EFFECT OF INFLUENZA VIRUS ON CILIA AND EPITHELIAL CELLS IN THE BRONCHI OF MICE*

By CARL G. HARFORD, M.D., AND ALICE HAMLIN

(From the Division of Infectious Diseases of the Department of Medicine, Oscar Johnson Institute for Medical Research, Washington University School of Medicine, St. Louis)

PLATES 4 TO 7

(Received for publication, October 10, 1951)

A study of bronchial cilia in mice was carried out in order to obtain evidence as to how influenza viral infection of the mouse lung lowers resistance to secondary pneumococcal pneumonia. Earlier work had shown that this effect is associated with the viral lesion rather than the presence of the virus *per se* and had suggested that viral infection damages antibacterial mechanisms of the lung (1). Particular attention was given to cilia because of the histologic evidence that influenza viral infection of the mouse causes necrosis of the bronchial epithelium (1–8). In addition, it has been shown that ciliary action removes foreign particles from the respiratory passages and therefore probably serves as an important mechanism of native resistance to bacterial infection of the respiratory tract (9–20).[1]

When severe infection with influenza virus was induced in mice, it was found that active ciliary beat persisted in the bronchi of involved lungs and that cilia could be shown to be present in stained sections of the viral lesion.[2] These findings were unexpected because it was thought that this viral infection causes destruction of bronchial epithelial cells. For this reason, further experiments were done to clarify the nature of the viral lesion. The result of this investigation has revealed no evidence that actual destruction of the epithelium takes place. Instead of nuclear changes in the early viral lesion, basophilic intracytoplasmic inclusion bodies have been found. Evidence has also been obtained to indicate that portions of the cytoplasm of non-ciliated cells become detached and are shed into the lumen.

EXPERIMENTAL METHODS AND RESULTS

Wave-Like Movements of the Bronchi[3]

In order to ascertain the effect of viral infection on broncial cilia, it was necessary to have a method for evaluation of ciliary action. A technique frequently

* This work was supported by the Commonwealth Fund.

[1] Further references concerning ciliary function in the respiratory tract can be obtained from several sources (9, 11, 17, 19, 20).

[2] Preliminary reports of this finding have already been made (21, 22).

[3] The term "wave-like movements" is used in order to designate the phenomenon without implying its nature.

used for this purpose consists of the visualization of wave-like movements on the surface of respiratory epithelium (23–28, 17), and adaptation of this method to the bronchus of the mouse was readily made.

Method.—The mouse was killed with ether and, immediately after death, the left lobe of the lung was excised and examined under a dissecting microscope with magnifications of 10 to 45 diameters.[4] By illumination with strong reflected light, dissection with fine needles was started at the hilum and carried out in such a manner that branches of the intrapulmonary bronchi were laid open for observation. It was necessary to manipulate the tissues with needles so that points of light reflecting from the surface of the epithelium could be seen. When the proper position was obtained, the rapid wave-like movements could be seen as reflected points of light in a manner which resembled a simmering fluid or the waves of a wheat field in miniature.[5] The distribution of the movements in the bronchial tree appeared to depend chiefly on the parts that were successfully illuminated at the correct angle, and most preparations showed vigorous activity in several places. The movements were often seen on the cut end of the bronchus at the hilum before dissection was begun or through the transparent walls of small bronchi. The appearance of the wave-like motion was striking and there was no doubt that it was a manifestation of an intrinsic property of the bronchus. In contrast, the alveolar portion of the lung showed no movement. After exposure to the air, drying of the surface of the tissue took place within a few minutes and movement ceased. Activity was seen in bronchi of lungs removed within approximately 30 minutes after death of mice but not thereafter. Examination of the bronchi of rats showed similar movements but none were seen in the bronchi of guinea pigs or rabbits.

Effect of Virus.—To determine the effect of influenza viral infection on these wave-like movements of the bronchi, lethal doses of virus were injected intrabronchially by techniques previously described (1, 8). At daily intervals after inoculation, the infected left lungs of the mice were excised and examined in the same manner for wave-like movements and it was found that activity was readily demonstrated during the first 3 days of infection but was slight or absent on the 4th and 5th days.

Effect of Anesthesia with Chloral Hydrate.—Since intrabronchial injection was done under anesthesia with chloral hydrate, observations were made to determine the effect of this drug on the wave-like movements. The results showed a marked inhibition of motion in mice under deep anesthesia with chloral hydrate in that waves were either completely absent or were very weak and localized.

Anesthesia with chloral hydrate was induced by intraperitoneal injection of 0.3 to 0.5 ml. of a 2.5 per cent aqueous solution of the drug and after various periods of time the mice were killed with ether. During approximately the first 30 minutes of anesthesia, wave-like movements could still be seen, but after this time were absent or extremely weak as long as anesthesia was maintained by further injections of the drug. Mice killed with ether immediately

[4] First observations were made with a microscope equipped with a vertical illuminator (Ultrapak) kindly loaned by Dr. Theodore Walsh.

[5] Similar wave-like movements have been well described by Proetz (17, 25, 27) and illustrated by him with motion pictures.

after recovery from anesthesia with chloral hydrate showed strong activity. In contrast with chloral hydrate, no inhibition of movement was detected after narcotization for 6 hours with ethyl alcohol, sodium pentothal, or sodium phenobarbital.

Cardiovascular Wave-Like Movements.—The observation of wave-like movements on the bronchial epithelium and their diminution or disappearance during the development of the lesion due to influenza virus appeared consistent with the concept that this activity was a manifestation of ciliary action and that viral infection destroyed the cilia-bearing cells of the bronchial epithelium. However, in the course of other experiments, the posterior mediastinum of mice was examined under a dissecting microscope in the same manner and it was surprising to find that movements apparently identical with those on the bronchi were present on the exposed surfaces of major blood vessels. Inasmuch as blood vessels do not have cilia, it was evident that movements on the blood vessels were not due to ciliary action and the possibility arose that the wave-like motions observed in the bronchi were also not manifestations of ciliary action. Further search showed that activity of the same appearance could be seen not only on blood vessels but also on the surface of the heart[6] and even on the freshly cut surface of the myocardium. The presence of wave-like movements on the heart and blood vessels suggested that this activity was a manifestation of fibrillary movements of muscle and therefore similar examinations were made of other surfaces of the body such as stomach, intestines, liver, spleen, kidneys, and bladder. However, the wave-like movements were found only in the bronchi and cardiovascular system.

Direct Observation of Ciliary Beat

The discovery of wave-like movements on the surfaces of the heart and blood vessels raised the possibility that such movements in the bronchi of mice are not manifestations of ciliary action and thereby rendered the method inconclusive for us as a criterion of ciliary beat. Therefore, in order to obtain valid evidence of ciliary movement, the beat was visualized directly in fresh microscopic preparations by a modification of methods described by others (29).[7]

Method.—The mouse was killed with ether and immediately thereafter, the left lobe of the lung was excised. With a razor blade, fine slices of the lung were made in such a way that cross-

[6] The presence of wave-like movements on the surface of the heart of the mouse was confirmed by Dr. Arthur Proetz who noted their similarity to those demonstrated by him on the trachea of the rabbit. Hearts of mice were examined in microscopic sections stained with iron-hematoxylin but cilia were not found.

[7] As a further criterion of ciliary action, mice were subjected to the inhalation of powdered carbon particles or fine droplets of India ink, after which the bronchial epithelium was observed with a dissecting microscope in the same manner as for wave-like movements. Although evidence was obtained that carbon particles were transported up the bronchi, as would be expected of ciliary action, the observations were not consistent enough to use this method as a criterion of the efficiency of the ciliary mechanism.

sections of the larger intrapulmonary bronchi could be recognized macroscopically. The slices were washed in a dish with physiologic saline to eliminate blood, teased into a flat unfolded position, and then mounted flat on microscopic slides in physiologic saline with coverslips. The 4 mm. objective (44×) and 10× ocular were used with transmitted light reduced by means of the iris diaphragm of the condenser. When the objective was focussed on the edge of a bronchus, ciliary beat was readily seen as a very rapid rhythmic movement. Immediately after preparation, individual cilia could not be seen because of the rapidity of movement but their identity was unmistakable because of their size, location, and beat. Occasional red blood cells coming into contact with the moving cilia could be seen to be propelled by them. After this movement had been observed for about 20 minutes, slowing occurred and actual fibers of cilia became visible.[8] Ciliary fibers could also be seen after the movement had stopped altogether.

Ciliary Beat in Lungs Infected with Virus.—This direct visualization of ciliary beat appeared to offer an unequivocal method for determining the effect of influenza viral infection on the ciliary mechanism of the intrapulmonary bronchi. Therefore, intrabronchial injections of large doses (dilution 10^{-3}) of the Weiss, PR8, and swine strains of influenza virus were done by the technique described previously (1, 8) and direct observation of ciliary beat in the bronchi was carried out at 1, 2, 3, and 5 day intervals after injection. Particular attention was given to ciliary beat 2 days after injection of virus because the appearance of the epithelium in stained sections indicated maximal destructive action of the virus at this time. Ciliary beat was observed with especial care also 5 days after injection of the virus because it had already been shown that the viral lesion at this interval is associated with decreased resistance to secondary pneumococcal infection (1, 30). The results of these observations of ciliary beat in lungs infected with virus were unexpected and showed ciliary action as actively and extensively as in normal mice.

Effect of Chloral Hydrate on Ciliary Beat.—Since chloral hydrate caused such a striking diminution of wave-like movements in the bronchi, and in view of observations indicating that general anesthesia inhibits ciliary action (31), it was important to determine the effect of chloral hydrate on ciliary beat as observed directly. Therefore, mice were anesthetized deeply by injection of chloral hydrate as before and anesthesia maintained for 1 to 6 hours by further injections of 0.1 ml. of the drug as needed. After varying periods of anesthesia, the mice were killed with ether or more chloral hydrate for direct microscopic examination of ciliary beat in fresh slices of lungs. Under these conditions, ciliary beat was as active as in the normal mouse.

When anesthesia with chloral hydrate failed to inhibit ciliary beat, further trials were made using relatively much larger amounts of the drug directly in contact with the tissue. Slices of fresh normal mouse lung were prepared as before, but, instead of saline, solutions of chloral

[8] Similar preparations were viewed also with an oil immersion objective (2 mm., 90×) and by phase microscopy, but the visualization of cilia under these conditions did not appear to be better than that described.

hydrate in saline were used to immerse and mount the slices of lung for microscopic study. It was found that ciliary beat was weak or absent in 1:100, 1:200, and 1:300 dilutions of chloral hydrate but that the activity of cilia in dilutions of 1:400, 1:500, and 1:1000 of chloral hydrate was as great as with saline alone. Since it is unlikely that concentrations even as high as 1:1000 can be obtained in living mice, it is apparent that these *in vitro* tests gave further evidence that anesthesia with chloral hydrate did not significantly inhibit ciliary motion. The striking inhibition of wave-like movements in the bronchi by anesthesia with chloral hydrate coupled with the failure of this anesthesia to stop ciliary beat provide further evidence that the wave-like movements are not due to motion of cilia.

Histopathologic Observation of Bronchial Epithelium

The presence of active ciliary movement in the bronchi of mice with severe influenza viral infection was difficult to reconcile with previous findings of others (2–7) and ourselves (1, 8) indicating that influenza virus causes necrosis of the bronchial epithelium. For this reason, it was necessary to study stained sections for confirmation of the fact that cilia are not destroyed by the viral infection and also to define the nature of the viral lesion of the epithelium that would permit sparing of ciliated cells.

Methods for Staining Cilia.—In sections stained with hematoxylin and eosin or other similar techniques, bronchial cilia of the mouse were either faintly visible or not seen at all. Therefore special stains were used.

Iron-hematoxylin has been recommended for demonstration of cilia (32) and we have used this stain successfully for bronchial cilia of the mouse. At first, orange G was used as a counterstain but later it was found that the cilia were more clearly visualized without any counterstain. Best results were obtained by careful control of the degree of differentiation.

The use of Bodian's silver stain (33) for demonstration of cilia was suggested by seeing well stained cilia in a section of Eustachian tube to which this stain has been applied for study of nervous elements.[9] Application of Bodian's stain to the mouse lung resulted in a remarkably selective deposition of silver on the bronchial cilia. There was little staining of the cytoplasm and the clumps of cilia could be seen clearly even when cut in cross-section (Fig. 1). However, a serious difficulty arose in the use of Bodian's stain for this purpose when it was found that satisfactory results could be obtained only with two old samples of protargol.[10] New samples of protargol were not successful even with modifications of the technique (34–36).

In spite of the fact that Bodian's stain gave excellent results with the old samples of protargol, the general unavailability of protargol suitable for the purpose of this work made it necessary to seek another stain for cilia. After unsuccessful trial of various silver stains, the technique described by Gomori as modified by Burtner and Lillie (37) was found to be capable of showing them. With this method, good results were not always obtained; however, some of

[9] This section was called to our attention by Dr. Catherine Smith of the Department of Otolaryngology and she also kindly supplied the two samples of protargol that gave satisfactory results.

[10] These samples of protargol were from the Winthrop Chemical Co., Inc., were labelled "albumose silver" and bore the numbers 4898 and 4948. Other samples of protargol of the same firm failed to give positive results including protargol S which is prepared solely for histologic purposes. In correspondence with Dr. M. Tainter of the Winthrop Chemical Co., Inc., it has not been possible to learn the method of preparation of this silver albumose since the foreign source of the material has been lost.

the unpredictability of the stain for this purpose appeared to be obviated by buffering the 10 per cent formalin fixative to an acid pH.

After use of the foregoing methods for some time, we were informed that a modification of Holmes's silver stain (38) was as satisfactory as the Bodian stain for study of nerve cells.[11] Accordingly, this method was applied to sections of mouse lung and it was found that cilia were stained clearly. In fact, the results with Holmes's stain were very similar to those obtained with Bodian's stain in that the cytoplasm was stained lightly while cilia and nuclei were stained intensely. Furthermore, reagents for Holmes's stain were all readily available. Since the first trial, we have used this stain exclusively for cilia and consider it the most reproducible and valuable method that we have tried.

The Holmes method as carried out in this laboratory is as follows:—Impregnating solution: A boric acid buffer solution is prepared by mixing 55 ml. of boric acid solution (12.4 gm. in 1 liter of water) with 45 ml. of sodium borate solution (19 gm. of $Na_2B_4O_7 \cdot 10H_2O$ in 1 liter of water) and diluting to 494 ml. with water. Add 1 ml. of a 1 per cent aqueous solution of silver nitrate and 5 ml. of a 10 per cent aqueous solution of pure pyridine. Mix thoroughly.

Reducing solution: Hydroquinone 1 gm., sodium sulfite 10 gm., water 100 ml. This solution can be used repeatedly but should not be kept more than a few days.

The lungs are fixed in unbuffered 10 per cent formalin,[12] and paraffin sections prepared in the usual manner. After removal of paraffin and passage through alcohols to distilled water, the sections are placed in 20 per cent aqueous silver nitrate in the dark at room temperature for 1 to 2 hours. The solution can be used again if no deposit of black material is observed. Sections are then washed in 3 changes of distilled water for 10 minutes each and placed in the impregnating solution (not less than 20 ml. of solution per slide) at 37°C. for 18 to 24 hours. After this time, the excess solution is shaken off and the sections are placed in the reducing solution for 2 minutes. The reducing solution is removed by washing in running tap water for 3 minutes followed by a rinse in distilled water. Toning is carried out by immersion for 3 minutes in a 0.2 per cent aqueous solution of gold chloride (1 gm. of gold chloride in 500 ml. of water) after which the sections are rinsed briefly in distilled water. The sections are then immersed individually in 2 per cent oxalic acid for 3 to 10 minutes each. The duration of treatment with oxalic acid is controlled by microscopic examination at frequent intervals and the process discontinued when the cilia and nuclei develop a bluish black color. Sections are again rinsed in distilled water and then immersed for 5 minutes in 5 per cent sodium thiosulfate. Following this treatment, the sections are washed for 10 minutes in tap water, rinsed in distilled water, dehydrated as usual, and mounted.

Throughout all of the present study of bronchial cilia, the lungs have always been reexpanded with fixative (39) in order to avoid the folding and compression of the epithelium which occur when the lung collapses after opening the thorax. For the silver stains, the fixative was 10 per cent formalin; for iron-hematoxylin the fixative was Zenker's fluid containing either 10 per cent formalin or 5 per cent glacial acetic acid. Because cilia seemed to be more clearly visible in thin sections, many of the sections were cut at a thickness of 2 to 3 microns by imbedding in hard paraffin (melting point 75°C.).

Bronchial Cilia in Viral Lesions.—To confirm the observation that bronchial cilia of the mouse were not destroyed by severe infection with influenza virus, lethal doses of virus were administered intrabronchially as before and sections were prepared at daily intervals. Again, most of the sections were taken 2 days

[11] The Holmes's stain was suggested by Dr. E. H. Polley, then of the Department of Anatomy of St. Louis University School of Medicine.

[12] Zenker's fluid gave unsatisfactory results.

or 5 days after inoculation. In these sections, cilia-bearing cells of the bronchial epithelium were demonstrated readily with iron-hematoxylin and all three silver stains. In fact, cilia in the viral lesion seemed even more easily visible than in normal mice. Cilia in viral lesions are illustrated in Figs. 2 to 4.[13] In Fig. 4, it can be seen that the whole ciliated epithelium is intact in spite of the fact that a maximal lesion of the epithelium is present judging from the cellular debris in the lumen. This cellular debris was examined carefully in order to detect some ciliated cells which might have been shed into the lumen and it is important to note that such cells were not found.[14]

Regeneration of the Epithelium.—Although it is known that rapid proliferation of bronchial epithelium takes place after infection with influenza virus (2, 5–7, 40), it was not clear whether the epithelium in our preparations was present because of rapid regeneration or because it had not been destroyed in the first place. In order to obtain evidence concerning this question, colchicine was used because it is known that administration of this drug causes division of cells to be arrested in mitosis so that the rate of proliferation of cells can be estimated from a microscopic determination of the frequency of mitotic figures (43).

Tablets of colchicine were powdered by grinding in a mortar and sufficient saline was added to give a concentration of 1 mg. per ml. This solution was injected intraabdominally into mice in a dose of 0.1 ml. In preliminary administration of colchicine to normal mice, it was found that only rare mitotic figures could be found in the bronchial epithelium indicating that multiplication of the cells was occurring at only a slow rate. To study the relative rate of multiplication in lungs with viral infection, mice were injected intrabronchially with a lethal dose of the Weiss strain of influenza virus and sections were prepared at daily intervals. In both normal and infected mice, colchicine was administered 9½ hours before each mouse was killed for section.

Microscopic examination showed no proliferation of epithelial cells 2 or 3 days after injection of virus but rare foci of mitotic figures became visible after about 4 days and increased progressively thereafter. Fig. 5 shows numerous mitotic figures in a portion of a bronchus 5 days after inoculation of virus. These findings indicated that considerable hyperplasia of bronchial epithelium occurs by the 5th day after injection of virus so that the presence of ciliated

[13] In the evaluation of bronchial cilia, it is important to note that the ratio of ciliated to non-ciliated cells diminishes with the subdivision of the bronchi into the smaller branches (40, 41).

[14] Because of the possibility that cilia might disappear immediately upon death of epithelial cells, some normal mice were killed with ether and their intact bodies allowed to stand for 7 hours at room temperature, after which sections of the lung were prepared and stained with iron-hematoxylin. Well defined cilia were found to persist under these conditions. Additional evidence that the morphologic characteristics of cilia persist after death of epithelial cells has been furnished by Bryan and Bryan (42) who have shown cilia on degenerating cells in smears from the human nasopharynx.

cells at this time may be due partially to growth occurring after action of the virus. On the other hand, absence of mitotic figures from the viral lesion of 2 days duration showed that ciliated cells present at this early stage must escape destruction by virus.

Globular Projections of Bronchial Epithelium.—Loosli (40) has reported that portions of the cytoplasm from cells of the bronchial epithelium are shed into the lumen during infection with influenza virus. In reexpanded sections of normal mouse lung, numerous globular projections of the bronchial epithelium are seen to extend into the lumen beyond the cilia (Fig. 6) and we have been able to confirm Loosli's observation in that the globular projections are absent in the viral lesion (Figs. 2 to 4).

In order to determine whether the globular projections are composed of mucous secretions, sections of normal lung were stained for mucous by mucicarmine (44), by toluidine blue for metachromasia (45), and by the periodic-Schiff reaction (46). Negative reactions were obtained with all three tests and indicated that the projections do not consist of mucous secretions.[15]

To obtain evidence as to whether the globular projections are portions of cytoplasm, thin sections of normal mouse lung were prepared and stains for mitochondria were carried out (44, 48). As can be seen in Fig. 7, mitochondria were readily demonstrated in the globular projections establishing the fact that these structures are portions of the cytoplasm.[16]

Polymorphonuclear Leucocytes in the Bronchial Lesion.—When lethal doses of virus are injected intrabronchially, the maximal lesion of the bronchial epithelium occurs approximately 2 days after inoculation. At this time, large amounts of basophilic material are present in the cellular debris within the lumen and also within the bronchial epithelium itself. Inasmuch as polymorphonuclear leucocytes are known to migrate through respiratory epithelium (52)

[15] Loosli (40) did not find mucous or mucous glands in the bronchi of the mouse and goblet cells were few in number. The terminal bronchioles of human beings are also stated to be lacking in mucous and mucous-secreting cells (41, 47).

[16] Cells of the bronchial epithelium of the mouse with globular projections are called "villus cells" by Macklin (49–51). He also found mitochondria in the globular projections and considered these structures to be portions of the cytoplasm.

In the terminal bronchioles of human beings and rabbits, Clara (47) has described and illustrated non-ciliated cells with similar globular projections into the lumen. He found mitochondria in the ciliated cells but only granules in non-ciliated cells and secretion interpreted his findings to mean that the non-ciliated cells produce a non-mucous aprocrine secretion.

In the bronchial lumen of the normal mouse, we have found round bodies unattached to the epithelial cells but having the same size and appearance as the globular projections. Such bodies are often present in large numbers at the narrow ends of ovoid sections of bronchi and have been interpreted as globular projections that appear separated because the epithelium was cut at such an angle that the cell bodies of those particular projections were not included in the section. It seems unlikely that these bodies are non-mucous secretions of cells since mitochondria were found in them also.

and since these leucocytes are present in the bronchial lumen adjacent to the epithelium 2 days after injection of virus, the question arose of whether this basophilic material is derived from degenerating polymorphonuclear leucocytes. In order to obtain evidence on this point, mice were exposed to large doses of roentgen rays, and then injected intrabronchially with influenza virus so that observation of the bronchial lesions in microscopic sections could be carried out when the polymorphonuclear leucocytes had practically disappeared from the body.

For irradiation, 10 to 12 mice at a time were placed in a flat covered cardboard box approximately 15 cm. square and exposed to 1000 roentgen units.[17] Since very few polymorphonuclear leucocytes are present in the blood of mice 4 days after severe whole body irradiation (53), the mice were injected intrabronchially with a lethal dose of influenza virus 2 days after irradiation. Four days after irradiation (2 days after inoculation of virus), sections of the lung were prepared in the usual manner and stained with hematoxylin and eosin.

For control, it was necessary to show that irradiation did not interfere with viral infection. Therefore, 2 days after irradiation, mice were injected intrabronchially with a lethal dose of virus and at the same time non-irradiated mice were injected for comparison. One day after inoculation of the virus, the left lungs of the irradiated and non-irradiated mice were removed for titration of their viral content. Titrations were done by pooling the infected left lungs in each group and preparing serial tenfold dilutions as before (1). These dilutions were injected intrabronchially into mice and the macroscopic lesions of the left lungs were observed 5 days after inoculation. Results of these titrations did not show any inhibitory effect of irradiation on viral multiplication.

It was necessary to show also that irradiation was effective in preventing the migration of polymorphonuclear leucocytes into the bronchi. For this purpose, a 5 per cent suspension of aleuronat in saline was injected intrabronchially into mice irradiated 3 days previously and also into normal mice. One day after injection of the aleuronat, sections were prepared and stained with hematoxylin and eosin. In the normal mice, large numbers of polymorphonuclear leucocytes could be seen to have migrated through the bronchial epithelium and to have infiltrated the aleuronat lodged in the bronchi. In contrast, polymorphonuclear leucocytes were almost completely lacking in the bronchi of irradiated mice.

Sections of lungs from mice irradiated in the same manner but not infected with virus showed no basophilic material in the lumen.

Observation of the viral lesion in irradiated mice showed large amounts of basophilic material in the bronchial lumen demonstrating that a major source of this material must be other than degeneration of polymorphonuclear leucocytes.

Cytoplasmic Inclusion Bodies in the Viral Lesion.—Since it could not be shown that the basophilic material of the viral lesion is due only to degeneration of polymorphonuclear leucocytes, it seemed likely that much of this material is derived from degenerating nuclei of epithelial cells. However, if epithelial cells are destroyed by viral action, it would be necessary to assume that only the non-ciliated cells become necrotic since it had already been shown that ciliated

[17] Irradiation of the mice was kindly carried out by Dr. W. B. Seaman and staff of the Mallinckrodt Institute of Radiology.

ones survive. For these reasons, it appeared desirable to study the earliest phases of the viral lesion in order to demonstrate that non-ciliated cells contain nuclei undergoing pyknosis.

It will be recalled that influenza virus multiplies in the lung within 24 hours after intrabronchial inoculation and that lesions due to viral infection could not be shown at this interval of time (1). On the other hand, the epithelial lesion is at its height 48 hours after inoculation and it seemed probable that the earliest lesion due to viral infection could be seen at an intermediate period and accordingly sections were prepared between 28 and 36 hours after injection.

At this early stage of the viral lesion, it was found that the nuclei of cells in the bronchial epithelium retained their vesicular character and appeared intact. However, in the cytoplasm of the epithelial cells, a profusion of basophilic inclusion bodies was found. These bodies were striking in appearance and could be readily distinguished from pyknotic nuclei especially when small in size and occurring singly (Figs. 8 and 9). Inclusions were often particularly noticeable in the globular projections and were shown to be present in ciliated cells as well (Figs. 10 and 11).

Most of the cytoplasmic inclusion bodies were round or ovoid but crescentic forms were seen frequently. In many instances, a crescentic basophilic rim surrounded an apparently round acidophilic body (Fig. 12). There was great variation in size of inclusions; some were only a fraction of the size of nuclei (Fig. 8) while others were as large as the nuclei (Fig. 13). Most of the inclusions were strongly basophilic[18] but similarly shaped cytoplasmic bodies were often seen that were acidophilic (Fig. 14). Sometimes basophilic inclusions appeared to be surrounded by envelopes of acidophilic material. Conclusions were drawn only from the basophilic inclusions because their presence and location could be more definitely ascertained than the acidophilic bodies. Inclusions were often found to be in the same plane of focus as the nuclei of respective cells. This location in the same plane as the nucleus indicated that the inclusions were actually within the cytoplasm and not merely adhering to the surfaces of cells. Staining with iron-hematoxylin was advantageous not only because cilia and nuclei were visualized but also because the cell membranes could usually be seen so well that the location of inclusions in individual cells could be easily shown. The nuclei of inclusion-bearing cells retained their vesicular character but were often indented by the inclusions or seemed slightly distorted in shape. No other abnormalities of nuclei were recognized. At this stage of the lesion, the epithelial cells retained their normal regular arrangement and no basophilic cellular debris was present in the lumen. An occasional polymorphonuclear leucocyte could be found in the lumen.

At later intervals after injection of virus, severe changes of the bronchial epithelium were often seen although areas characterized only by inclusions were still to be found. These severe lesions were thought to be more advanced stages of the viral process and here the inclusions were numerous and frequently large in size. The epithelial cells often contained multiple inclusions so that great disorganization of the cytoplasm appeared to exist and yet the nuclei continued to show their normal vesicular character. Also, the epithelial cells no longer showed the regular alignment found in the normal epithelium and showed a very irregular and distorted pattern. Ovoid nuclei were often found to have their long axes parallel to the basement

[18] And gave a positive Feulgen reaction (44).

membrane rather than in the usual perpendicular position. In the whole epithelium at this stage, the changes were severe and it was easily possible to understand that such a lesion would appear to be composed chiefly of necrotic epithelial cells. Nevertheless, no definite pyknosis or karyorrhexis of epithelial nuclei could be demonstrated and the basophilic masses resembled closely the cytoplasmic inclusion bodies observed earlier.

Much of the basophilic material in the lumen resembled the cytoplasmic inclusions to a striking degree and often revealed the crescentic shape frequently seen in intracellular inclusions (Fig. 15). The basophilic material resembling inclusions was often free in the lumen or was surrounded by acidophilic material as if inclusion-laden globular projections had become detached from the non-ciliated cells (Fig. 15). Basophilic bodies could be seen in the lumen adjacent to the epithelial cells (Fig. 16) and inclusion bodies were found close to the cell membrane as if in the process of extrusion (Fig. 17).

Polymorphonuclear leucocytes in various stages of degeneration were seen in the lumen and it was not always possible to ascertain whether a particular particle of basophilic material originated in a leucocyte or an epithelial cell. Small clusters of basophilic material within a rounded matrix were sometimes found amongst the epithelial cells and were interpreted as polymorphonuclear leucocytes which had degenerated during migration through the epithelium.

When inclusion bodies were first definitely identified in sections taken 28 to 36 hours after viral injection, they were sought and recognized in 48 hour lesions as well. Although numbers of inclusions diminished gradually in the later stages of the viral lesion, they were still present in some of the epithelial cells 5 days after inoculation.

The development of inclusions as an integral part of the viral lesion as described gave evidence as to their specific association with the viral infection. Nevertheless, for further evidence of this relationship the following types of controls were examined. Bronchial epithelium of normal mice and of mice injected 30 hours previously with sterile broth were carefully examined but inclusions were not found. Because of reports that bronchial epithelial cells may be phagocytic,[19] heavy suspensions of pneumococci were injected intrabronchially into mice, and sections prepared at intervals within the first 6 hours were stained by the Giemsa method. Numerous bacteria were found in the lumen adjacent to the epithelial cells but no evidence of intracellular location of pneumococci could be obtained and inclusion bodies were not present within the epithelial cells. In order to determine whether non-specific irritation or the presence of large numbers of polymorphonuclear leucocytes could induce the appearance of inclusions, examination was made of the epithelium of bronchi with intense exudate of polymorphonuclear leucocytes in the lumen due to intrabronchial injection of aleuronat as described above. Inclusions were not found in these sections. Mice subjected to lethal doses of roentgen rays as described above and infected with virus showed numerous inclusions while mice treated with roentgen rays alone showed no inclusions.

In a few of the control sections, after prolonged search, it was possible sometimes to find one or two small round basophilic cytoplasmic granules which resembled the smallest viral inclusions. This rare finding was not considered significant since bodies associated with viral infection were present in very large numbers, often in nearly every cell. Also, the viral inclusions were of various sizes and were often quite large (Fig. 12).

In sections stained with iron-hematoxylin, Clara (47) has described some large granules in epithelial cells of bronchi from rabbits and human beings which might possibly be confused with influenza viral inclusions. Although fine acidophilic granules are present in the corresponding cells of mice when stained with iron-hematoxylin, the distinction between normal granules and viral inclusions has not been found to be difficult. In sections of normal mouse

[19] These reports have been reviewed by Robertson (54).

lung stained with hematoxylin and eosin, the cytoplasm of bronchial epithelial cells has a homogeneous and non-granular appearance.

As noted above, most of the observations have been made with the Weiss strain of type A influenza virus. Confirmatory histopathologic studies have also been carried out with the PR8 strain and a swine strain of influenza virus with similar results.

DISCUSSION

That cilia persist in the bronchi of mice infected with influenza virus has been demonstrated clearly in the present experiments. Survival of cilia has been established by direct observation of their movement and by visualization in stained sections of fixed tissue. Furthermore, it has been shown that the presence of cilia, at least in the early maximal lesion, could not be due to rapid regeneration of ciliated epithelium but that the ciliated cells must have escaped destruction by viral infection. It might be suggested that some slight impairment of ciliary action might not have been detected by these methods, but, if such a mild effect took place, it would seem to be insignificant because the animals were inoculated with many lethal doses of a virus which causes pneumonia of maximal severity and which has a particularly extensive effect on bronchial epithelium. Under these conditions, one would expect that viral infecion would completely eliminate cilia from the bronchi and yet no lack of cilia was found.[20]

Cilia were demonstrated particularly well on the bronchial epithelium in the viral lesion of 5 days duration under the same conditions in which previous experiments showed that resistance of the lung to inhaled pneumococci has become impaired (1). Active cilia in this lesion indicate that the means by which viral infection of the lung lowers resistance to pneumococcal infection is not by destruction of ciliated cells and consequent interference with the ciliary mechanism for removal of bacteria. On the other hand, persistence of cilia supports the theory previously advanced (30) that resistance of the lung to secondary pneumococcal infection is lowered by the presence of edema fluid in the viral lesion. According to this concept, the fluid enables inhaled pneumococci to grow rapidly enough to overcome ciliary, phagocytic, and other defense mechanisms of the lung.

Wave-like movements have been accepted by numerous workers as visible manifestations of ciliary beat (23–28, 17) and have frequently been used as a test of whether application of drugs to respiratory epithelia does harm to the ciliary mechanism. In view of this general opinion, it is necessary to realize that such movements, as observed in the bronchus of the mouse, were not found to be due to cilia. The wave-like movements were strikingly inhibited by anesthesia with chloral hydrate and yet this drug had no apparent effect on movement of cilia as observed directly. Also, wave-like movements were present on

[20] Francis and Stuart-Harris (55) have reported that influenza virus causes necrosis of the nasal epithelium in ferrets. Our observations of cilia in viral infection have not been extended to this species.

cardiovascular structures which are not ciliated and therefore may be fibrillary movements of muscle. That wave-like movements in the bronchus of the mouse are not due to cilia does not mean that these movements are not caused by cilia when viewed elsewhere. However, this evidence does indicate that wave-like movements cannot be used as unequivocal evidence of ciliary movement without some different type of supporting observations.

Although cytoplasmic inclusion bodies are found in cells infected with numerous viruses (56), their association with influenza virus appears to have been mentioned only once before (40). As in the case of fowl pox (57), these influenzal inclusions may consist of colony-like aggregations of viral particles but the present evidence does not exclude the possibility that they are reactions of the cell to viral infection. However, regardless of their nature, the inclusions have been shown to be present only in lungs infected with the virus and not in normal lungs or lungs subjected to non-viral irritation. Because of this specificity, presence of these inclusions within a particular type of epithelial cell would appear to give evidence that such cells are infected with influenza virus. Therefore, since ciliated cells were shown to contain inclusions and yet survive, another example is given of viral infection of cells without resulting necrosis (58–60).

When it was found that ciliated cells survive in spite of severe viral infection, it seemed likely that viral infection caused selective necrosis only of non-ciliated cells leaving the ciliated cells intact. The results of further study, however, have caused us to favor the idea that the non-ciliated cells survive also, since many vesicular nuclei always persist in the severe viral lesion and no definite early stages of nuclear degeneration have been recognized. Instead of nuclear changes in the early viral lesion, we have found cytoplasmic inclusion bodies.

The presence of inclusions in the early stages of the viral lesion and their appearance in the lumen at later periods indicate that they become separated from the cells in which they are formed. In the case of the non-ciliated cells, separation of inclusion-laden globular projections appears to account for this phenomenon, especially when the inclusions are crescentic in shape since the visible structure of the inclusions in these instances is distinctive. The manner of separation of inclusions from ciliated cells is not clear but basophilic material closely resembling inclusions and devoid of any cytoplasmic material is often found adjacent to the epithelium and appears to have been simply extruded from the cytoplasm. If the inclusions are composed, at least partly, of viral particles and become separated from the epithelial cells as discussed, a mechanism for viral growth and spread would exist which would not necessitate dissolution of the whole cell. In this connection, it may be noted that small cytoplasmic protrusions of chick embryo cells have been observed during infection with influenza virus (61), and that electron microscope observations

of cells in tissue culture infected with the virus of equine encephalomyelitis have shown what appear to be viral particles growing in portions of cytoplasm which subsequently become detached from the cell (62, 63). Growth and liberation of the virus without necrosis of the cell would also fit well with the idea that the damage done to the lung by influenza virus is the result of a toxic action of the viral particle rather than the destruction of cells from viral growth within them (64–66).

From these observations in mice, the conclusion cannot be drawn that influenza or other respiratory viruses do not destroy ciliated cells of human respiratory epithelia. On the other hand, information obtained by study of human material seems inconclusive (67–71, 42). It is difficult to obtain tissues from human beings suffering from respiratory viral infections under circumstances in which the diagnosis of a known viral infection free of secondary bacterial invaders can be definitely established. Tissues removed at autopsy almost invariably have secondary bacterial infection and do not show the early stages of the viral infection. In addition, the present work indicates that special methods for demonstration of cilia may render them visible although general histologic study of the epithelium might lead one to believe that such extensive destruction of epithelial cells had occurred that cilia could not have survived.

SUMMARY

In order to determine the effect of infection with influenza virus on bronchial cilia of the mouse, ciliary beat has been visualized directly by microscopic examination of the bronchi in slices of fresh lung. Cilia have been shown also in sections of fixed tissue by the use of special silver staining methods. The results have shown persistence of the cilia in spite of severe viral infection and indicate that the lowered resistance to secondary pneumococcal infection which occurs in influenzal pneumonia of the mouse is not due to interference with the ciliary mechanism. By a process of exclusion, the findings give further support to the theory that lowered resistance to pneumococcal infection in influenzal pneumonia is due to edema fluid in the viral lesion furnishing a culture medium for inhaled pneumococci.

A widely used method for evaluation of ciliary activity on respiratory epithelia has been the microscopic observation of wave-like movements in reflected light. This activity was observed readily in the bronchi of mice but evidence was obtained showing that at this site it was due to something other than ciliary beat.

Further histopathologic observations were made in order to define the lesion of the bronchial epithelium that would permit sparing of ciliated cells. In addition to usual techniques, mice were injected with colchicine for estimation of the rate of cellular proliferation and were exposed to a large dose of roentgen rays to eliminate polymorphonuclear leucocytes. Stains for mucous and for

mitochondria were done also. The evidence obtained favors the theory that the viral infection does not destroy any of the cells of the bronchial epithelium. Inclusion bodies were found in the cytoplasm, making it seem likely instead that viral particles grow in colony-like aggregations and that liberation of virus into the lumen takes place not only by simple extrusion of inclusions but also by detachment of inclusion-laden globular portions of the cytoplasm.

In this investigation, capable assistance has been given successively by Virginia Leidler, Mary Hara, Francis McCarthy Edinger, Carolyn Reitter, and Esther Parker.

BIBLIOGRAPHY

1. Harford, C. G., Leidler, V., and Hara, M., *J. Exp. Med.*, 1949, **89,** 53.
2. Straub, M., *J. Path. and Bact.*, 1937, **45,** 75.
3. Dal, M. K., *Arkh. biol. nauk*, 1938, **52,** 107 (English summary).
4. Nelson, A. A., and Oliphant, J. W., *Pub. Health Rep., U. S. P. H. S.*, 1939, **54,** 2044.
5. Straub, M., *J. Path. and Bact.*, 1940, **50,** 31.
6 Oliphant, J. W., and Perrin, T. L., *Pub. Health Rep., U. S. P. H. S.*, 1942, **57,** 809.
7. Dubin, I. N., *Am. J. Path.*, 1945, **21,** 1121.
8. Harford, C. G., Smith, M. R., and Wood, W. B., Jr., *J. Exp. Med.*, 1946, **83,** 505.
9. Hilding, A., *Arch. Otolaryngol.*, 1932, **15,** 92.
10. Hilding, A., *Ann. Int. Med.*, 1932, **6,** 227.
11. Lucas, A. M., *in* Cowdry, E. V., Special Cytology, New York, Paul B. Hoeber, Inc., 2nd edition, 1932, **1,** 415.
12. Lucas, A. M., and Douglas, L. C., *Arch. Otolaryngol.*, 1934, **20,** 518.
13. Hermann, *Z. Hals-, Nasen-, u. Ohrenh.*, 1934, **36,** 279.
14. Barclay, A. E., Franklin, K. J., and Macbeth, R. G., *J. Physiol.*, 1937, **90,** 347.
15. Barclay, A. E., and Franklin, K. J., *J. Physiol.*, 1937, **90,** 482.
16. Barclay, A. E., Franklin, K. J., and Macbeth, R. G., *Am. J. Roentgenol.*, 1938, **39,** 673.
17. Proetz, A. W., Essays on the Applied Physiology of the Nose, St. Louis, Annals Publishing Co., 1941.
18. Proetz, A. W., *Proc. Roy. Soc. Med.*, 1948, **41,** 793.
19. Tremble, G. E., *Laryngoscope*, 1948, **58,** 206.
20. Negus, V. E., *Thorax*, 1949, **4,** 57.
21. Harford, C. G., and Hara, M., *J. Clin. Inv.*, 1949, **28,** 787 (abstract).
22. Harford, C. G., *Washington Univ. Med. Alumni Quart.*, 1951, **14,** 58 (abstract).
23. Lucas, A. M., *Proc. Soc. Exp. Biol. and Med.*, 1932, **30,** 501.
24. Proetz, A. W., *Ann. Otol., Rhinol., and Laryngol.*, 1932, **41,** 1117.
25. Proetz, A. W., *Ann. Otol., Rhinol., and Laryngol.*, 1933, **42,** 778.
26. Lucas, A. M., *Arch. Otolaryngol.*, 1933, **18,** 516.
27. Proetz, A. W., *J. Laryngol., Rhinol. and Otol.*, 1934, **49,** 557.
28. Frenckner, P., and Richtnér, N. G., *Acta Otolaryngol.*, 1939, **27,** 668.

29. Lucas, A. M., *J. Morphol.*, 1932, **53,** 243.
30. Harford, C. G., and Hara, M., *J. Exp. Med.*, 1950, **91,** 245.
31. Ernst, A. M., *Arch. internat. pharmacod. et de thérap.*, 1938, **58,** 208.
32. Cowdry, E. V., Laboratory Technique in Biology and Medicine, Baltimore, The Williams & Wilkins Company, 2nd edition, 1948, 67.
33. Bodian, D., *Anat. Rec.*, 1936, **65,** 89.
34. Bodian, D., *Anat. Rec.*, 1937, **69,** 153.
35. Davenport, H. A., *Stain Technol.*, 1948, **23,** 219.
36. Silver, M. L., *Anat. Rec.*, 1942, **82,** 507.
37. Burtner, H. J., and Lillie, R. D., *Stain Technol.*, 1949, **24,** 225.
38. Greenfield, J. G., and Daniel, P. M., *in* Lee's, The Microtomist's Vade-Mecum, (J. B. Gatenby, and H. W. Beams, editors), Philadelphia, The Blakiston Co., 11th edition, 1950, 537.
39. Loosli, C. G., *Arch. Path.*, 1937, **24,** 743.
40. Loosli, C. G., *J. Infect. Dis.*, 1949, **84,** 153.
41. Miller, W. S., The Lung, Springfield, Charles C. Thomas, 2nd edition, 1947, 38.
42. Bryan, W. T. K., and Bryan, M. P., *Laryngoscope*, 1950, **60,** 523.
43. Bullough, W. S., *J. Endocrinol.*, 1950, **6,** 340.
44. Lillie, R. D., Histopathologic Technic, Philadelphia, The Blakiston Co., 1948, 149, 93, 88.
45. Wislocki, G. B., Bunting, H., and Dempsey, E. W., *Am. J. Anat.*, 1947, **81,** 1.
46. Hotchkiss, R. D., *Arch. Biochem.*, 1948, **16,** 131.
47. Clara, M., *Z. Mikr. anat. Forsch.*, 1937, **41,** 321.
48. Harman, J. W., *Stain Technol.*, 1950, **25,** 69.
49. Macklin, C. C., *Canad. J. Research, Sect. D., Zool. Sc.*, 1949, **27,** 50.
50. Macklin, C. C., *Anat. Rec.*, 1949, **103,** 550 (abstract).
51. Macklin, C. C., *Rev. Canad. Biol.*, 1949, **8,** 328 (abstract).
52. Andrew, W., and Burns, M. R., *J. Morphol.*, 1947, **81,** 317.
53. Brecher, G., Endicott, K. M., Gump, H., and Brawner, H. P., *Blood*, 1948, **3,** 1259.
54. Robertson, O. H., *Physiol. Rev.*, 1941, **21,** 112.
55. Francis, T., Jr., and Stuart-Harris, C. H., *J. Exp. Med.*, 1938, **68,** 789.
56. Rivers, T. M., *Am. J. Path.*, 1928, **4,** 91.
57. Woodruff, C. E., and Goodpasture, E. W., *Am. J. Path.*, 1930, **6,** 713.
58. Florman, A. L., and Enders, J. F., *J. Immunol.*, 1942, **43,** 159.
59. Melnick, J. L., and Godman, G. C., *J. Exp. Med.*, 1951, **93,** 247.
60. Enders, J. F., *Fed. Proc.*, 1949, **8,** 625.
61. Hoyle, L., *J. Hyg.*, Cambridge, Eng., 1950, **48,** 277.
62. Bang, F. B., and Gey, G. O., *Bull. Johns Hopkins Hosp.*, 1951, **88,** 278 (abstract).
63. Bang, F. B., and Gey, G. O., *Fed. Proc.*, 1951, **10,** 350 (abstract).
64. Sugg, J. Y., *J. Bact.*, 1950, **60,** 489.
65. Davenport, F. M., *Fed. Proc.*, 1951, **10,** 405 (abstract).
66. Ginsberg, H. S., *J. Exp. Med.*, 1951, **94,** 191.

67. Parker, F., Jr., Jolliffe, L. S., Barnes, M. W., and Finland, M., *Am. J. Path.*, 1946, **22,** 797.
68. Straub, M., and Mulder, J., *J. Path. and Bact.*, 1948, **60,** 429.
69. Mulder, J., and Verdonk, G. J., *J. Path. and Bact.*, 1949, **61,** 55.
70. Hers, J. F. P., and Mulder, J., *J. Path. and Bact.*, 1951, **63,** 329.
71. Hilding, A., *Arch. Otolaryngol.*, 1930, **12,** 1933.

EXPLANATION OF PLATES

The Weiss strain of influenza virus was used in all experiments illustrated. Photomicrographs were made by Cramer Lewis, Department of Illustration, Washington University School of Medicine.

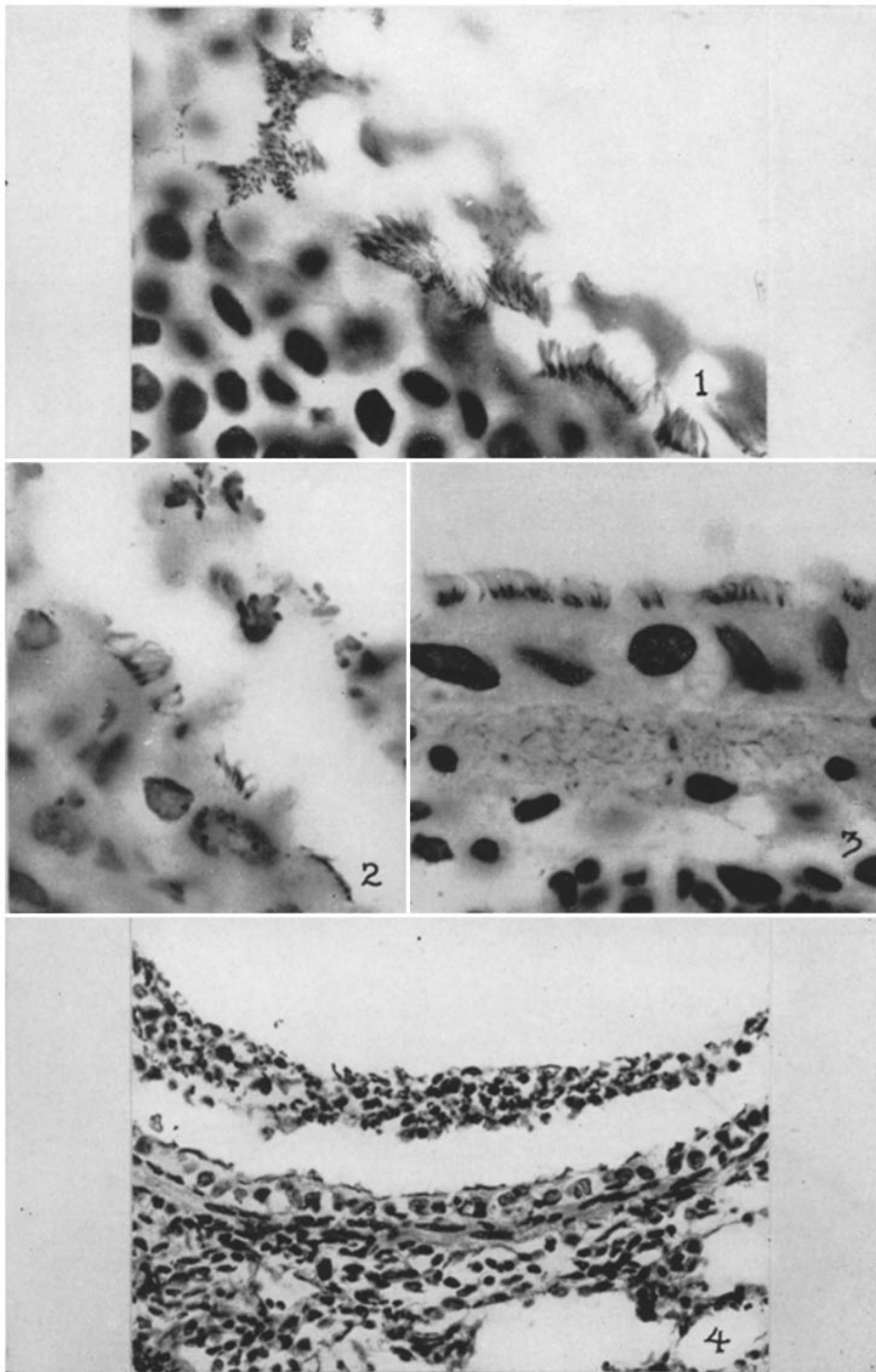
PLATE 4

FIG. 1. Clumps of cilia on normal bronchial epithelium of the mouse. Photograph taken near the bend of a bronchus and includes a clump of cilia cut in cross-section. Bodian's stain. × 1530.

FIG. 2. Cilia during the time when viral changes of the epithelium are most severe (2 days after injection of virus). Bodian's stain. × 1400.

FIG. 3. Cilia of the bronchial epithelium in the fully developed viral pneumonia (5 days after injection of virus). There is only one layer of cells here so that probably no proliferation of cells has taken place in this portion of the epithelium. Bodian's stain. × 1400.

FIG. 4. Low power view of the bronchial epithelium from the same section as Fig. 2. The dark line on the surface of the epithelial cells was shown to be cilia at a higher power. This section shows that cilia are present as in the normal bronchus during the time when the viral changes of the epithelium are most severe. The material in the lumen has become separated from the epithelium to a greater extent than in most other sections but this separation allows a better view of the ciliary line. Bodian's stain. × 400.



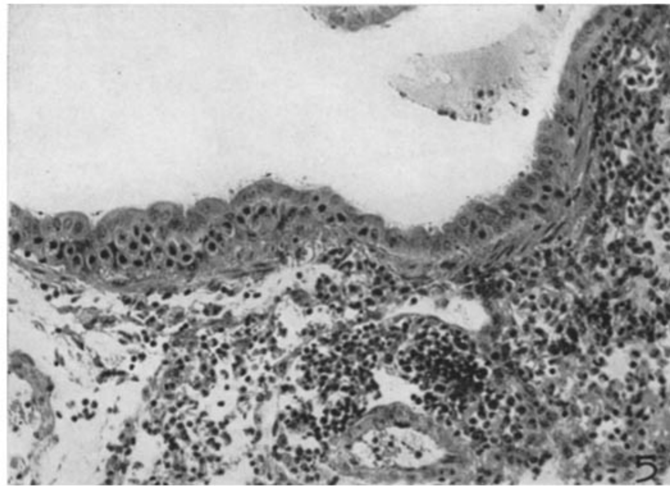
(Harford and Hamlin: Influenza virus and ciliated epithelium)

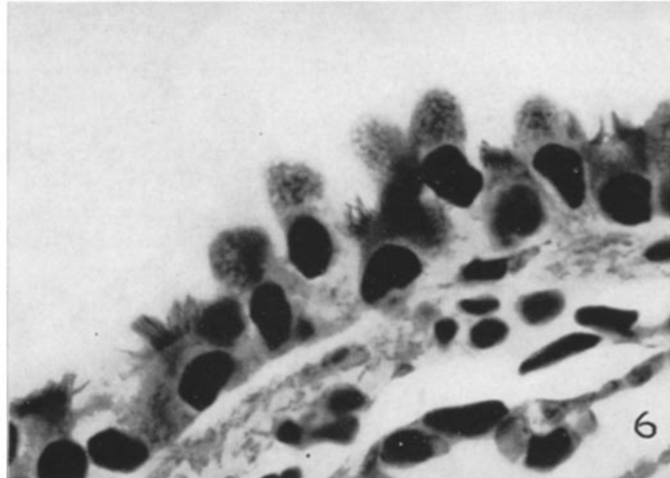
PLATE 5

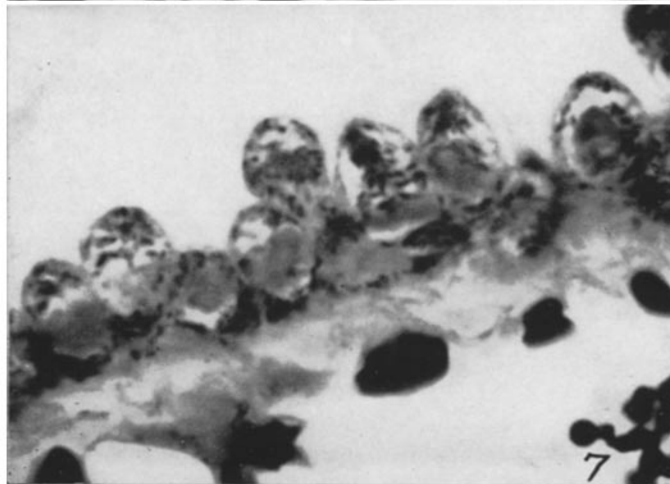
FIG. 5. Focus of mitotic figures indicating rapid proliferation of cells in this portion of the bronchial epithelium. In the photograph, mitotic figures appear as deeply staining masses in the epithelial cells. Under high magnification, chromosomes were clearly visible. Five days after injection of virus. Hematoxylin and eosin. × 190.

FIG. 6. Normal bronchial epithelium showing the globular projections of the cytoplasm and their relation to the cilia. Fixed in 10 per cent formalin buffered to pH 5.3 and stained by the technique of Gomori modified by Burtner and Lillie. × 1400.

FIG. 7. Mitochondria in the globular projections of the cytoplasm. Stain of Bensley modified by Cowdry. × 1800.







(Harford and Hamlin: Influenza virus and ciliated epithelium)

Plate 6

Fig. 8. Small basophilic inclusion body in the cytoplasm between the nuclei of two cells. Cilia are faintly visualized. 31 hours after injection of virus. Hematoxylin and eosin. × 1620.

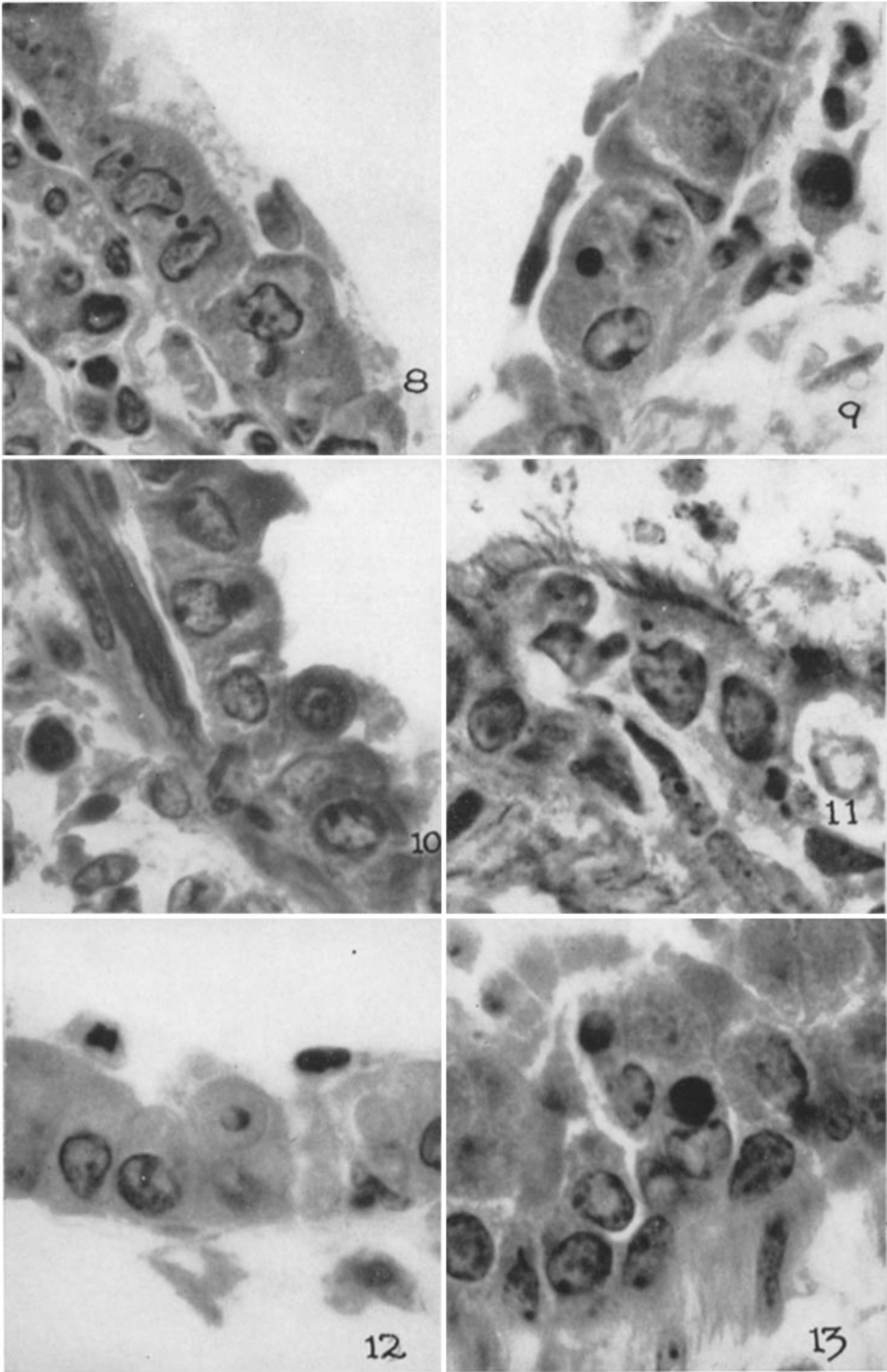
Fig. 9. Medium sized basophilic inclusion body in the cytoplasm. 31 hours after injection of virus. Hematoxylin and eosin. × 1620.

Fig. 10. Inclusion body within the cytoplasm of a ciliated cell. The cilia are only faintly visualized but were easily identified with further focussing of the microscope. The nucleus appears slightly indented by the inclusion. 31 hours after injection of virus. Iron-hematoxylin. × 1620.

Fig. 11. A small inclusion body within the cytoplasm of a ciliated cell. 30 hours after injection of virus. Holmes's silver stain. × 1620.

Fig. 12. A crescentic inclusion body in the cytoplasm. The curved line adjacent to the inclusion may be an early stage of the separation of the globular projection containing this inclusion. 31 hours after injection of virus. Hematoxylin and eosin. × 1620.

Fig. 13. Large inclusion body, approximately equalling the nucleus in size. This inclusion apparently indents the nucleus. A crescentic inclusion body is also visible. 31 hours after injection of virus. Hematoxylin and eosin. × 1620.



(Harford and Hamlin: Influenza virus and ciliated epithelium)

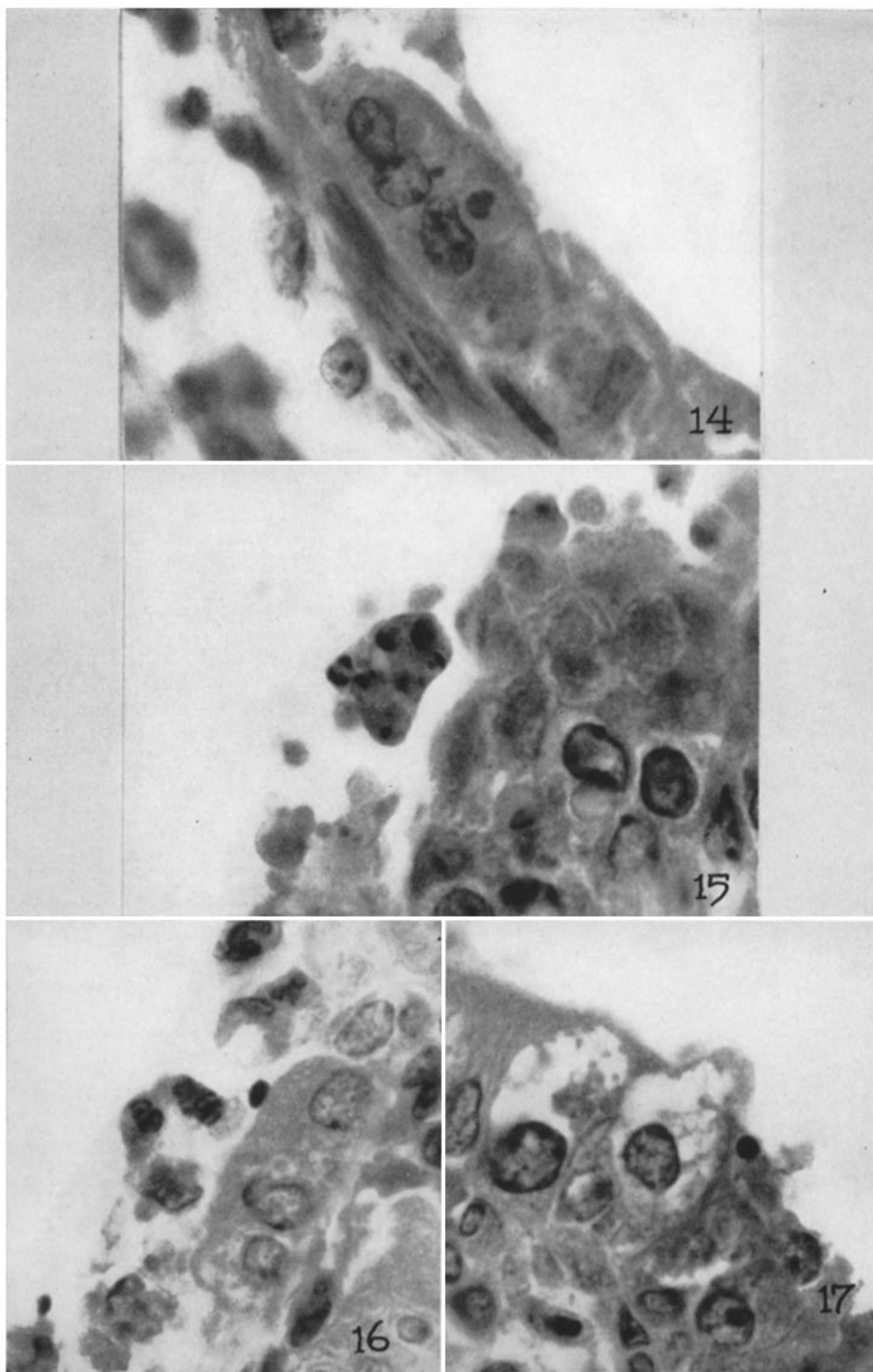
Plate 7

Fig. 14. Basophilic and acidophilic inclusion bodies in cytoplasm of epithelial cells. 33 hours after injection of virus. Hematoxylin and eosin. × 1620.

Fig. 15. Several crescentic basophilic bodies in the bronchial lumen. The bodies are enclosed in a matrix of acidophilic material as if they consisted of inclusion bodies shed into the lumen by the separation of one or more globular projections. 31 hours after injection of virus. Hematoxylin and eosin. × 1620.

Fig. 16. An ovoid basophilic body in the bronchial lumen adjacent to an epithelial cell as if it had just been extruded. Several polymorphonuclear leucocytes are seen nearby. 33 hours after injection of virus. Hematoxylin and eosin. × 1620.

Fig. 17. A cytoplasmic inclusion body so close to the cell membrane that it appears to be in the process of extrusion. Two halo cells are also shown (1). 34 hours after injection of virus. Hematoxylin and eosin. × 1620.



(Harford and Hamlin: Influenza virus and ciliated epithelium)