Replacement of Virus-Destroyed Epithelium by Keratinized Squamous Cells in Vitamin A-Deprived Chickens[1] (34146)

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Current experiments show that in vitamin A-deprived (VAD) chickens intranasally inoculated with Newcastle disease virus (NDV), locally destroyed epithelium of the middle turbinates is replaced not by mucociliated but by keratinized squamous cells. Previously we had found that in chickesn intranasally inoculated with laryngotracheitis virus (LTV) (1), or with NDV (2), initial lesions were in mucous cells in the inner (under) surface of the middle turbinate where the mucous membrane is shallowest. Further, in chickens deprived of vitamin A for 3 weeks after hatching, the membrane showed advanced preliminary signs of keratinizing metaplasia in the same area, and mucociliary flow rate of surface mucus was selectively retarded on reaching that epithelial area (3).

We report here that in VAD chickens, keratinizing squamous cells first begin to replace mucociliated cells in this same area, and that in NDV-infected VAD chickens, mucociliated cells are rapidly destroyed, then replaced by keratinizing squamous cells in this quite localized epithelial area. In normally fed birds, desquamated epithelial cells are replaced by mucociliated cells.

Materials and Methods. Beginning at the age of 11 days, hatchery stock chickens were fed commercial mash lacking vitamin A for 3 weeks. A total of 16 VAD and 16 normally fed chickens the same age were inoculated intranasally, without anesthesia, with 1 drop/nostril of crude Beaudette ("B") strain NDV in normal saline. This is a mesogenic strain of NDV which produces local mucosal lesions and viremia but does not kill chickens. Six VAD and 6 normally fed controls were not inoculated. Each day for 6 days, two inoculated and one control were killed, and nasal capsules were fixed in 10% neutral formalin; 2 VAD and 2 normally fed controls were killed on alternate days. After decalcification in 2% nitric acid, 6-μ histological sections through the turbinates were stained with H & E.

Results (histology). 1. VAD controls. In contrast with normally fed controls, (Fig. 1), VAD birds showed reduced numbers of mucous cells per acinus in the middle turbinates, mucociliated cells were replaced by keratinized squamous cells along the free inner margin of the turbinate scroll, and the respective relationships of mucous, ciliated and basal cells were diagnostic of incipient metaplasia (Fig. 2).

2 NDV-inoculated and VAD + NDV. Day 1 postinoculum. NDV only—mucosa and submucosa of inner (and part of outer) turbinate swollen and heavily infiltrated with inflammatory cells; acini partly obliterated, and epithelial sloughing moderately advanced (Fig. 3).

VAD + NDV—moderate infiltration of small lymphocytes in loose shallow submucosa and in single layer of mucociliated (no acini) epithelium overlying basal cell layer: entire distal 2/3 of inner surface of turbinate (Fig. 4).

Day 5 postinoculum. NDV only—inner turbinate mucosa and submucosa heavily infiltrated with inflammatory cells, disorganized, postcapillary venules packed with inflammatory cells and RBC, mucosal surface largely destroyed and almost no normal acini visible (Fig. 5); surface repair beginning.

VAD + NDV—entire mucosa of inner turbinate surface is squamous, as well as part of outer surface. Underlying the keratinized

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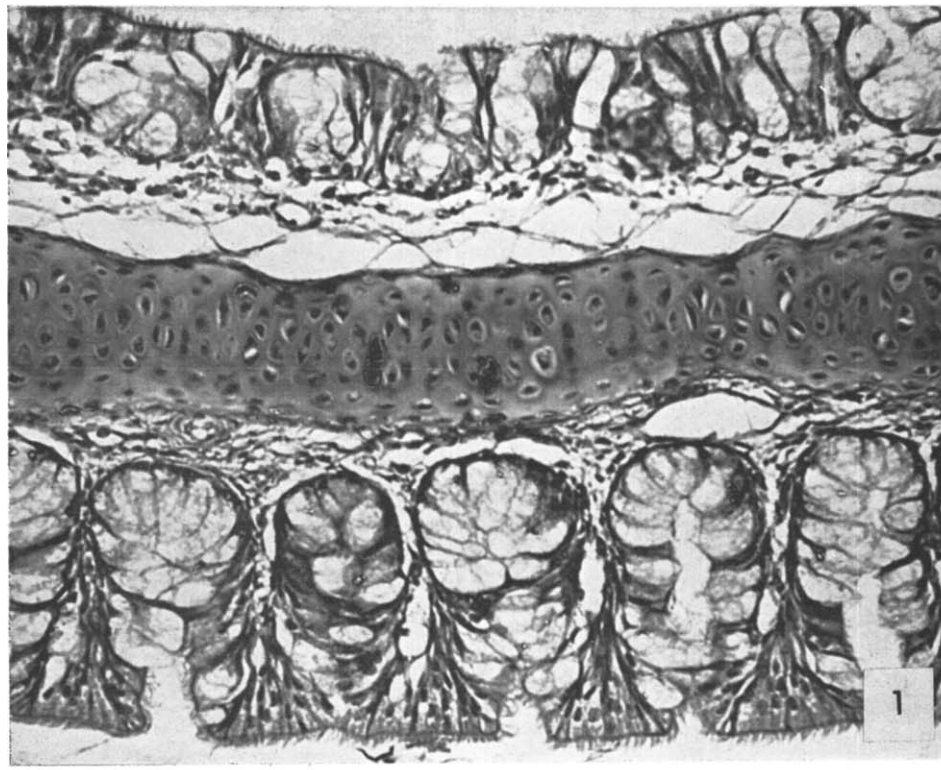


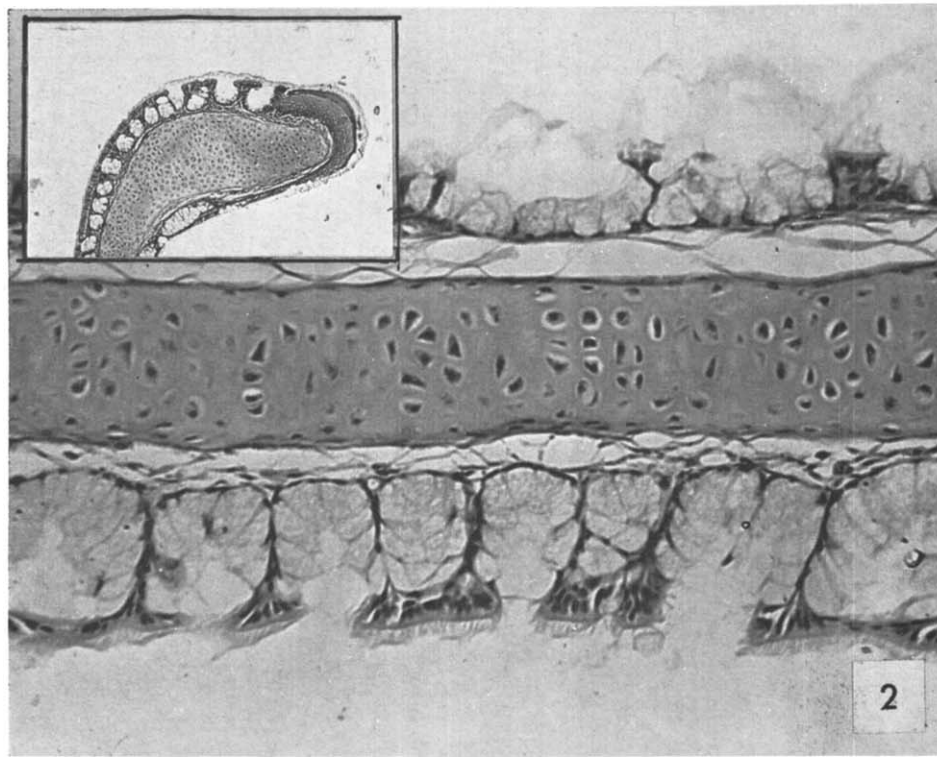
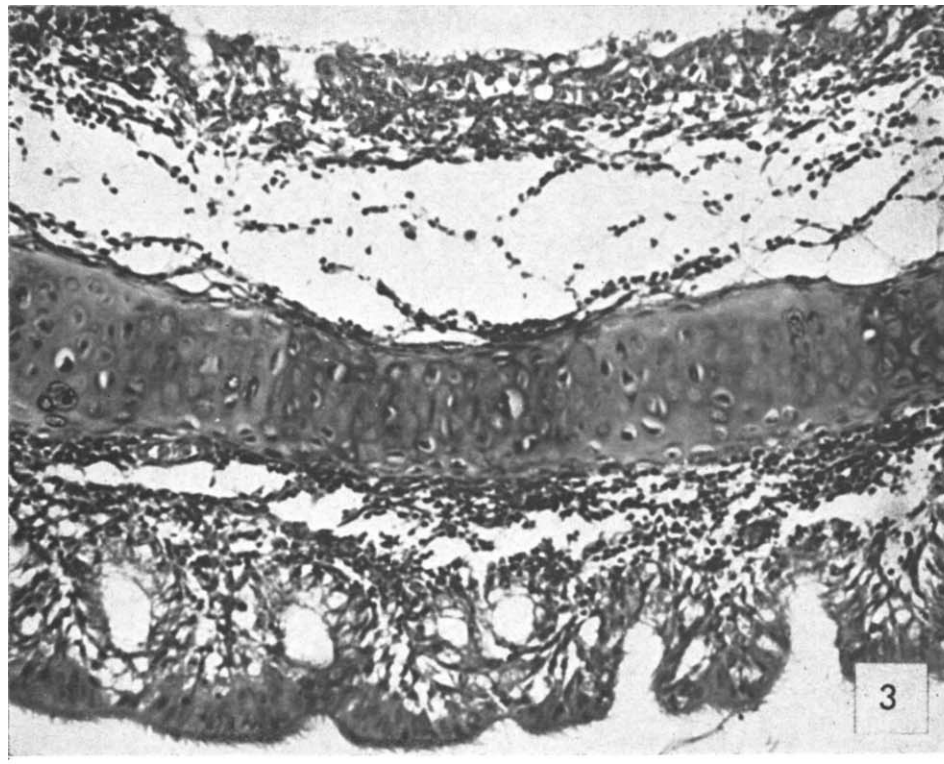


FIG. 1. Portion of maxillary turbinate of normally fed control chicken; mucosae and submucosae normal. In Figs. 1–7, the inner, shallower surface of the turbinate is at the top; the outer surface at the bottom. All stained with H & E, all ×300; inset ×52. Each set of chickens is the same age.

FIG. 2. Turbinate of control vitamin A-deprived (VAD) chicken showing reduced numbers of acinar gland cells, reduced mucosal depth. Inset: beginning keratinization of tip of same concha.



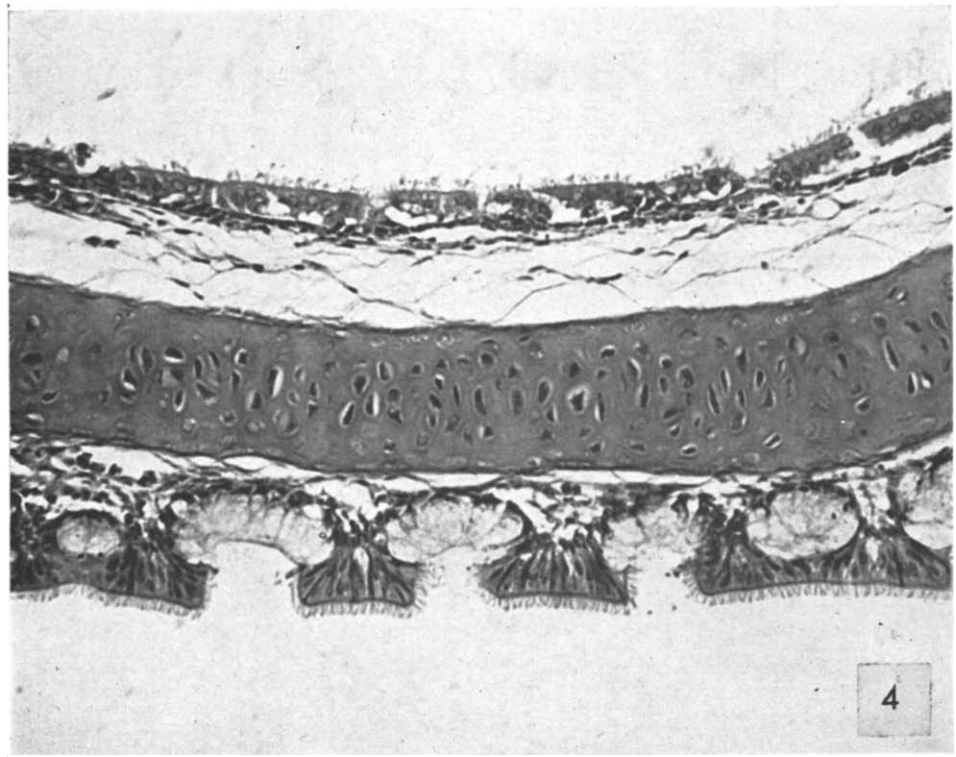
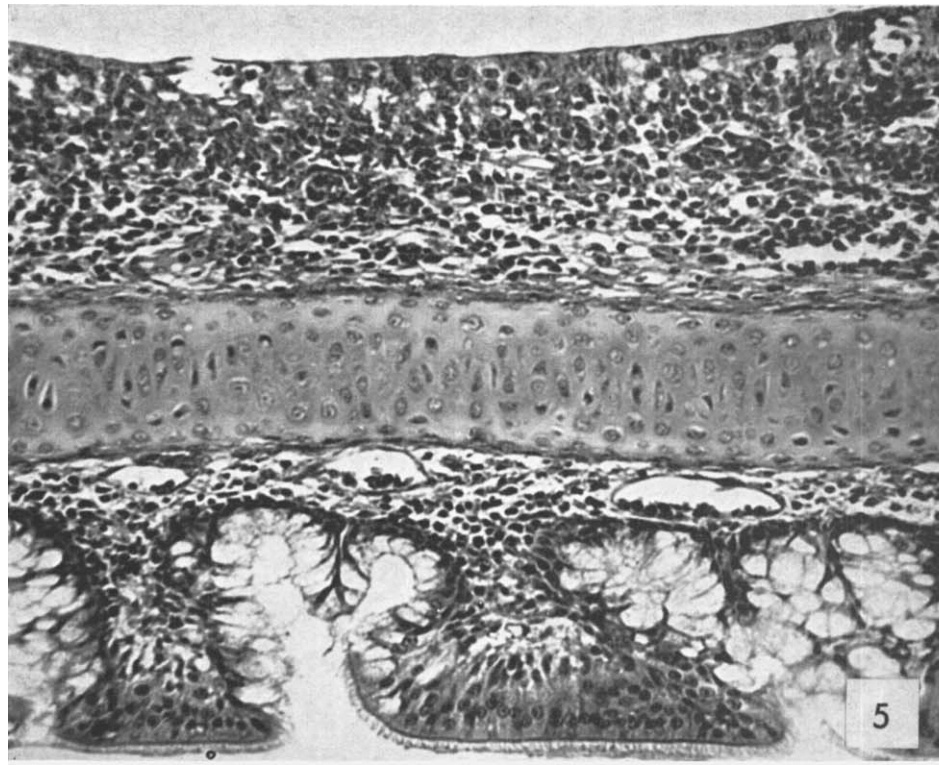


FIG. 3. Normally fed chicken 1 day after inoculation with NVD: Infiltration of inflammatory cells, submucosal edema, mucosal invasion and destruction.

FIG. 4. VAD chicken 1 day postinoculum: moderate inflammatory response, submucosal edema, and early desquamation, inner surface.



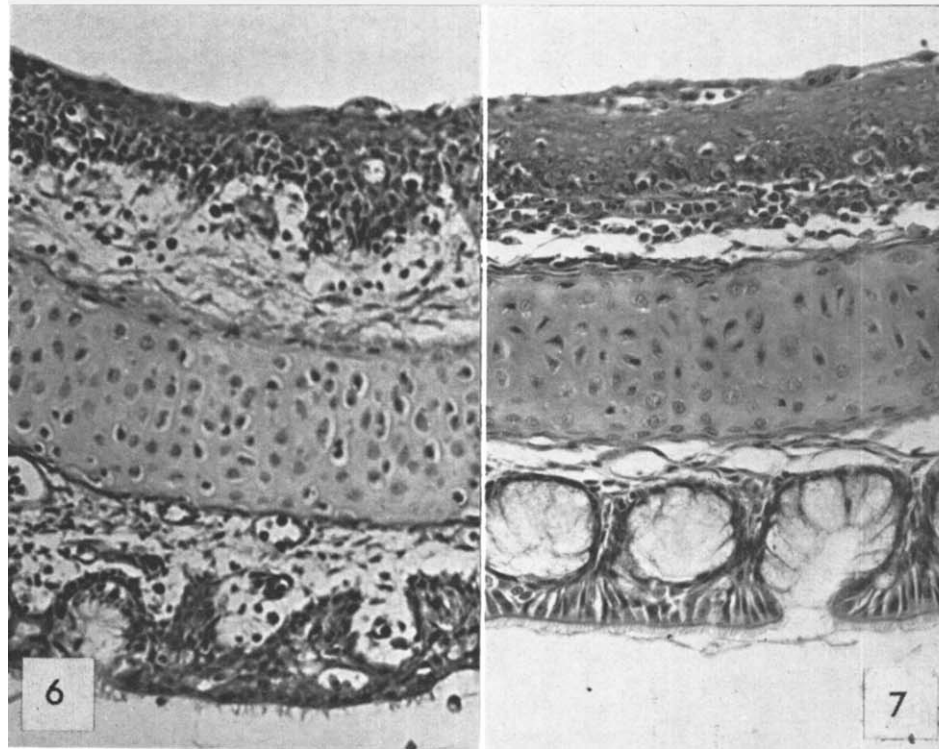


FIG. 5. Normally fed chicken 5 day postinoculum: beginning repair of mucosal surface, intensive infiltration of submucosa, dilated venules crowded with inflammatory cells (inner surface).

FIG. 6. VAD chicken 5 days postinoculum: several superficial layers of squamous cells, several layers of basal cells, inner surface; and beginning keratinization of outer surface which was also infected.

FIG. 7. VAD chicken 7 days postinoculum: keratinized layers of squamous cells have replaced mucociliated epithelium; mild inflammation remains.

squamous layer of 4–5 cells, basal layer is 2–3 cells deep instead of normal single row. Heavy infiltration of inflammatory cells in submucosa, and modest numbers in postcapillary venules (Fig. 6).

Day 7 postinoculum. NDV only— while similar to day 5, a few areas of normal surface repair are evident.

VAD + NDV—surface layer of keratinized squamous cells remains 4–5 cells deep, basal layer now chiefly one cell deep; markedly reduced submucosal inflammation (Fig. 7).

Discussion. A succession of reports since the twenties has shown that degrees of keratinizing epithelial metaplasia are produced by low concentrations of vitamin A in animals (4–6). Excess amounts of vitamin A suppress normal keratinization and induce mucociliated metaplasia in chickembryo skin cultures (7). The mechanism for selective production of squamous, mucous, or ciliated cells doubtless acts on the basal cell layer (7, 8). Chickembryo skin cultures in which mucous metaplasia is induced by excess vitamin A lose susceptibility to vaccinia virus and become susceptible to influenza (9). In relation to infections in living populations: chickens with low (borderline) A levels have higher mortality from NDV infection than do normally fed birds (10); and in humans with latent A-deficiency, acute clinical hypo-A is precipitated by infection (11, 12). Further, death rates in children with combined protein calorie malnutrition PCM and clinical signs of A-deficiency are 65% higher than in equally malnourished children without signs of hypo-A (13). So consistent synergism of A-deprivation and infection is well recognized, but the mechanism is not clear.

Post infectious keratinizing metaplasia of respiratory mucosa has evidently not been reported in animals or man. While we have not yet investigated degrees of A-deficiency required to induce postinfectious metaplasia, the present experiments raise several questions in relation to upper tract infection in vitamin A-deficient populations: is there differential absorption of virus in respiratory tissues early in course of infection in hypo-A individuals? Are they more susceptible to, and/or more efficient excretors of, virus? Once a local mucosal site is subjected to rapid basal-cell mitosis, might repeated insult stimulate irreversible focal hyperplasia or metaplasia?

Malnutrition and respiratory infections often coincide in human populations. In such conditions, if, in a high proportion of individuals, one particular area of respiratory mucosa was especially susceptible to repeated infection with different agents (myxoviruses, herpesviruses, poxviruses, etc.), the stage would be set for a cycle of desquamation and repair at that site. A means of studying these combined factors seems to be offered in an experimental model.

Summary. One localized area of nasal mucous membrane in chickens was selectively susceptible to initial infection with a myxovirus (NDV) and a giant-cell-forming herpesvirus (LTV). The same quite extensive mucosal area shows earliest signs of incipient keratinizing metaplasia in vitamin A-deprived chickens, and complete conversion to keratinized epithelium following NDV infection. Specific questions of interplay of A-deficiency and respiratory infection are open to study in this whole-animal model.

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