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

I. A Model for the Study of Environmental Factors on Upper Respiratory Tract Infection

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Acute Newcastle disease virus infection following intranasal inoculation of chicks with a mesogenic strain of the virus produced a localized infection of the middle turbinate which was histologically demonstrable 18 hours after inoculation. There was destruction of mucous cells of individual acini in the under surface of the middle turbinate, and the infection rapidly spread to ciliated and goblet cells and to neighboring acini. By day 2 there was simultaneous remodeling of the mucosa, continued destruction and inflammatory infiltration and frequent loss of cartilage basophilia. By day 3 polymorphonuclear cells almost disappeared, epithelial mitoses commenced, and lymphocyte infiltration intensified; the plasma cells normally present along the lateral nasal gland ducts were often destroyed, very occasionally the glands themselves were destroyed. By days 5 and 6 inflammation greatly decreased, and by day 8 the mucociliated epithelium was essentially normal. The infection is sequentially comparable to acute mild rhinitis of man (Am J Pathol 76:333–348, 1974).

ACUTE VIRAL DISEASE of the upper respiratory tract is a major cause of morbidity the world over. It is not limited to temperate and cold zones and competes with and even outdistances gastrointestinal disease in many tropical countries.[1] Since it is caused by a complex of viral agents [2] which vary in their capacity to destroy tissue, to persist and to provoke an immune response, there is a need to compare responses of the same host to agents which belong to different virus groups and to determine some of the nutritional factors which influence the immune responses of the host. This paper is concerned with uncomplicated pathogenesis of infection in the upper respiratory tract, and with anatomic and functional features relevant to the localization and pathogenesis of a myxovirus, Newcastle disease virus (NDV), in the tissues of the chicken upper tract. A forthcoming paper [3] will ex-

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amine the effect of diet, especially of lack of vitamin A, on the progress of two strains of NDV in these tissues.

Functional Anatomy

The functional anatomy of the chicken oculonasal tissues and the mucociliary flow patterns within the nose have been described in details elsewhere.[4,5] In the context of pathogenesis it is important to recall the following features.

As in the human nose, the mucociliary blanket moves chiefly away from the olfactory membrane and over the surfaces of the septum and maxillary turbinates into the nasopharynx to be swallowed. However, a small part moves anteriorly and is deposited at the mucocutaneous junction of the nasal vestibule. This deposit of mixed serous and mucous secretions may give some protection to the very anterior mucous membranes against infection.

The rate of ciliary motion of the sheet of mucus varies in different parts of the nose, as do the depth and mucous composition of the acini.[5] The mucociliated membranes of the inner surfaces of the scrolled maxillary turbinates are the shallowest and most delicate part of the nasal mucosa; mucociliary flow in this area is the most susceptible to deceleration and the most susceptible to initial virus infection.[4,6]

The large bilateral gland of Harder and its ducts serve the nictitating membrane of the eye. It is apparent that this organ has a further important role in the immune system;[7,8] there is a large population of plasma cells in the intralobular spaces of the gland body and a large population of small lymphocytes—sometimes with germinal centers—in the subepithelium of the duct leading to the eye. In the newly hatched chick, the area which is later occupied by plasma cells is filled with polymorphonuclear granulocytes, which are then gradually replaced by plasma cells during about the first 2 weeks of life.[7]

Paired lateral nasal glands are present in most mammals and in nearly all birds.[9] The ducts discharge droplets of serous fluid into the nasal vestibule, and upon in-breathing these droplets are apparently atomized and provide the saline vapor essential for the function of the olfactory and respiratory membranes.[10] The gland in chickens has a long primary duct and many collecting ductules within the gland; a closely aligned single row of plasma cells intrudes between and remains in position among the basal cells along the entire duct system. In normal chickens a few of these are usually seen histologically to be passing between the duct epithelial cells into the duct lumen.[7] Russell bodies are not

uncommonly found in the plasma cells of both the Harderian and lateral nasal gland systems, more in the former than in the latter, and more in birds over 2 months old than in those under 1 month.[11]

A curious characteristic which has been found to obtain histologically in most commercial, "Rous-free" [12] and gnotobiotic chicken stocks is significant infiltration of the lacrimal duct submucosa by a large population of small lymphocytes suspended in a delicate fibrous reticulum; in chickens over 3 to 4 weeks of age these primary foci often contain several germinal centers. While these incursions are always at the expense of the normal mucous acini, histology shows that the ciliated surfaces of the lacrimal ducts are usually intact, though areas where lymphocytes break through into the duct lumen are moderately frequent. Rarely, individual chickens lack these incursions, and they may be scant in particular batches of chicks. Whether such infiltrations represent an autochthonous infection is not known, but they are an undesirable complication in assessing the effects of intranasal infection.[7]

Materials and Methods

White Leghorn commercial chickens were maintained in 24 × 18 × 6 inch brooders from 1 day of age until ready for experimental use. For most experiments, chicks were from 1 to 3 weeks of age. At age 3 weeks they were transferred to larger brooders (36 × 24 × 9 inches); thus birds were caged according to the design of a given experiment, since age at inoculation varied from 3 to 26 weeks. Undiluted allantoic fluid from stocks of Newcastle disease virus (B strain) [12] was used for intranasal inoculation. The stock viruses were from the 11th, 13th or 15th allantoic fluid passages. Nasopharyngeal swabs were done with single Q-tip® applicators, which were then placed in 1 ml of buffered saline containing 100 units of penicillin and streptomycin. These vials were tested (fresh or frozen) for virus by inoculation of 10- or 11-day-old embryos on the chorioallantoic membrane. When an experiment was terminated, chicks were killed either by cardiac bleeding or decapitation. Specimens for histology were fixed immediately in 10% buffered formalin. Histologic technics have been previously described.[13,14,15]

Results

Infection with Newcastle Disease Virus

Day 1

In experiments in which more than 10^6 LD_{50} doses of virus were given, none of the chicks failed to show histologic evidence of infection during the week following inoculation. The most remarkable characteristic of infection with NDV following intranasal inoculation is the uniform localization of the earliest lesions in the epithelium of

the inner surface of the middle turbinate (Figures 1–3). The histologic lesions appeared within this limited part of the nasal mucosa during the first week of infection in all but a few of several hundred chicks. The earliest visible lesion was destruction of the mucous cells in individual acini within 18 hours of inoculation;[14,15] thereafter there was rapid spread to other portions of the mucous membrane.

There was considerable variation from chicken to chicken in the first 24 hours of infection. Although the most striking lesion was the acinar one, some of the chicks at 24 hours showed cyst formation in and destruction of the ciliated cells between the acini. The cystic areas often contained polymorphonuclear granulocytes and bulged slightly above the mucosal level (Figure 3). There were no mitotic figures in the epithelium on day 1. There was often inflammatory infiltration of the anterior portion of the lateral nasal gland ducts.

Day 2

Day 2 of infection was characterized by a remodeling of the whole mucosa concomitantly with further destruction and continued polymorphonuclear and monocytic infiltration. In areas where destruction and inflammation were greatest, the remaining epithelial cells were stretched very taut over the surface, appearing histologically as wafer-thin flattened cells. There was destruction and leukocytic infiltration of the sinus epithelium in several chicks (Figure 11). Mitotic figures in the nasal epithelium were rare on day 2, but a few were found after 33 hours of infection. During this acute phase if virus infection was severe on both the inner and the outer surface of a given turbinate, the underlying cartilage was seen, histologically, to have lost all normal basophilia from the matrix (Figures 13 and 14). This same effect was produced by another acute chicken virus infection, laryngotracheitis (LTV), and biochemical study has shown that it is due to virus-induced loss of lysosomal enzymes from the affected area.[16] The cartilage effect lasted only during the acute phase of NDV infection. In a study of thymidine uptake during acute infection,[17] cartilage basophilia was lost between 48 and 72 hours after infection in 22 of 64 turbinates in 32 one-week-old chickens infected intranasally with NDV. If infection was unilateral, there was normal acinar secretion and normal cartilage staining on the unaffected side (Figures 4 and 5).

Day 3

By day 3 most polymorphonuclear infiltration disappeared, and heavy lymphocytic infiltration (Figure 6), beginning mitoses of epi-

thelial cells and a predominance of cell types were involved in the process of mucosal remodeling. There were many areas in which infiltrating large lymphocytes and rounded pavement epithelial cells were mixed in a disorderly mass with mitotic figures among them.

In most chicks on about the third day after inoculation, the anterior part of the lateral nasal gland ducts became acutely disorganized (Figure 12) and most of the plasma cells along the basal layer were shed into the lumen of the duct; some remained in place but became pyknotic. Shedding plasma cells were often associated with polymorphonuclear granulocytes. Normal duct organization and normally located populations of plasma cells were usually restored by 12 to 14 days after the original inoculation, but there was occasional partial or complete destruction of one or both lateral nasal glands. Sections of different individuals on different days postinfection indicated that destruction began with an overwhelming incursion of polymorphonuclear leukocytes into the gland substance.

Days 4 and 5

Inflammation was greatly decreased, and in most regenerating areas a multilayered pavement epithelium overlay the lamina propria (Figures 7–9). Mitotic figures (Figure 9) were apparent in epithelial cells at 4 days and became common at 5 days. They were found at all levels from the basal layer to the surface. Some surface epithelial cells were much larger than normal cells and had a clear pale cytoplasm and enlarged nucleus. Mucous acini were rare in regenerating areas. At this stage, the tubuloacinar salivary glands in the roof of the mouth were often in acute stages of infection, destruction and polymorphonuclear infiltration. Sinusitis with polymorphonuclear infiltration was still present.

Days 6 and 7

Most of the inflammatory changes had disappeared, though lymphoid infiltration sometimes persisted in the lamina propria. The epithelium of the inner surface of the maxillary turbinate uniformly lacked cilia, acini were absent, and the several layers of pavement epithelium were essentially devoid of mitotic figures. Infection of the lateral nasal gland ducts was common; again there was occasional polymorphonuclear infiltration in and destruction of the gland body.

Days 8 through 13

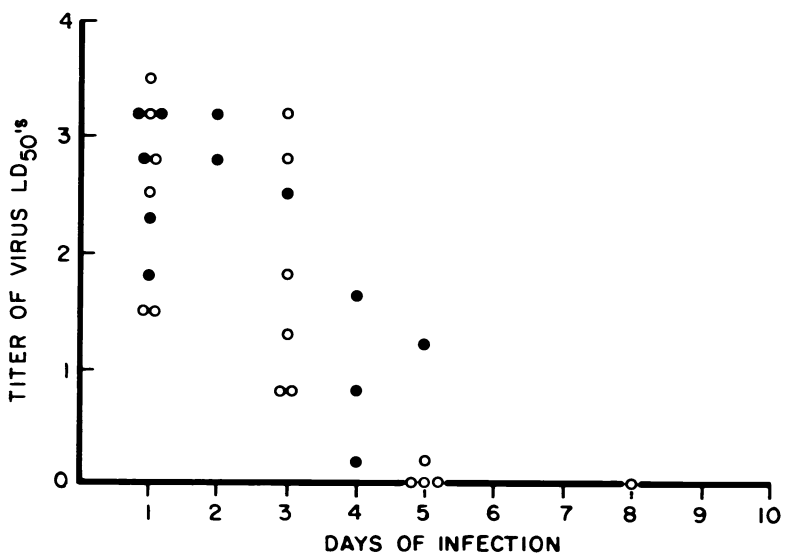
In five separate experiments in which typical lesions had been found in nearly all of the chicks killed during the first 7 days, those examined

on days 8 through 13 appeared quite normal. Minor exceptions included focal persistence of submucosal inflammation, a few patches which still lacked cilia (Figures 8 and 10), and some structurally abnormal acini.

Prevalence of Virus in Upper Respiratory Tract

Partly to make a more valid comparison between NDV infection of chickens and acute upper respiratory infection in man, most of the data on the chicken virus infection has been obtained by throat swabs. It is a convenient method by which virus titers in individual animals may be followed over time, and it furnishes unequivocal evidence of infection. Text-figure 1 shows that good virus recovery was obtained from all animals sampled on days 1 through 3 and that on days 4 and 5 the titer had dropped. In other experiments we have found virus present in normal animals on days 6 and 7 but never beyond day 8.

The question whether an individual chicken consistently maintained a low or high titer of virus was tested by comparing the titer of individual chickens on day 1 with the titer of the same chicken on day 2; that of day 2 with day 3, and again for day 3 and 4. No correlation was found, and we therefore conclude that high virus yielders and low virus yielders were not separable by this method.



TEXT-FIG 1—Amount of virus in throat swabs from 3-week-old chicks following intranasal inoculation. (Experiment 1, *open circles;* experiment 2, *solid circles*).

Effect of Virus Infection on Immune Cells

We have elsewhere described the presence of plasma cells intercalated between the epithelial cells of the lateral nasal gland ducts, and of masses of plasma cells in the Harderian glands of normal chickens.[7] It was therefore of interest to see whether the NDV infection, which is usually limited to the nasal area proper, would affect the plasma cells of these two organs. Review of five separate experiments showed that disappearance of plasma cells began on day 3 and extended through day 6 to 8 after infection. Since uninfected chickens killed at the same time had the usual infiltrations of plasma cells, absence of these cells in individual birds on days 5 through 8 is significant. There was no significant effect of infection on the plasma cells in the Harderian glands.

Discussion

The histopathogenesis of viral infection in the human upper respiratory tract cannot be conveniently studied in man. It is difficult to infect most experimental animals with human viral agents, and the common human myxoviruses have been studied principally in nonprimate mammals whose complex nasal turbinate structure is vastly at variance with the human nose. The chicken's simpler structure and more relevant acinar system are a closer facsimile and have proved an excellent system in which to study the comparative responses of nasal mucous membranes to infection with unrelated viruses.

Francis and Stuart-Harris [18-20] 35 years ago detailed the effects of influenza A on ferret nasal mucosae. This is still the most thorough study of viral destruction and regeneration of nasal mucosa. Table 1 lists the sequence of changes described in their classic paper and a companion list of changes found in the chicken middle turbinate mucosa. The similarity of the sequence and nature of the lesions is striking. The changes seem to evolve more rapidly in the chicks, which were usually 3 weeks old, than in the ferrets, which were 8 months or older. Francis and Stuart-Harris [18-20] noted that repair was more rapid after repeated infection with influenza. There is an obvious structural difference between the usually single layered ferret mucosa and the pseudostratified chick mucosa, in which mucous gland cells are interspersed with ciliated cells in the form of both goblet cells and shallow or deep acini. The vulnerable inner surface of the chicken turbinate most closely resembles the ferret turbinate mucosa. The effect of the infection on the cartilage is apparently different. Francis

Table 1—A Comparison of Acute Viral Lesions of the Nasal Mucosa

Influenza in ferrets (Francis and Stuart-Harris[17–19])	Newcastle in chicks (this study)
1. Goblet cells empty	1. Disappearance of mucus from acinar cells and goblet cells
2. Necrosis of columnar epithelium; leukocytic infiltration	2. Destruction of epithelium; polymorphonuclear cell infiltration into acini; epithelial blisters and cysts with polymorphonuclear cells inside
3. Beginning regeneration with mitotic figures in thickened epithelium; plasma cells, lymphocytes, fibroblasts	3. Remodeling and repair, associated with continuing destruction; beginning mitosis, heavy lymphoid infiltration
4. Epithelium in full repair; surface cells thin, flat	4. Inflammation reduced; thin surface epithelium, cilia mostly absent
5. Cartilage change; chondroblast destruction	5. Increased mitosis of epithelium; loss of cartilage basophilia
6. Epithelial blisters and polymorphonuclear cells	
7. Developing cilia; goblet cells still poorly developed	
8. Fully ciliated, hyperplastic; intraepithelial cavities lined by cilia.	8. Complete regeneration of ciliated cells, mucous cysts lined with ciliated epithelia; plasma cells associated with acini

and Stuart-Harris described a sequence in which multinucleated chondroclasts appeared to attack the cartilage and break it up into strips during repair. The chicken infection was characterized by loss of metachromasia from the cartilage matrix, both in NDV and in laryngotracheitis, and concurrent limpness of the cartilage itself. It may be that if studies were done on animals of similar relative age and with similar methods of cartilage staining, the effects would be more comparable.

NDV infection is also of considerable immunologic interest, for it destroys the system of plasma cells which line the lateral nasal gland ducts. The localization of infection in the middle turbinate, though it is by no means limited to that area, is so regular that it offers peculiar advantages for study of the interaction of infection with other factors such as specific nutritional and immunologic deficiencies and exposure to particular environmental toxins or other insults. This turbinate is a discrete organ system in which virus localization can be identified by fluorescent antibody, and the effects of infections on mucosal cell kinetics can be followed by thymidine uptake.[17,21]

To return to the established similarity of the sequential changes in

the mucosa (and leaving out the propensity for NDV to produce a viremia and to attack glandular tissue) the sequence of destruction, leukocyte infiltration, epithelial blebs, remodeling of mucosa with frequent mitotic figures, the formation of a thin epithelial surface cell covering, then regeneration of cilia on the surface cells and the later differentiation of mucous cells, all suggest that the pattern of regeneration following acute destructive virus infection is under the control of a few specific factors involved in cell recognition. The earliest destruction of cells attracts first polymorphonuclear cells, then lymphoid cells. Mitosis is either depressed at the start [17] or is not stimulated until there is significant loss of cells. Following the increase in the number of new epithelial cells which have come from a reservoir of undifferentiated epithelial cells, a reorientation towards the surface precedes the subsequent differentiation first of ciliated cells, then of mucous cells. Evidently the information involved in the process of regeneration is not always perfect even after relatively mild sloughing, since there were scattered ciliated and mucous cysts in the epithelia following both infections. After rigorous sloughing there may be permanent derangement of respiratory mucosae, and permanent metaplasia of olfactory mucosae.[4] The fundamental pathobiologic problems of inflammation, destruction, mitoses of undifferentiated cells, rearrangement and redifferentiation may be studied in one localized area of the chick nasal mucosa.

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[*Illustrations follow*]

Legends for Figures

Figs 1–5—Inner layer of maxillary turbinate in a series of chicks on normal (Growena) chick diet. Varying degrees of changes induced by NDV strain B.

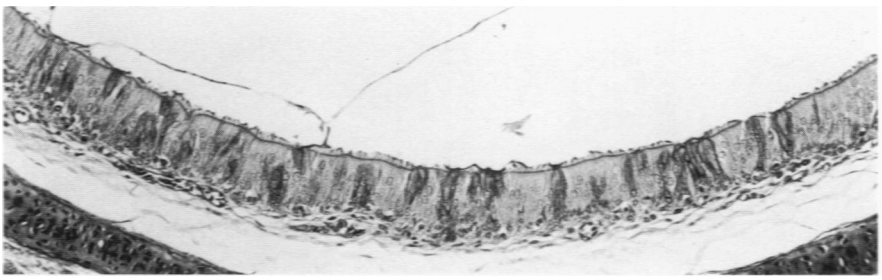
Fig 1—Normal uninfected 23-day-old chick. Ciliated cells with mucous cells interspersed (H&E, × 150).

Fig 2—Early infection (33 hours) of same area. Some areas show lack of cilia, minimal leukocytic infiltration, edematous area between mucosa and cartilage (H&E, × 150).

Fig 3—Two-day infection. Cystic destroyed areas alternate with patches of intact cilia (H&E, × 150).

Fig 4—Normal uninfected 23-day-old chick. AB-PAS staining brings out areas of mucus secretion (× 150).

Fig 5—Two-day infection. Note minimal amounts of mucus, some remaining at bottom of acini.



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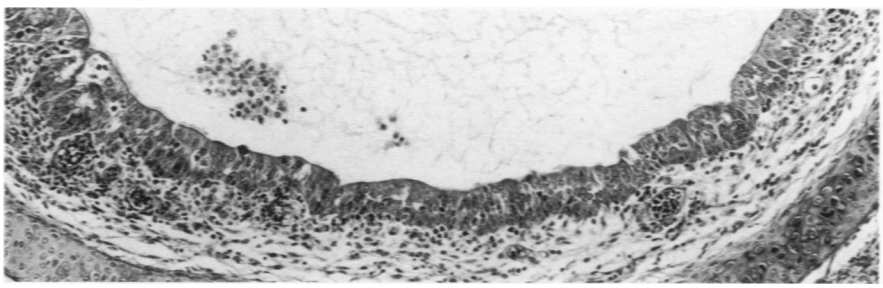

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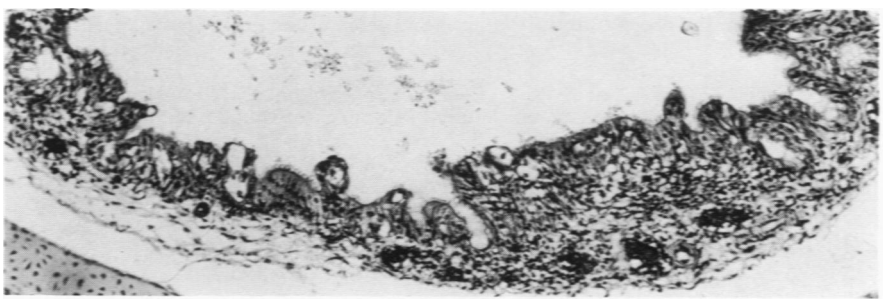

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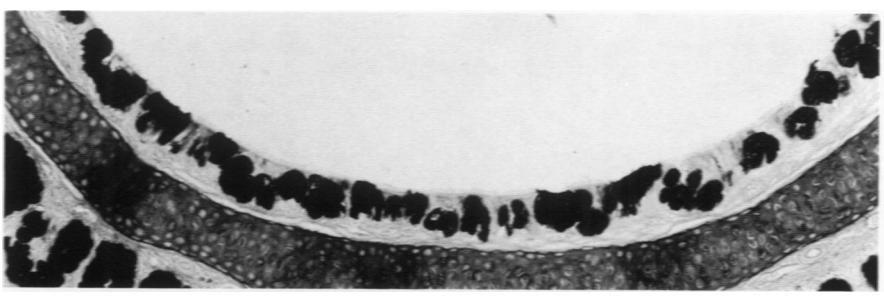

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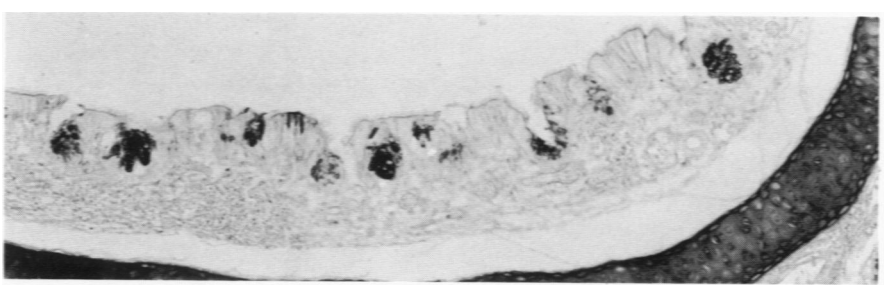

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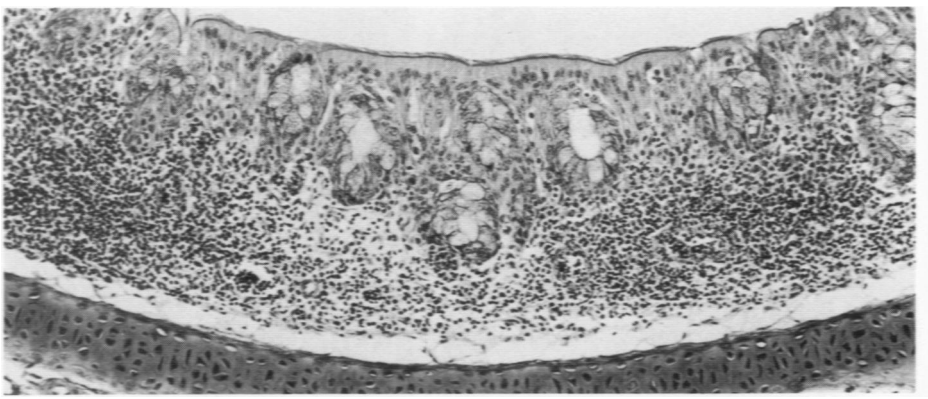
Fig 6—NDV infection at 3 days. Intact mucociliary epithelium with heavy inflammatory infiltration between cartilage and epithelium. This illustrates the irregular distribution of the early destruction of ciliated epithelium, although mucus acini were apparently destroyed in the areas both to the right and left of the center (H&E, × 150).

Fig 7—NDV infection at 5 days. Regenerating epithelium completely lacking in cilia. Reorganization has taken place. Minimal amounts of mucus secretion (H&E, × 150).

Fig 8—Late (13 days) stage of recovery. Mucus acini present, but distorted. Patches of ciliated epithelium on left but absent from most of the central portion (H&E, × 150).

Fig 9—Higher magnification of same. One mitotic figure seen deep in the epithelium in upper portion (H&E, × 600).

Fig 10—Higher magnification of same. Several cells show tufts of cilia. Dead cells extruded on the surface of mucosa (H&E, × 600).



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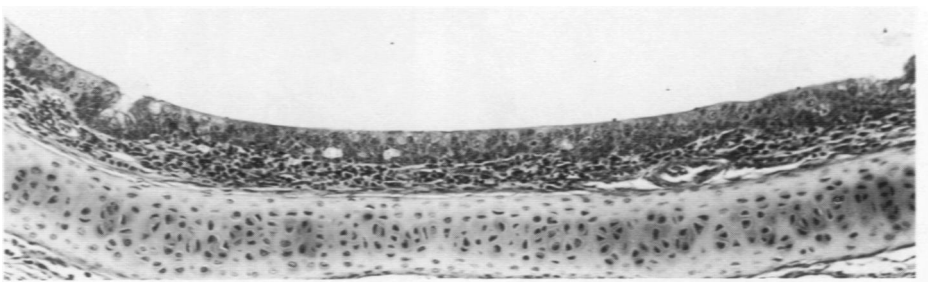

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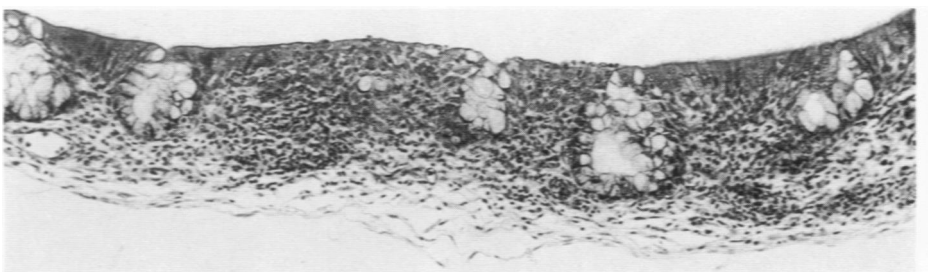

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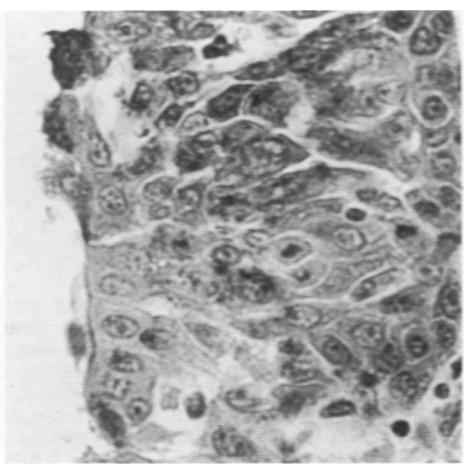

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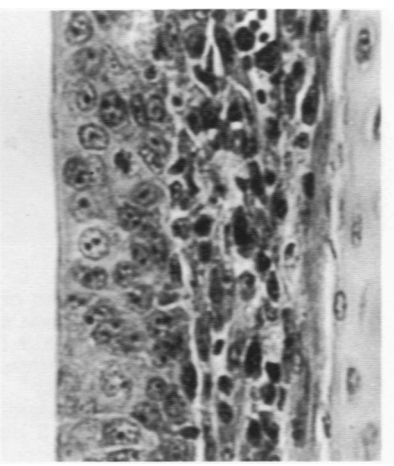

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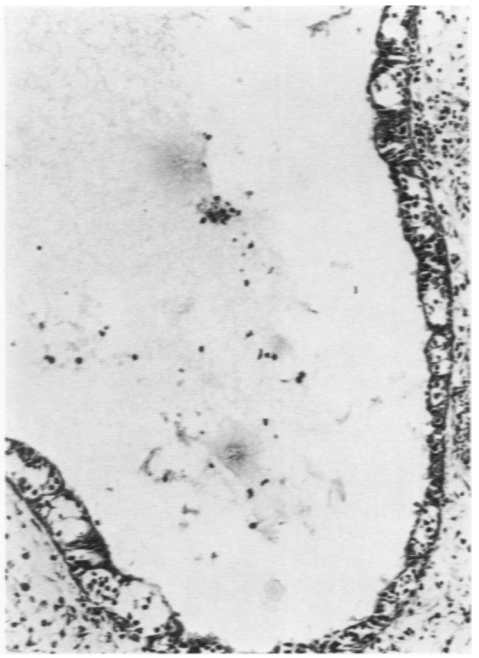

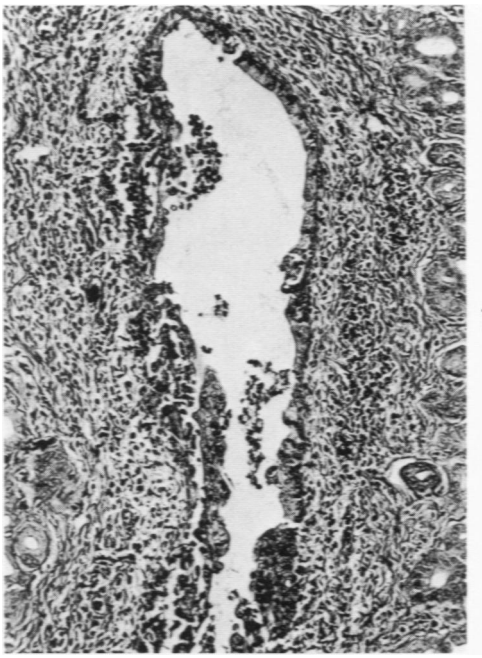

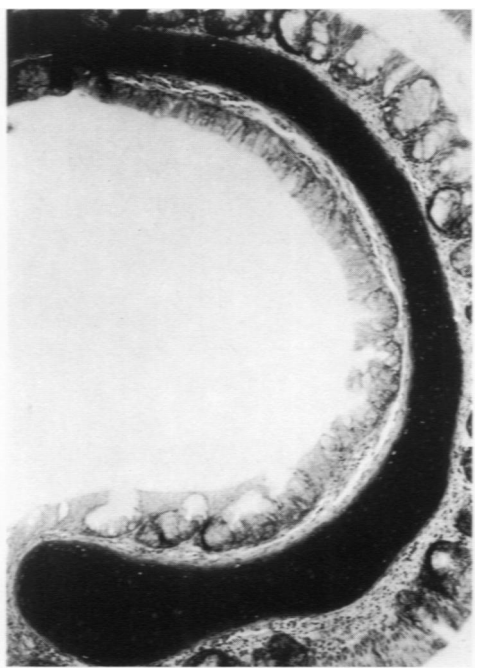

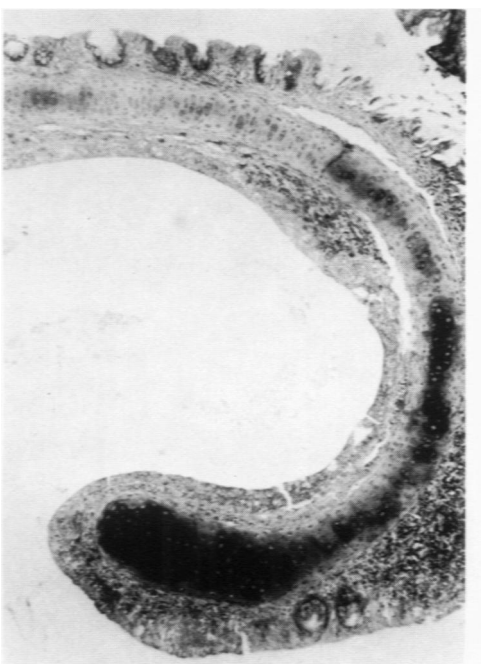

Fig 11—Thirty-three-hour infection of 22-day-old chick. Destruction of mucosa of maxilliary sinuses by NDV. Cystic appearance caused by beginning repair associated with continued destruction. Compare with Figure 3 (H&E, × 150). **Fig 12**—Destruction of mucosa of duct of lateral nasal gland. Inflammatory cells apparent between the surrounding acinar tissue and the duct (H&E, × 150). **Fig 13**—Normal turbinate of 3-week-old chick. Heavy staining of cartilage apparent (Toluidine blue, × 150). **Fig 14**—Turbinate from 3-week-old chick infected with NDV for 3 days. Note poor staining of cartilage, especially in the curved area where infection has occurred in overlying mucosa (Toluidine blue, × 150).