STUDIES ON THE NASAL HISTOLOGY OF EPIDEMIC INFLUENZA VIRUS INFECTION IN THE FERRET

I. The Development and Repair of the Nasal Lesion

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Plates 36 to 39

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During the past few years a considerable body of evidence has accumulated which suggests that immunity to influenza virus infection in man, in swine, and in ferrets is closely related to the presence of neutralizing antibodies to the virus in the serum. In man, examination of sera during the acute stage and during convalescence from influenza has revealed a constant rise of antibodies as a result of the infection (1–6). Further, evidence has been brought forward to suggest that individuals who escape infection after direct inoculation (7) or during an epidemic (3, 5) possess on the average a somewhat higher titer of antibodies than do those who acquire infection. In both the pig and the ferret it is readily demonstrable that the serum of a normal susceptible animal has no neutralizing antibody for influenza virus but that the serum of an animal convalescent from influenza virus infection has a high titer of neutralizing antibody (8, 9). However, in the case of the ferret it is difficult to accept the view that immunity to influenza virus is solely related to the possession of humoral antibodies. Immediately after infection the animal is solidly immune to a second inoculation of virus, but within from 3 to 6 months after infection reinoculation of virus again induces clinical evidence of infection although at this time antibodies, while perhaps less than before, can still be demonstrated in the serum (9, 10). Again, it is difficult, if not impossible, to induce complete immunity to intranasal inoculation of virus in the ferret by subcutaneous vaccination, although this procedure causes the development of antibodies (10, 11). Nevertheless, a second response to intranasal inoculation with influenza virus or the response of vaccinated ferrets is of a modified type and is usually not accompanied by the development of lung lesions. It has been suggested (6) that a high level of antibodies in the ferret assures immunity but that below this level susceptibility exists, and Hoyle and Fairbrother (3) are of the opinion that a similar state of affairs may hold in man.

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There are, however, certain difficulties in the general acceptance of this view of the mechanism of immunity to influenza. Soon after an infection by human influenza virus ferrets and mice are immune not only to the original virus but to other serologically different strains of influenza virus either of human or porcine origin, and at this time heterologous antibodies may be either low or unrecognizable in the serum (6, 10, 12). Moreover, pigs inoculated with swine influenza virus are subsequently immune to an inoculation with human virus although the serum after the first infection contains no antibody for the human virus (13, 14). On the other hand, while the majority of human patients have a very low level of antibody during the acute stage of influenza, some cases were encountered during the 1936–1937 epidemic with as high a level of antibody on the first day of the illness as was present in others during convalescence (4–6). Furthermore, certain individuals were observed (5) who, possessing little or no demonstrable antibodies against influenza virus, escaped the disease although subject to the same degree of exposure as others who incurred infection while retaining a comparatively high titer of circulating antibodies.

The foregoing data render it apparent that factors other than the mere presence of circulating antibodies as measured to date are required in order to explain the mechanism of immunity to influenza. The possibility immediately presented itself that some functional or anatomical factor in the respiratory tract itself played a rôle in the immunity to infection with influenza virus. With these facts in mind a study of the nasal mucosa of the ferret during infection and recovery from infection with influenza virus was begun.

Method of Examining the Nasal Structure in the Ferret

In order to compare the appearances in different ferrets it was necessary to use a standard method of histological examination. The ferret was sacrificed with carbon monoxide, the thorax opened, and the trachea ligatured. The great vessels were then cut, the head was severed from the neck, and the lower jaw cut off. The bony lateral walls of the nasal fossa were chipped away by bone forceps and the turbinates thus exposed on either side. A hacksaw was used to cut through the skull just posterior to the turbinates and the whole mass was then immersed in Zenker's solution to which glacial acetic acid had been added to a strength of 5 per cent. After 24 hours the mass was removed from fixative and by further chipping the turbinate system on each side of the nose was isolated. Fixing was then continued for another 24 hours. Dehydration and the embedding in paraffin were carried out in the usual manner. The turbinates from one side were cut horizontally and longitudinally; the other half was cut in two, the posterior portion discarded, and a transverse coronal section made. Sections were stained with hematoxylin and eosin, Giemsa, and Mallory's stain.

Normal Anatomy and Histology of the Ferret's Nose

A thorough study of the structure and variation in the nasal chambers of the normal ferret was an essential preliminary to the study of the variations and changes observed under the abnormal conditions of infection and repair.

The ferret's nose is filled with an intricate system of cartilaginous scrolls attached partly to the lateral wall of the nasal fossa and partly to the vertical wall separating the nasal fossa from the cranial cavity posteriorly. Anteriorly the turbinate scrolls are attached to a fibromuscular strand which continues forward in the form of a spiral uniting with the nostril and reducing the size of the nares to a narrow pore. Posteriorly the fossa communicates with the pharynx through a small oval opening. The nasal septum is cartilaginous anteriorly and bony posteriorly and is separated from the turbinates on either side by a mere chink. The accessory sinuses comprise the maxillary antra which form simple pockets on the lateral aspects of the turbinates and the floor of each antrum is occupied by a serous gland, the lateral nasal gland of the mammalia (15). In addition, numerous shallow outgrowths filled with cartilaginous scrolls are directed into the bone covering the posterior part of the roof of the fossa; these may represent frontal or ethmoid sinuses.

Fig. 1 shows above, the medial, and below, the lateral aspect of the turbinate system as exposed by the dissection employed. On the lateral aspect are visible the maxillary sinus, the lateral nasal gland, and the outlines of the turbinates still covered by the mucosa of the lateral wall of the fossa. The medial aspect which in the living animal faces the septum shows the division of the system into anterior and posterior portions by a well defined cleft. This can also be made out by reference to Fig. 2 which is an enlargement of a horizontal longitudinal section of one-half of the turbinates.

The anterior turbinates form a series of scrolls of cartilage covered by mucosa and attached both anteriorly and laterally to the walls of the fossa. Anterior to the lateral attachment the lateral wall of the fossa carries two ducts, the naso-lacrimal and nasal gland ducts which run forward to empty into the fossa between the anterior turbinates and the lateral wall. The posterior turbinate has a simpler structure and consists of parallel scrolls running horizontally from an attachment to the posterior wall of the fossa which is pierced by the olfactory nerve fibers. The anterior part of this turbinate undergoes an abrupt change of direction, gives off lateral branches, and may here be termed the middle turbinate (Fig. 2).

Histologically, respiratory and olfactory mucosae can be distinguished easily. The whole of the anterior turbinate, the middle turbinate, the antrum, the anterior half of the septum, and the lateral wall of the nasal fossa are lined by respiratory mucosa. Nearly all of the posterior turbinate is olfactory in structure except for occasional leaves which carry respiratory epithelium. Figs. 3 and 4 show the

detailed structure of the mucosa in the respiratory region through sections of the anterior turbinate and the mucosa covering the gland. The respiratory epithelium is composed for the most part of a superficial layer of columnar ciliated cells and a deeper layer of flattened cells with darkly staining nuclei forming a pavement layer closely applied to the basement membrane. Between these two layers intermediate cells may be interposed so that the epithelium is converted into a stratified columnar ciliated structure. Such an epithelium is found over the septum and the lateral wall of the nasal fossa and in patches elsewhere, but most of the turbinate epithelium is composed of two layers. In between the ciliated cells there are goblet cells which vary greatly in number in different ferrets. Furthermore, in the normal ferret occasional intra-epithelial leucocytes or remains of leucocytic nuclei are found, especially near the tip of the nose. The strand-like attachment of the anterior turbinate to the nostril is covered by a frankly stratified squamous though non-keratinized epithelium (Fig. 5), but such an epithelium does not occur in the normal ferret posterior to the junction of the attachment and the turbinate scrolls. The only other variation from the columnar epithelium in the respiratory area is due to a shortening of the long diameter of the ciliated cell which occurs at the bends of the scrolls and produces the appearance of a cubical yet ciliated epithelium.

The submucosa or tunica propria of the respiratory area is very thin and consists only of connective tissue and venous sinuses except over the septum and lateral wall. Here submucous glands occur but these are entirely absent from the turbinates proper. Beneath the submucosa in both anterior and posterior turbinates is a thin lamina of cartilage.

The olfactory epithelium has a superficial columnar ciliated layer and beneath this are many cells with round nuclei, the olfactory cells (Fig. 6). The submucosa of the olfactory area includes the glands of Bowman and also nerve fibers.

The lateral nasal gland is a compound tubulo-alveolar gland with serous secreting cells normally packed with secretion granules. Accumulations of lymphocytes are sometimes found in the interstitial tissue of the gland and beneath the glandular epithelium. The ducts of this gland and also of the lacrimal gland are lined by a simple columnar epithelium except near their anterior ends where stratified columnar epithelium is present. The air passages between the turbinate scrolls constitute the turbinal passages and these are usually free from secretion but may occasionally contain a little mucus or cell debris with leucocytes.

The Nasal Lesion of Influenza Virus Infection in the Ferret

The material available for histological study was provided by a series of 26 ferrets 8 to 9 months of age, anesthetized with ether and inoculated intranasally on the same day with 2 cc. amounts of the same preparation of the PR8 strain of epidemic influenza virus in the form of a 1 per cent emulsion of infected ferret lung. These animals were then sacrificed in pairs at various intervals up to 4

weeks after infection. There were, in addition, a few ferrets inoculated for routine passage or other purpose with 5 or 10 per cent preparations of the PR8 strain of epidemic influenza virus, and 1 ferret similarly infected with the WS strain of virus.

In general the 1st day of fever corresponded with the 1st day after inoculation, the 2nd day of fever with the 2nd day after inoculation, and so on. Figs. 8 to 26 represent the microscopical appearances in this group of ferrets. They are all taken from coronal sections of the anterior turbinates.

Day 1.—2 ferrets were sacrificed 24 hours after inoculation before either had developed fever. The appearance was normal in the gross, but microscopically a slight increase in the amount of fluid in the turbinate passages was seen (Fig. 8; compare with Fig. 7 taken from a normal ferret and viewed at the same power of magnification). The epithelium also presented a slight increase in granularity and the cell outlines, including the cilia, were slightly blurred. The goblet cells were seen to be emptying their contents into the fluid which formed a thin film over the epithelium.

Day 2.—The 2 ferrets sacrificed on this day had been febrile for 18 hours and macroscopically had injected, glistening turbinates. Microscopically the turbinates showed necrosis of the respiratory epithelium, congestion, edema, and cellular infiltration of the submucosa, and the formation of an exudate in the turbinal passages (Fig. 9). Almost the whole of the nasal respiratory area showed a complete desquamation of the ciliated columnar cells leaving only the basal "pavement" layer and basement membrane (Fig. 10). Those parts of the respiratory mucosa, such as the lateral wall and the nasal septum, which were covered by stratified columnar epithelium showed in some areas a less complete desquamation, and one or two layers of polyhedral or cubical cells remained on the surface of the basal layer. Elsewhere the basal layer formed the sole covering for the submucosa, although in each of the 2 ferrets a tiny pocket of unaffected ciliated columnar epithelium was found; in one instance this was in the middle turbinate region and in the other it was covering part of the gland in the region of the antrum. The olfactory epithelium, the stratified squamous epithelium of the attachment of the anterior turbinates, and the columnar epithelium of the nasolacrimal and lateral nasal gland ducts were intact. Those laminae in the posterior turbinates which were covered partly by respiratory and partly by olfactory epithelium showed an abrupt transition between the necrotic zone of the former and the normal structure of the latter. The submucosa of the respiratory area showed a moderate congestion of the venous sinuses, edema of the interstitial tissue, and a mild infiltration of the latter with polymorphonuclear leucocytes and mononuclear cells. The exudate in the nasal passages consisted of leucocytes, desquamated and necrotic epithelial cells, mucus, and debris. The lateral nasal gland showed a discharge of secretion granules from the cells of the alveoli but no inflammatory changes.

Day 4.—The 2 ferrets were still febrile when killed on this day, and the gross appearance of their turbinates was the same as that of the previous ferrets. Mi-

croscopically also the turbinates showed little change except that no ciliated columnar epithelium was present anywhere in the respiratory area. There was still exudate in the nasal passages although this was less dense than before. The respiratory area was still covered only by the basement membrane and basal cells. Here and there, however, commencing regeneration was seen, particularly over the lateral nasal gland, on the lateral wall of the nasal fossa, and along the epithelial stalk of the anterior turbinates (Fig. 11). In these areas flattened cells were beginning to accumulate so as to form a layer two or three cells thick. Mitotic figures were frequent in this layer and two cells in a state of anaphase are seen in Fig. 12, which is a high power view of the thickening epithelium. Elsewhere on the anterior turbinates the absence of a reparative process was conspicuous. The submucosa showed a dense cellular infiltration with polymorphonuclear leucocytes, mononuclear cells—lymphocytes, plasma cells, and monocytes—and fibroblasts. The venous sinuses were still engorged. The lateral nasal gland showed a slight increase in lymphocytes in the interstitial tissue and in the submucosa, and the alveolar cells were in a state of active secretion.

Day 6.—The ferrets sacrificed on the 6th day were already convalescent with normal temperature, and the nasal turbinates were almost normal macroscopically. Microscopically, repair of the epithelium was in full progress, although there was still considerable exudate in the nasal passages and the submucosa was still densely infiltrated. The turbinates of one ferret (Fig. 13) appeared to be slightly less advanced in repair than that of the other (Figs. 14 and 15). Both showed, however, substantially similar changes. The epithelium throughout the respiratory area was two, three, or four cells thick, and formed a structure similar to the transitional epithelium of the urinary tract. The deep cells of the layer were cubical or polyhedral in shape, but the superficial cells were elongated and flattened, and their cytoplasm seemed to form a continuous layer bounding the free surface of the epithelium. The nuclei of this epithelium were hyperchromatic when compared with those of normal columnar cells. Occasional leucocytes and fragments of the nuclei of leucocytes were seen in between the epithelial cells.

In a few pockets of the anterior turbinates of the ferret showing the more advanced repair a remarkable type of epithelium was present. This seemed to be the result of increased flattening of the superficial cells. As a result, the epithelium presented an appearance suggesting stratified squamous epithelium. The deep cells were arranged in a palisade fashion, the intermediate cells were polygonal in shape, and the superficial cells were flattened but non-keratinized squamae. It was not possible to decide definitely whether the repair of the turbinate epithelium had been accomplished by cell migration from the squamous epithelium at the tip of the nose or by multiplication of the basal layer, but the uniform character of the repair and the absence of any advancing edge of epithelium were considered to favor the latter process.

Beneath the epithelium two changes were evident. Fibroblasts were replacing

the mononuclear and polymorphonuclear cells in the interstitial tissue and were accumulating on either side of the cartilage as though to enclose it in a fibrous capsule. The cartilage itself was also undergoing change. Here and there throughout the course of the laminae there were multinucleated chondroclasts which appeared to be attacking the cartilage and breaking it up into strips.

Days 7 and 8.—The turbinates of the 2 ferrets sacrificed when convalescent on the 7th and 8th days respectively were covered by a stratified squamous epithelium of ovoid cells lying parallel to the surface, only a few laminae being still covered by an epithelium with polygonal superficial cells. Figs. 16 and 17 from the 7th day ferret show the striking appearance of the squamous type of epithelium. The superficial layers were undergoing desquamation partly by flaking off and partly by the formation of epithelial blisters such as those from the 8th day ferret seen in Figs. 18 and 19.

This latter ferret showed in addition, however, occasional laminae where the repair had reached a more advanced stage. Here the deepest layer of cells had flattened and the superficial cells were becoming differentiated so that their cytoplasm was ranged at right angles to the basement membrane, thus producing a columnar epithelium mounted on several layers of cells (stratified columnar). The nuclei of the superficial cells were less hyperchromatic than before. It was not possible to decide exactly how this stage was reached. Possibly the casting off of the superficial layers was simultaneously accompanied by a differentiation of the cells beneath into the columnar type of cell, or it may be that both processes were due to the increase in pressure occasioned by the accumulation of cells in the developing mucosa.

The three stages, however, of transitional, stratified squamous, and stratified columnar epithelium were clearly visible side by side at the 8th day although most of the epithelium was of the stratified squamous type. The repair of the submucosa had also reached a more advanced stage by this day. Fibroblasts were becoming converted into chondroblasts and these cells bordering on the cartilage can be clearly seen in Fig. 18. Elsewhere fibroblasts were scattered throughout the interstices of the submucosa and beneath the basement membrane of the epithelium. There was still a loose exudate composed of mucus and leucocytic debris in the nasal passages. Leucocytes were still visible between the epithelial cells but not to any extent in the submucosa. There was still an infiltration of mononuclear cells in the gland, particularly in the submucosa.

Day 10.—By the 10th day (Fig. 20) most of the respiratory epithelium showed a columnar layer of superficial cells which in places were developing cilia. However, there were still areas of stratified epithelium in the anterior turbinates and in other areas several abnormalities were noted: (*a*) Near the tip of the nose the epithelium had an irregular outline with fronds of hyperplastic cells projecting outwards; (*b*) in many areas there were oval or round cavities in the epithelium often containing a few leucocytes (Fig. 21); (*c*) beneath the newly formed columnar

cells there were one, two, or three layers of polygonal or cubical cells with hyperchromatic nuclei so that the epithelium was a stratified columnar one; and (*d*) goblet cells were still undeveloped. The submucosa was more normal and the fibroblasts lining the cartilage were being converted into chondroblasts by a process of encapsulation. There was still a scanty leucocytic exudate in the nasal passages, but there were fewer intra-epithelial leucocytes than formerly.

Days 13 and 14.—Macroscopically the turbinates at this stage appeared velvety, and microscopically the epithelium was fully ciliated, but hyperplastic and stratified columnar in type. Goblet cells were beginning to reappear. However, near the tip of the anterior turbinate there were still epithelial fronds, and intra-epithelial cavities were still prominent in many parts of the mucosa although not in the area shown in Fig. 22. Many of the intra-epithelial cavities still contained leucocytes; others, however, showed a reorientation of their surrounding cells as though to form an alveolus, and cilia were growing on the inner rim of the alveolus. There were other areas which resembled superficially intra-epithelial cavities composed of three or four goblet cells massed together, a formation occasionally seen in the normal ferret's mucosa. The intra-epithelial alveoli, however, have never been seen in normal ferrets. There was still an exudate in the nasal passages but this was much scantier than before. Many fibroblasts still persisted (Fig. 22).

Days 16 and 17.—Fig. 23 shows that the epithelium was much more normal in appearance by the 17th day. This was due to disappearance of the intermediate cells between the columnar and basal cells and to a flattening out of the basal layer. Fronding of the epithelium was still present at the tip of the nose, however, and at the extreme tip of the laminae of the anterior turbinate there was a non-ciliated pseudostratified epithelium two cells thick in which both superficial and basal cells were cubical in shape. Exudate had at this time disappeared. Cartilage regeneration was still active and with its formation the thickness of the submucosa had diminished. The nasal gland was secreting less actively and the mononuclear infiltration of its submucosa was replaced by fibroblasts.

Days 21 and 28.—The noses of the ferrets sacrificed 21 days after infection might almost be considered normal. The epithelium was ciliated columnar, the submucosa was thin, the gland was perfectly normal, and the cartilage was well formed. More careful search, however, revealed certain abnormal appearances which were still visible both in these ferrets and in those studied 28 days after infection. The epithelium at the extreme tip of the anterior turbinates retained a pseudostratified non-ciliated appearance with two or three layers of polygonal or cubical cells. Passing from the attachment of the anterior turbinates backwards, one encountered first a continuous layer of stratified squamous epithelium, next a pseudostratified layer two cells deep, and then a ciliated columnar layer. Nevertheless, occasional patches of pseudostratified epithelium could be made out some distance away from the tip (Fig. 24, taken from a 28 day ferret). Epithelial irregularities persisted with stretches of stratified ciliated columnar cells, epithelial fronds, and intra-epithelial cavities lined by cilia. The submucosa was still

slightly thicker and more fibrous than normal. Cartilage formation was still active and a peculiar staining effect was noted with hematoxylin, the exact significance of which was doubtful. Parts of the cartilage, often near the center of the laminae, but sometimes at the side (Fig. 25), were a deeper blue in color than the rest. There was a sharp line at the junction of the two types of cartilage and it seems likely that this effect was due to the reformation of cartilage which occurred during the repair process. The line may be analogous to the growth lines which occur in the bones of children as a result of arrested formation of bone during an acute infection. It has not been possible to determine how long such lines in the cartilage persist, but they were not seen in ferrets killed 4 months or 6 months after an attack of influenza, although they were visible in a few ferrets killed 3 months after an attack. While the possibility that they represent restoration of normal growth cannot be excluded, the lines have not been observed in normal ferrets but are practically pathognomonic of the process of repair.

The above findings in the series of 26 ferrets infected with the virus of epidemic influenza provided a picture of the orderly development and repair of the lesion produced in the nose by this strain of virus. The remaining few ferrets which were studied provided additional data relating to the acute lesion and its repair. 3 ferrets aged 18 to 20 months, which were a year older than the ones previously studied, were sacrificed on the 3rd day of an infection with the PR8 strain of virus. The histological appearances did not differ significantly from those seen in the younger ferrets, so that within the range studied the histology of the acute lesion of influenza did not seem to vary with the age of the ferret.

One ferret in the series had been inoculated with the WS strain of human influenza virus and was sacrificed on the 3rd day of the infection. The respiratory mucosa of this animal showed the same changes as those seen in ferrets infected with the PR8 strain of virus. However, there was slightly more exudate to be seen and an important difference was the fact that a necrosis of the olfactory epithelium occurred on a few of the laminae (Fig. 26). As the PR8 strain of virus caused no demonstrable damage to the olfactory mucosa, while the one ferret infected with WS strain showed partial necrosis of this tissue, it is suggested that different strains of epidemic influenza virus may vary somewhat in their specificity and in the extent of the damage they produce.

DISCUSSION

The foregoing observations have revealed an orderly progression of changes in the nasal mucous membrane of ferrets after intranasal inoculation of relatively large doses of epidemic influenza virus. After 24 hours little but irritative phenomena is observed while after 48 hours complete destruction of the respiratory epithelium has occurred. At that time the respiratory area is covered only by the thin basement membrane; the submucosa is edematous and infiltrated with inflammatory cells; there is a rich exudate chiefly polymorphonuclear in type in the air passages. Illustrating the sharp specificity of the lesion the olfactory mucous membrane, on the other hand, exhibits practically no evidence of injury. In the next 2 days no further damage occurs and one gains the impression that a phase between injury and repair has been reached.

By the 6th day reparative processes have definitely begun and over the respiratory area a transitional type of epithelium composed of polyhedral cells three to four layers deep is comparatively uniform. In the next 48 hours a rapid advance results in the development of a further differentiated epithelium predominantly stratified squamous in type, with desquamation of superficial squamae and the formation of epithelial blisters. In some places the deeper cells are flattening and the superficial cells are suggesting the return of a columnar epithelium. Moreover, hyperplastic papillary folds of the regenerating epithelium are being thrown up. 2 days later the epithelium approaches a stratified columnar type and cilia have begun to appear. The papillary fronds are much more marked and intra-epithelial nests of columnar cells are caught in the hyperplastic epithelium. At this time the exudate is decreased in amount, the inflammation of the submucosa is subsiding, and a marked acceleration of cartilage formation has begun.

By the 13th or 14th day ciliated epithelium occupies most of the affected area. Hyperplasia is still prominent, however, and islands of transitional epithelium remain. Goblet cells are seen for the first time. Only a scanty exudate is present. On the 16th to 17th days the sections reveal a still closer approach to normal. The epithelium is columnar in type and the basal cells have become

flattened. Goblet cells are extremely numerous and active. Exudate is no longer present. It is interesting, however, that the anterior tip of the respiratory area is still non-ciliated. In the 3rd and 4th weeks the respiratory epithelium is essentially normal except for pseudostratified non-ciliated patches of cells which remain here and there directly adjacent to normal ciliated columnar epithelium. Moreover, hyperplastic fronds and intra-epithelial cavities are still observed. The submucosa is somewhat thicker and more fibrous than normal while the laminal cartilage shows dark staining zones of accelerated cartilage formation. After 4 to 6 months nothing more than suggestive residues is noted.

The damage produced by epidemic influenza virus in the nose of the ferret is striking in its specificity for the respiratory epithelium and in the totality with which it destroys that structure. Only rarely in the previously untreated animal is any respiratory epithelium left, while the olfactory epithelium is essentially untouched. Despite the severity and the completeness of the damage, repair is well under way by the 6th day after infection, and between the 6th and 14th days the regenerating epithelium progresses from a relatively undifferentiated transitional epithelium of polygonal cells, through a well organized stratified squamous type resembling the normal lining of the nasal vestibule, to a hyperplastic stratified columnar epithelium which rapidly develops cilia. The character of the regenerating respiratory membrane is so manifestly different from that of the normal tissue as to suggest immediately the possibility of different functional reactions such as described by MacNider (16–22) in the liver and kidneys of dogs previously subjected to chemical injury. Moreover, the cycle of epithelial changes observed in the present experiments is somewhat paralleled by the observations of Boling (23) upon repair after traumatic damage to the nasal mucous membranes of sheep. In the experiments of MacNider and Boling the alteration in cell types resulted in resistance to subsequent injury by chemical agents and it seems not unlikely that a similar resistance might be exhibited by the regenerating respiratory membrane of ferrets following influenza virus infection. The second paper of the series deals with experiments designed to test this hypothesis.

SUMMARY

A study has been made of the nasal histology in normal ferrets and in ferrets during and after infection with epidemic influenza virus. During the acute stage of infection the respiratory epithelium of the nasal mucous membrane undergoes necrosis with desquamation of the superficial cells and exudation into the air passages, and an inflammatory reaction occurs in the submucosa. Repair begins on the 4th day after infection, and from the 6th to the 14th day the respiratory area is covered successively by a transitional, a stratified squamous, and finally a stratified columnar epithelium. By the 21st day after infection the epithelium has been largely restored to normal but repair in the submucosa and cartilage is still in progress. The respiratory mucosa is substantially normal in structure 1 month after infection although minor abnormalities of cellular arrangement and type can still be distinguished.

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EXPLANATION OF PLATES

All sections stained with hematoxylin and eosin.

PLATE 36

FIG. 1. The turbinate system in a normal ferret exposed from the medial aspect (above) and from the lateral aspect (below). Approximately × 2. The tip of the nose lies to the right and the roof of the nasal fossa above each half. The medial aspect shows the anterior turbinate to the right and the posterior turbinate to the left separated by a cleft. The lateral aspect shows the antrum and lateral nasal gland on the surface of the turbinates. A bristle has been inserted through the torn roof of the antrum.

FIG. 2. Horizontal section of left turbinate system in a normal ferret with the tip of the nose pointing upwards. Approximately × 3.

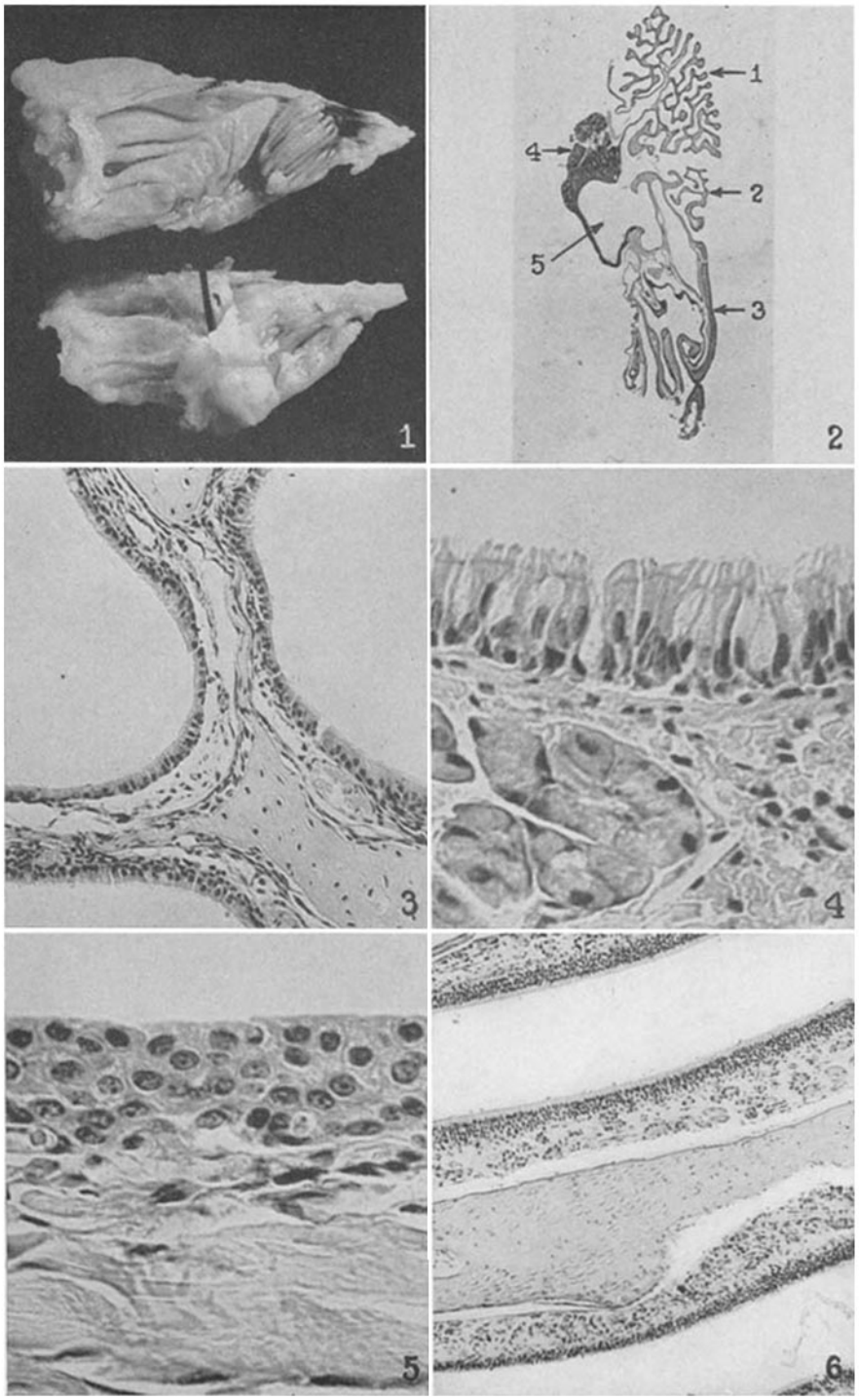
1. Anterior turbinate } respiratory epithelium.
2. Middle turbinate }
3. Posterior turbinate, olfactory epithelium.
4. Lateral nasal gland.
5. Cavity of antrum.

FIG. 3. Normal respiratory epithelium in the anterior turbinate. × 170. The lamina of cartilage is covered by a ciliated columnar epithelium resting on a thin submucosa containing venous sinuses.

FIG. 4. Normal respiratory epithelium covering the lateral nasal gland. × 570. Ciliated columnar and goblet cells comprise the superficial layer of the epithelium. The gland is composed of serous acini.

FIG. 5. Normal epithelium covering the anterior attachment of the anterior turbinate. × 570. A stratified squamous epithelium without keratinization.

FIG. 6. Normal olfactory epithelium covering the posterior turbinate. × 80. A multilayered epithelium with superficial ciliated cells and deeper olfactory and supporting cells. Nerve fibers run in the submucosa.



Photographed by Joseph B. Haulenbeek

(Francis and Stuart-Harris: Nasal histology of influenza infection. I)

PLATE 37

FIG. 7. Normal anterior turbinate. × 80. Regular ciliated columnar epithelium. Air passages free from exudate.

FIG. 8. Influenza. Day 1, 24 hours after infection. Anterior turbinate. × 80. Ciliated columnar epithelium covered by a thin film of exudate in the air passages (mucus and leucocytes). Inclusion bodies were not seen in specially stained preparation.

FIG. 9. Influenza. Day 2. Anterior turbinate. × 80. The epithelium has been desquamated. The air passages are filled with exudate. The submucosa is wider than normal. Inclusion bodies were not seen in specially stained preparation.

FIG. 10. High power view of Fig. 9. × 570. The epithelium is a single layer of flattened cells incorporated with the basement membrane. The submucosa is edematous and infiltrated with polymorphonuclear leucocytes and mononuclear cells. The exudate in the air passages is composed of leucocytes and cell debris.

FIG. 11. Influenza. Day 4. Anterior turbinate. × 80. The epithelium is still a single flattened layer except along the stalk of the anterior turbinate shown to the right where accumulation of cells is in progress. The submucosa shows a richer cell infiltration and is still acutely inflamed. Inclusion bodies were not demonstrated.

FIG. 12. High power view of Fig. 11. × 570. Shows the earliest sign of regeneration of epithelium on one side of a lamina (below). The arrows point to two nuclei showing mitosis (anaphase). The epithelium on the other side of the lamina is probably a single layer of cells but has been cut obliquely.

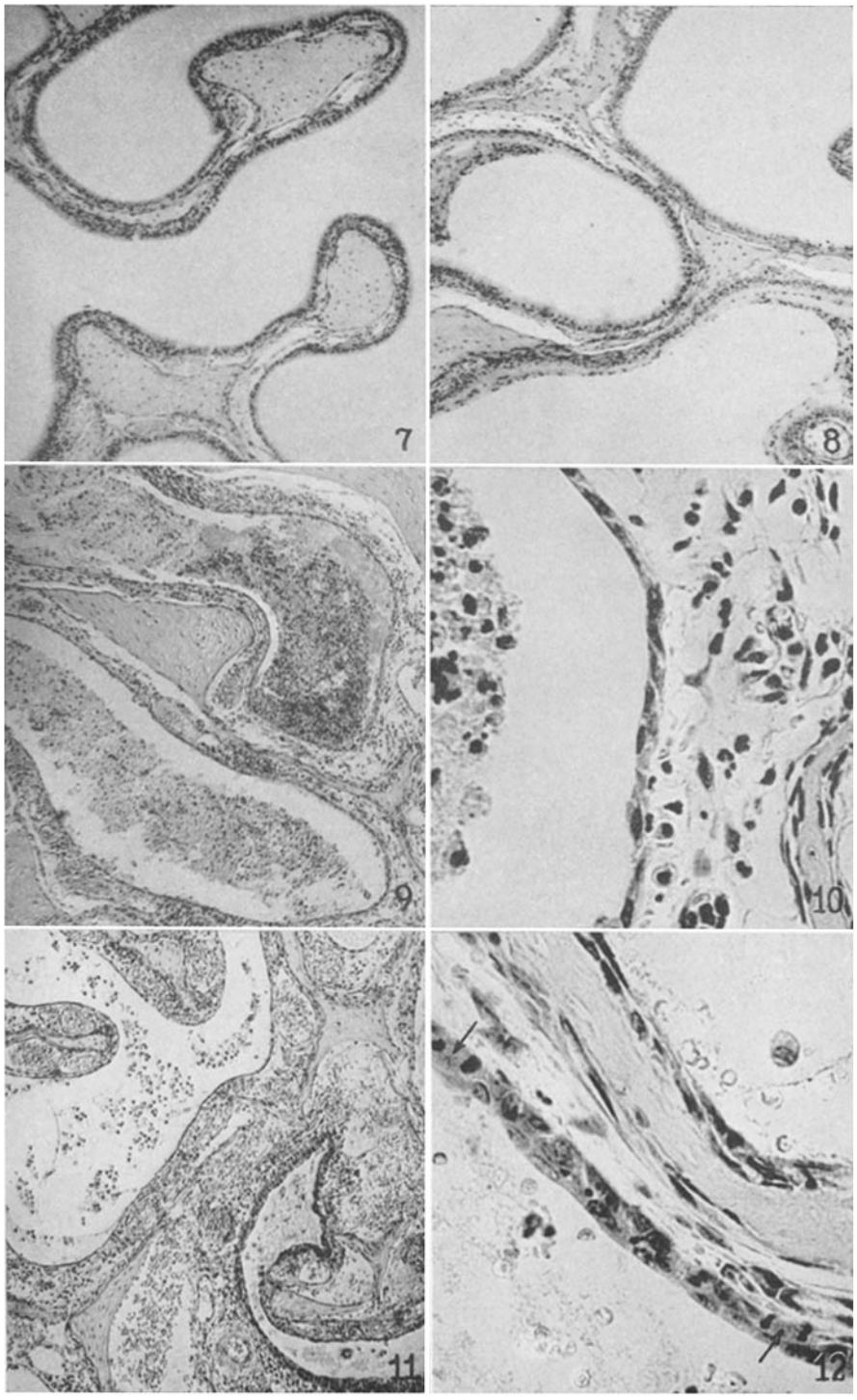


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PLATE 38

FIG. 13. Influenza. Day 6. Anterior turbinate. × 80. Epithelial regeneration is in progress, the submucosa is densely cellular, and fibroblasts are forming a layer alongside the cartilage which is being split by chondroclasts. Exudate still in the air passages.

FIG. 14. Influenza. Day 6. Anterior turbinate. × 80. Slightly more advanced stage of epithelial regeneration.

FIG. 15. High power view of Fig. 14. × 570. The epithelium is composed of polyhedral and cubical cells with hyperchromatic nuclei. It is a stratified transitional type of structure.

FIG. 16. Influenza. Day 7. Anterior turbinate. × 80. The epithelium is now a stratified squamous structure, the superficial layers of which are being desquamated.

FIG. 17. High power view of Fig. 16. × 570. Note the desquamation of the superficial squamae and hyperchromatic nuclei.

FIG. 18. Influenza. Day 8. Anterior turbinate. × 80. Most of the epithelium in this turbinate was stratified squamous as in Fig. 16 but this area was chosen to illustrate the formation of epithelial blisters. Cartilage regeneration is beginning in the submucosa.

FIG. 19. High power view of Fig. 18. × 570. The blisters are composed of intra-epithelial cavities containing leucocytes. Note the palisade type of formation in which the deep cells of the epithelium are arranged.

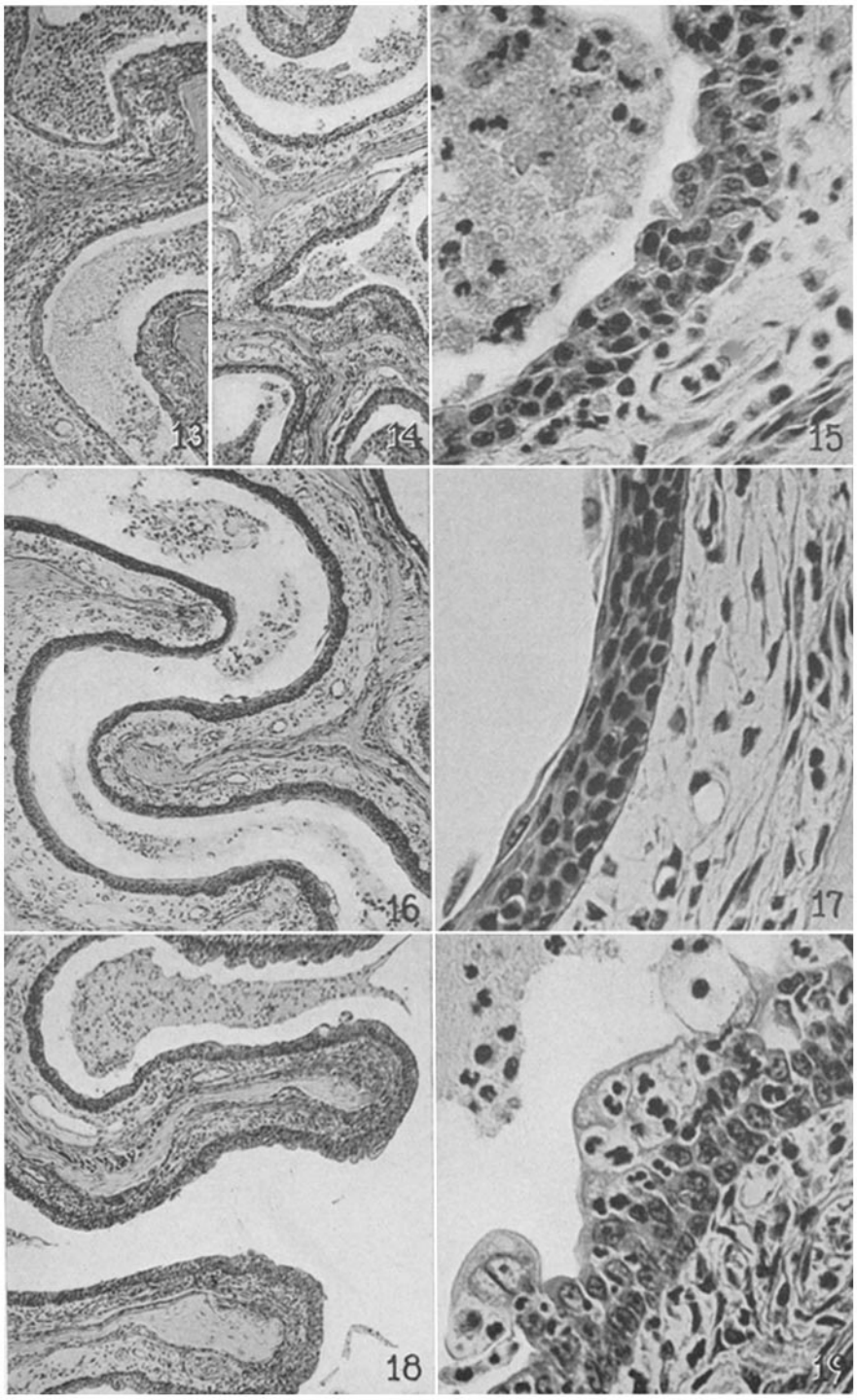

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PLATE 39

FIG. 20. Influenza. Day 10. Anterior turbinate. × 80. The epithelium is beginning to appear more normal. The first columnar elements are visible but there are still several layers of cells so that the epithelium is stratified columnar. There is still a small amount of exudate in the air passages.

FIG. 21. High power view of Fig. 20. × 570. Shows the earliest formation of columnar cells with young cilia sprouting from the free surface. Below, an intra-epithelial cavity is shown which contains leucocytes. The submucosa shows a layer of fibroblasts next to the cartilage and some of these are in the process of transformation into chondroblasts.

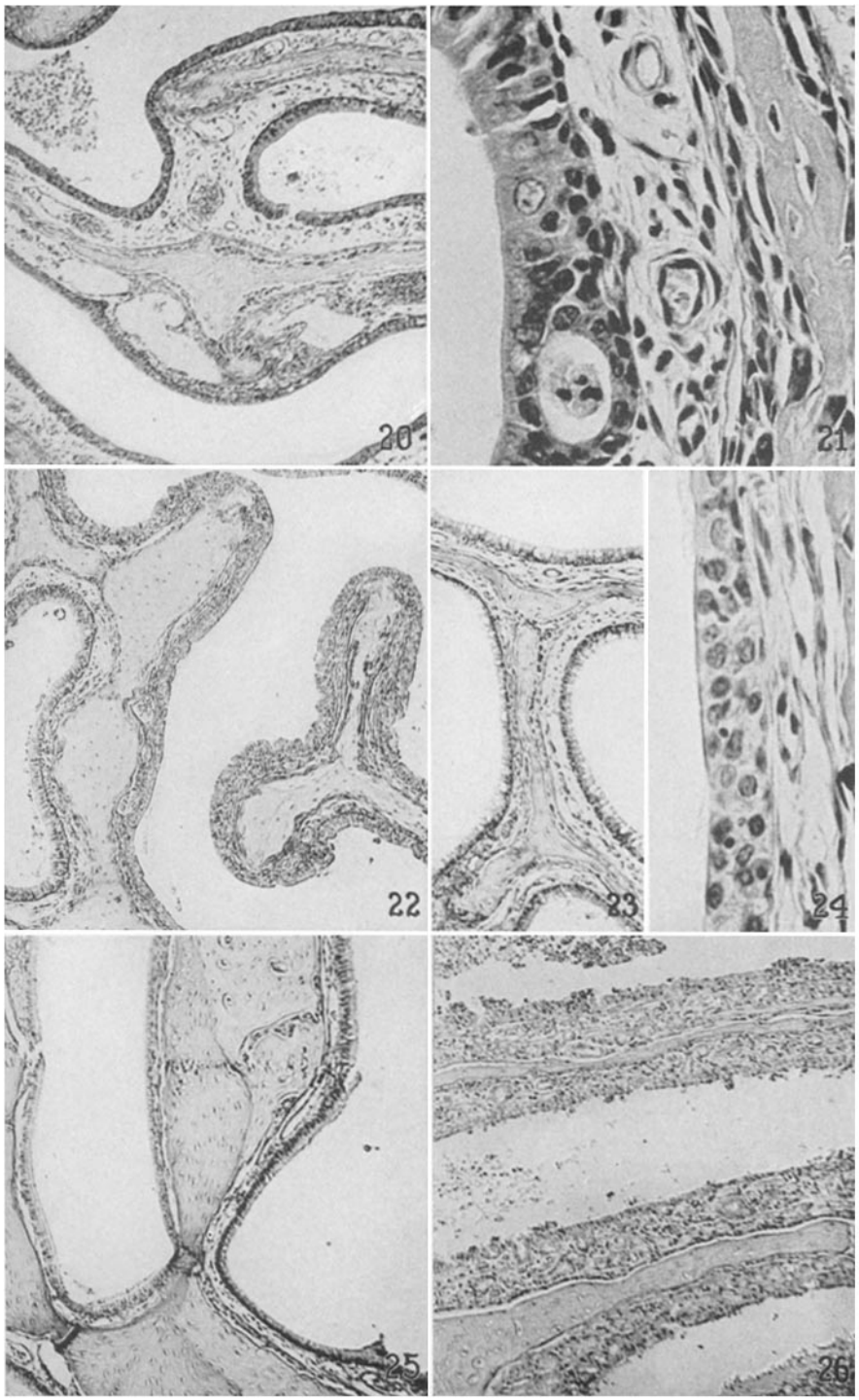
FIG. 22. Influenza. Day 14. Anterior turbinate. × 80. The epithelium is hyperplastic and stratified columnar ciliated in type. The laminae of cartilage are widening as new cartilage is laid down from fibroblastic transformation. The air passages are free from exudate.

FIG. 23. Influenza. Day 17. Anterior turbinate. × 80. The epithelium is simple columnar ciliated in type and contains abundant goblet cells.

FIG. 24. Influenza. Day 28. Anterior turbinate. × 570. A portion of pseudostratified non-ciliated epithelium is shown which was present near the tip of the anterior turbinate. The epithelium is mostly of a normal type, however.

FIG. 25. Influenza. Day 28. Anterior turbinate. × 80. Cartilage regeneration is a striking feature of this section. The deeply stained zone is apparently older than the lighter pink-staining area to the left of the section. Note the shrinking of the submucosa which has occurred as the cartilage has been laid down.

FIG. 26. Influenza. WS strain of virus. Day 3. Posterior turbinate. × 570. The PR8 strain of virus does not affect the olfactory epithelium but some of the laminae in the posterior turbinate of this ferret infected with the WS strain showed epithelial necrosis. The absence of a basement membrane in this area has led to the exposure of the nerve endings and glands of the submucosa.



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