficient host's cells are then unable to regenerate normally.[2] We suggest that, following destruction by virus (which then stimulates regeneration of the epithelium), the residual basal cells are unable to produce normal mucous cells or ciliated cells in the absence of vitamin A. Wilhelm [16] indeed has shown that the regenerating epithelial cells in the trachea of vitamin-A-deficient rats following cu-

rettage are squamous and keratinized even when the adjacent cells are still columnar. This alternation of squamous and mucous columnar cells is most clearly demonstrated in the *in vitro* experiments of Fell.[17]

Avitaminosis A in human infants, however, "is a disease of poverty and ignorance," [9] and it is often found in company with other dietary deficiencies. The marked effect of respiratory virus infections in chicks which were raised on the very nutritionally deficient vitamin-A-deficient rat diet are therefore of immediate concern because they offer certain crude analogies to the plight of infants in whom marasmus and vitamin-A-deficiency are associated who are liable to upper respiratory virus infections.

NDV infection produced striking focal keratinization in vitamin-A-deprived chicks but not in normal chicks. In our laboratory, influenza infection did not produce keratinization, and laryngotracheitis virus produced belated keratinized patches in vitamin-A-deprived chicks. Perhaps the rapid, abnormal production of keratinizing cells by basal cells is a characteristic response of vitamin-A-deprived epithelia to specific myxovirus infections.

Depletion of bone marrow stem cells may be a primary synergistic effect of vitamin A deficiency and NDV infection. The rapid loss of lymphocytes from thymus, bursa, Harderian gland and nasal gland ducts could result directly from failure of stem cells to divide due to lack of vitamin A. Other viruses are known to have an effect on bone marrow cells,[18,19] though the effect of lack of vitamin A on this has not been studied.

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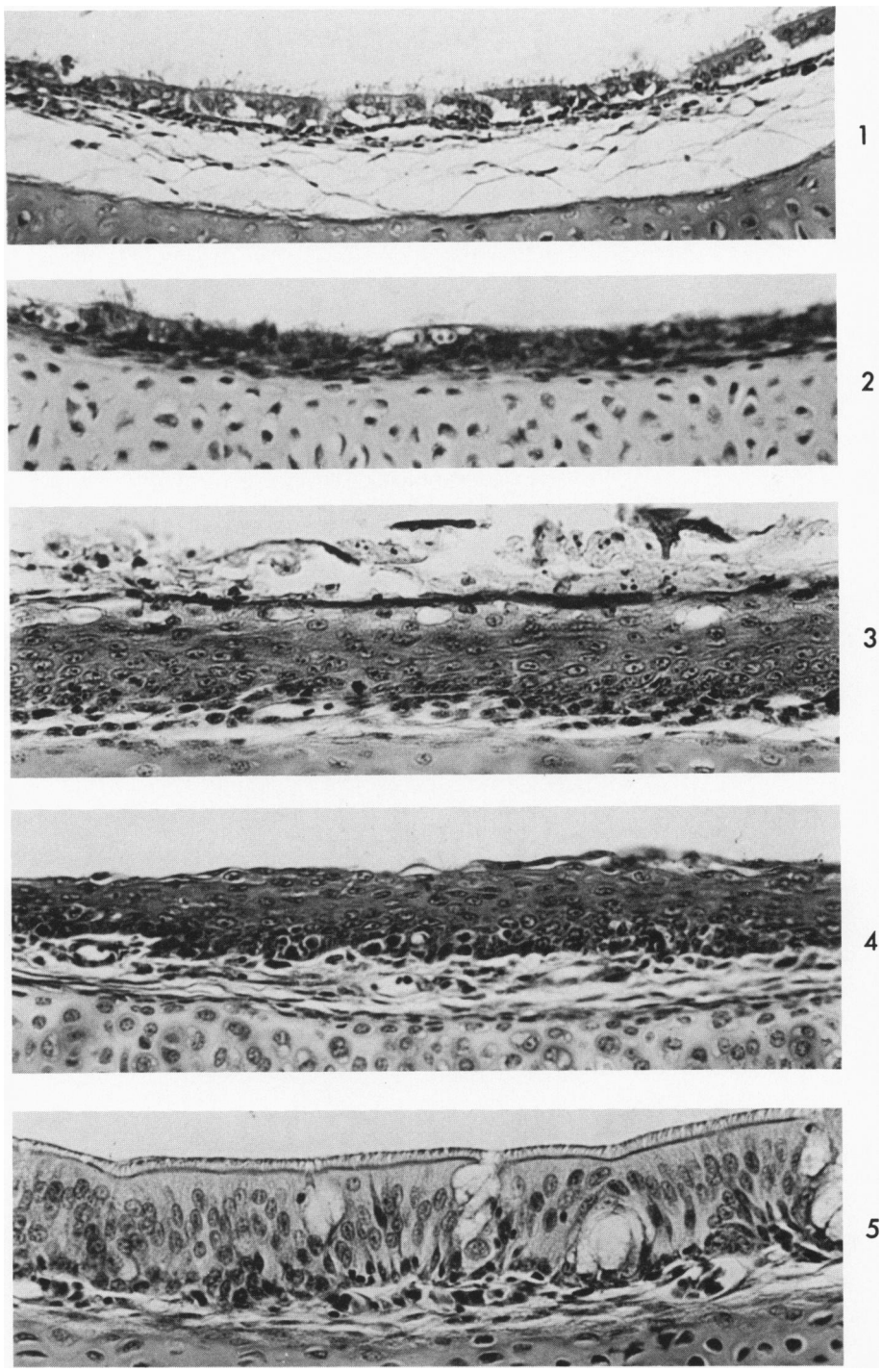
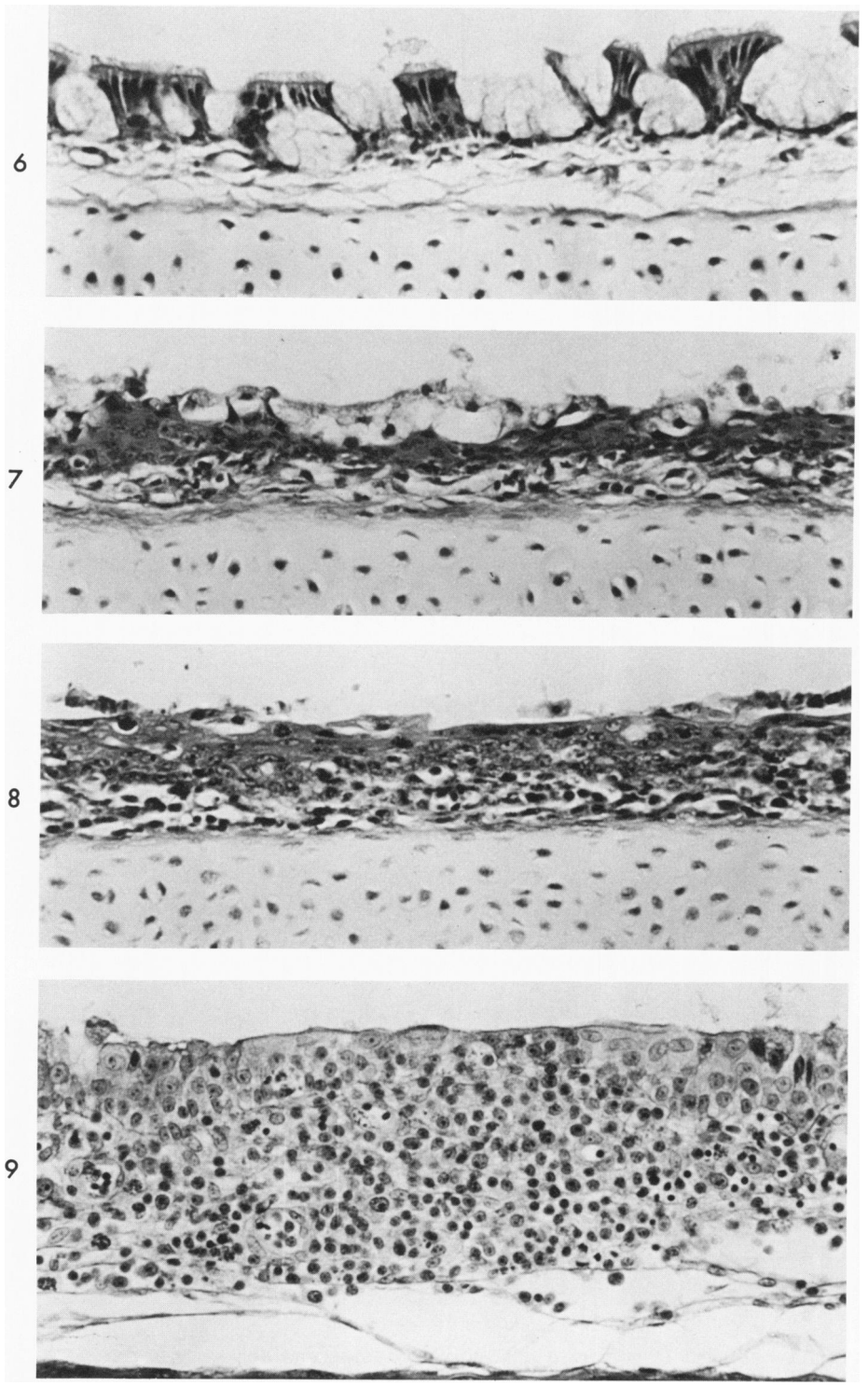


Figs 1–5—Effect of vitamin A deficiency and associated deficiency on response to NDV. **1**—Twenty-one-day-old chick fed multiply deficient diet at 1 day of infection (H&E, × 300). **2**—Twenty-three-day-old chick fed multiply deficient diet at 2 days infection with NDV-B strain (H&E, × 400). **3**—Thirty-one-day-old chick fed multiply deficient diet at 10 days infection with NDV-B strain (H&E, × 400). **4**—Forty-four-day-old chick fed multiply deficient diet at 13 days infection with NDV-vaccine strain (H&E, × 400). **5**—Fifty-day-old chick infected with NDV-vaccine strain. Complete recovery from keratinization is seen after 1 week on normal diet (H&E, × 400).



Figs 6–9—Effect of vitamin A deficiency and infection on keratinization (H&E, × 400). **6**—Twenty-five-day-old chick that was vitamin-deficient since hatching. No infection. **7**—Twenty-five-day-old chick at 3 days after infection with NDV-B strain. **8**—Twenty-five-day-old chick at 5 days of infection with NDV-B strain. **9**—Twenty-seven-day-old chick fed normal diet at 6 days after infection.