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THE HISTOPATHOLOGY OF THE RESPIRATORY TRACT IN HUMAN INFLUENZA

BY

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CONTENTS

	Introduction	1
I	Remarks on histology of the tracheo-bronchial tree in general	8
II	Methods and materials	11
III	Histopathology of acute cases of broncho-bronchiolitis and cases of pneumonia not associated with influenza virus but showing bacterial infection of trachea, bronchi, and (or) lungs other than *Staphylococcus aureus*	15
IV	Histopathology of cases of respiratory diseases associated with influenza virus complicated by bacterial infection of trachea, bronchi and lungs other than *Staphylococcus aureus*	19
V	Histopathology of cases of respiratory diseases associated with influenza virus and showing bacterial infections of trachea, bronchi and lungs caused by coagulase-positive *Staphylococcus aureus*	33
VI	Rapid tentative postmortem diagnosis of influenza through cytological preparations of the tracheal epithelium	48
VII	The cytology of the sputum of patients suffering from influenza	50
VIII	Histopathology of bronchiectatic lung lobes surgically removed from patients having suffered recently from influenza virus infection	53
IX	Histopathology of the respiratory tract epithelium in fatal cases of pandemic influenza in 1918	58
X	Histopathology of the respiratory tract in cases showing bacterial infections of the trachea, bronchi, and lungs associated with measles and varicella viruses	62
	Summary and conclusions	67

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INTRODUCTION

Histopathological examinations of changes of the epithelium of the human respiratory tract due to influenza virus infection in chronological relation to duration of illness and secondary bacterial invasion have been reported only twice (Straub and Mulder (1948) and Mulder and Verdonk (1949)). Accurate studies regarding the nature, extent, and spreading of the changes in the tracheo-bronchial tree are almost totally lacking. Also conspicuously absent are controlled virological studies definitely proving the respiratory tract alterations involved to be remnants of an influenza virus infection. Comparative studies of the influenza-induced alterations to the epithelium in fatally stricken victims of recent epidemics with those of the pandemic of 1918 have, at the present writing, not appeared in the literature.

An important question in this connection is whether it is indeed possible to demonstrate specific influenza virus lesions in the human respiratory tract, and further whether other epitheliotropic virus, *e.g.*, the measles virus, are capable of inducing the same surface changes to the mucosa of the respiratory passages. There are undoubtedly great possibilities here for electron microscopy with respect to localisation of the virus in the respiratory tract epithelium and conceivably in the lung.

Study of the histopathology in human respiratory tract epithelium following influenza is severely complicated, however, by the fact that a fatal course is almost invariably accompanied by secondary bacterial infection of the respiratory tract and lungs. Pyogenic cocci are the most frequent offenders in such cases, therefore the histological picture is composed of viral and bacteriotoxic lesions of the mucosa. It is self-evident that differentiation of the two is not without difficulty.

The majority of the patients from whom the respiratory passages and lungs have been analysed at the present writing had succumbed to *Staphylococcus aureus* pneumonias. The extensive necrotizing and purulent inflammation which arises in the respiratory passages hereby is never overlooked. However, it is important to investigate whether the epithelium of the respiratory tract exhibits, in addition to these bacteriological lesions, others which could be caused by the influenza

virus alone. Investigation must therefore be directed above all toward cases in which secondary infection in the respiratory tract is due to pathogenic micro-organisms other than *Staphylococcus aureus* and which, as control cases teach, do not necrotize the epithelium.
After the discovery of the influenza virus in 1933 it was especially the advances of experimental pathology that necessitated a review of the histopathology and therewith of the histopathogenesis of influenza virus infections in the respiratory tract in man.

The Histopathology of Influenza in Experimental Animals (literature table I)
The pathology of experimental influenza has revealed that influenza virus A after adaptation to the test animal (pig, mouse, ferret, or chick embryo) causes certain lesions of the ciliated epithelium of the nasopharynx, trachea, and bronchi, but no investigator has performed an accurate study as to the depths to which these changes descend in the bronchial tree. The lesions themselves are very well-known histopathologically. After two hours one can already observe a clear degeneration and necrosis of the ciliated epithelium. The basal cell layer remains intact, however, and it is from the latter that the epithelium regenerates after desquamation of the necrotic superficial cells. The first signs of regeneration are seen on the fourth day after inoculation. About the fourteenth day the air passages are lined by high stratified squamous epithelium. One month after infection the epithelium is again wholly replaced by normal ciliated epithelium. The explanation for this epithelial necrosis must be sought in intracellular multiplication of the influenza virus. Harford and Hamlin (1952), on the basis of a specific technique and the study of ciliary movements, are of the opinion that influenza virus does not destroy the ciliated epithelium of the respiratory tract in the mouse. The illustrations in their publication, however, are not very convincing. Concerning pathogenesis of the fatally progressing haemorrhagic oedematous pneumonia which can develop in mice and ferrets after intranasal inoculation with lung-adapted influenza virus strains, opinions differ widely. Most investigators believe on the basis of the haemorrhagic oedematous aspect and the peribronchial and interstitial lymphocytic infiltrates that this pneumonia is an influenza virus pneumonia. Correspondingly little is sure regarding the development of the adaptation process of human influenza virus to the lung tissue of the experimental animal. The photographic electron microscopic study of influenza virus pneumonia in the mouse by Eddy and

Wyckoff (1950) makes it probable that the lung-adapted virus destroys alveolar epithelium.

The Histopathology of Influenza in Man (literature table II)

The macroscopic anatomy of the respiratory tract and lung tissue in influenzal pneumonia has been well known since antiquity. The oldest descriptions were those of Sydenham (1675) and Morgagni (1761), but only in 1837 was the granular surface of the respiratory tract first recognized by Nonat as typical of influenza. During the pandemics of 1890 and 1918 the microscopic changes were described as a necrotizing purulent inflammation of the mucosa accompanied by severe hyperaemia and haemorrhages in the tunica propria. According to the bent of the investigators one spoke of necrotic purulent, fibrino-necrotizing, croupous or phlegmonous inflammation as typical for influenza. The fact that the necrotic, fibrin rich, and purulent coating was partially free of the surface, but locally adherent to the tunica propria caused further discussion of a pseudomembranous inflammation. Lubarsch (1918) and Kaufmann (1922) pointed out the great difference from the pseudomembranous inflammation in diphtheria. Nevertheless it appears from the microscopic descriptions of this period that a few investigators had noted epithelial lesions in the respiratory tract in addition to the necrotizing fibrino-purulent inflammation. These, rather than the necrotizing and purulent inflammations, were conceived by them to be specific for influenza without benefit of the knowledge that an intracellularly proliferating virus should be held responsible. The lesions were described by them as occurring at every level of the respiratory tract and involving the mucosa of the nose and trachea as well as that of the large and small bronchi. They laid emphasis on the superficial necrosis and desquamation of the ciliated epithelium as well as on the regenerative changes whereby the epithelium eventually came to be called "metaplastically" altered. There was even mention of a "metaplastic catarrh" as typical for influenza (Widal (1899) and Askanazy (1919)).

It is striking that in the publications subsequent to 1933, the year in which Smith, Andrewes, and Laidlaw discovered the influenza virus, no detailed histopathological investigation was reported. Only in 1947, 1948, 1949, and 1951 were respiratory tract changes found in virologically controlled interpandemic pneumonias with influenza which strongly resembled the earlier described epithelial degenerations and regenerations from the pandemic of 1918 and which corresponded with

lesions in the test animal. It was remarkable that the degenerations involved exclusively the ciliated and goblet cells, the basal cell layer remaining intact. The emphasis in those studies fell upon the trachea and large bronchi as localisation of the lesions. Detailed studies as to the extent over which these spread in the bronchial tree are lacking. The overall impression was that the epithelium of the smaller bronchi and bronchioli remains spared. It is consistently evident, however, that some doubt prevailed as to whether or not the epithelial lesions were of bacteriotoxic origin.

Through the publications of Pfeiffer (1893) in which *Haemophilus influenzae* was regarded as the instigator of influenza (despite the criticism in 1900 by Rosenthal in France and later from German and Anglo-Saxon investigators, and also the fact that Nicolle and Lebailly (1919) and Fejes (1919) demonstrated on monkeys the viral aetiology of influenza), there arose about 1918 great confusion which also extended over into pathological anatomy. This is the reason that publications appear in the influenza literature in which neither epithelial necrosis or regenerations nor necrotizing fibrino-purulent inflammations are described and which probably must be regarded as primary bacterial infections of the respiratory tract caused by *Haemophilus influenzae*. In addition to "metaplasia", the concept "catarrhal inflammation" also made its debut into the influenza literature.

We have serious objections to the use of either term in influenzal pathology as typical for this disease. If one confines himself to the definition and signification which since Virchow have been attached to "metaplasia" and especially to "metaplastic" bronchial epithelium, it is patently inapplicable to the regenerative changes which arise in an influenza virus infection since no permanent morphologic or functional transformation has been demonstrated in humans or observed in experimental animals. The term "catarrhal inflammation" refers only to a mucosal inflammation whereby an exudate appears "freely" on the mucosal surface (Tendeloo, 1938); it says nothing as to the nature, pathogenesis, or aetiology of the inflammation nor about the nature or composition of the exudate. Both terms shall therefore be avoided. To forestall any misunderstanding, it is hereby stated that any further mention in this study of influenza without further qualification will signify infection by the influenza virus established by virological methods except for the cases of 1918.

TABLE I

TABULATED SURVEY OF THE FOREMOST HISTOPATHOLOGICAL LITERATURE REGARDING EXPERIMENTAL INFLUENZA VIRUS INFECTION OF TEST ANIMALS

a. *Necrosis and "Metaplasia" of Surface Epithelium in the Respiratory Tract*

Author	Year	Test Animal
Shope	(1931)	Pig
Straub	(1937 and 1940)	Mouse
Francis, and Stuart-Harris	(1938)	Ferret
Dal	(1938)	Mouse
Nelson, and Oliphant	(1939)	Mouse
de Balogh	(1939)	Mouse
Behr, and Hadders	(1940)	Mouse
Burnet	(1940)	Chick embryo
Loosli	(1949)	Mouse
Wirth	(1950)	Chick embryo
van Tongeren	(1951)	Mouse
Westwood	(1952)	Chick embryo
Harford, and Hamlin	(1952)	Mouse

b. *Haemorrhagic-oedematous pneumonia*

Author	Year	Test Animal
Francis	(1935)	Ferret
Laidlaw	(1935)	Ferret
Brightman	(1936)	Mouse
Smith	(1937)	Ferret
Bieling, and Oelrichts	(1938)	Mouse
de Balogh	(1939)	Mouse
Nelson, and Oliphant	(1939)	Mouse
Straub	(1940)	Mouse
Behr, and Hadders	(1940)	Mouse
Taylor	(1941)	Mouse
Hoyle, and Orr	(1945)	Mouse
Loosli	(1949)	Mouse
Eddy, and Wykoff	(1950)	Mouse
van Tongeren	(1951)	Mouse

TABLE II

TABULATED SURVEY OF THE FOREMOST PATHO-ANATOMICAL, BACTERIOLOGICAL, AND HISTOPATHOLOGICAL LITERATURE OF INFLUENZA IN HUMANS

a. *Necrosis and "Metaplasia" of Surface layer of the epithelium in the Respiratory Tract*

Author	Year	Localisation
Widal	(1899)	Nose, trachea, and bronchi
Berblinger	(1918)	Trachea and bronchi
Askanazy	(1919)	Trachea and bronchi
MacCallum	(1919 and 1921)	Bronchi
Wätjen	(1919 and 1921)	Trachea and bronchi
Schmidtmann	(1920)	Bronchi and bronchioli
Winternitz *et al.*	(1920)	Trachea, bronchi, and bronchioli
Jaffé	(1927)	Bronchi
van Bruggen *et al.*	(1947)	Large bronchi
Straub, and Mulder	(1948)	Trachea and bronchi
Mulder, and Verdonk. . . .	(1949)	Trachea and bronchi
Hers, and Mulder	(1951)	Trachea and bronchi

b. *Fibrino-purulent inflammation of the respiratory tract with haemorrhagic purulent bronchopneumonias with special references to the staphylococcus aureus group*

Sydenham (1675), Morgagni (1761), Nonat (1837), Kundrat (1890), Ribbert (1890), Finkler (1890), Netter (1892), Leichtenstern (1896), Fraenkel (1918), Oberndorfer (1918), Lubarsch (1918), Stettner (1918), Borst (1918), Fischer (1918), Dietrich (1918), Goldschmid (1918), Hart (1915 and 1918), Gruber and Schädel (1918), von Hansemann (1918), Hannemann (1919), Marchand (1919), Meyer (1919), Walker (1919), Wätjen (1919 and 1921), Chickering and Park (1919), Opie *et al.* (1919), Stone and Swift (1919), MacCallum (1919 and 1921), Askanazy (1919), Versé (1918 and 1920), Koopman (1920), Winternitz *et al.* (1920), McIntosh (1922), Patrick (1923), Jaffé (1927), Habbe (1929), McCordock and Muckenfuss (1933), Scadding (1937), Stuart-Harris *et al.* (1938), Stokes and Wolman (1940), Finland *et al.* (1942), Solomon and Kalkstein (1943), Himmelweit (1943), Wollenman and Finland (1943), Parker *et al.* (1946), Burnet, Stone, and Anderson (1946), van Bruggen *et al.* (1947 and 1950), Straub and Mulder (1948), Mulder and Verdonk (1949), Maxwell *et al.* (1949), Bruins Slot (1950), Stuart-Harris *et al.* (1949 and 1950), Gibson and Belcher (1951), Hers and Mulder (1951), Tyrrell (1952), Stuart-Harris (1953), Escher and Löffler (1954).

c. *Hyperaemic and haemorrhagic pneumonia without fibrino-purulent or necrotic membranes in the respiratory tract with special references to bacterial groups other than staphylococcus aureus*

Mojon (1803), Nonat (1837), Duflocq (1890), Kundrat (1890), Weichselbaum (1890), Oberndorfer (1918), Lubarsch (1918), Mandelbaum (1918), Simmonds (1918), Berblinger (1918), Dietrich (1918), Fischer (1918), Borst (1918), Hannemann (1918), Lyon (1919), Goodpasture (1919), Kinsella (1919), LeCount (1919), Lord *et al.* (1919), Opie *et al.* (1919), Walker (1919), MacCallum (1919), Winternitz (1920), Wätjen (1921), Brannan and Goodpasture (1924), Parker *et al.* (1946), Maxwell *et al.* (1949), Stuart-Harris *et al.* (1949), Hers and Mulder (1951), Stuart-Harris (1953).

Chapter I

REMARKS ON HISTOLOGY OF THE TRACHEO-BRONCHIAL TREE IN GENERAL

The large variations possible and the gradual transitions from one type of bronchus into another make every classification more or less fictitious. Policard and Galy (1945), later followed by Engel (1950) and von Hayek (1953), eschew a rigid schema. In accordance with Policard we shall categorize the trachea and bronchi as that part of the bronchial tree equipped with cartilage and mucous glands, and bronchioli as that part of the respiratory tract in which no cartilage or mucous glands are present and which can be assumed to lie intralobularly. By limiting discussion to segmental bronchi and, within the lobule, distinguishing bronchioli and respiratory bronchioli, a subdivision is acquired which approximates a histologically and anatomically trustworthy localisation and determination of niveau. It should be kept in mind, however, that the transition from bronchus to bronchiolus occurs gradually, is extremely variable, and is therefore often hard to detect. So cartilage-containing bronchioli and cartilage-free bronchi are no rarities. The typical folding of the mucosa and the absence of mucous glands can aid in delimiting the bronchioli.

Accompanying acute inflammations of the bronchi dilatation of the bronchioli is the rule, so that the folding of the mucosa is not encountered. But by performing serial sections from the respiratory bronchioli toward the hilus one is always able to distinguish with reasonable assurance whether one has to do with a small bronchus or a cartilage-containing bronchiole. Measurement of the diameter of the bronchial lumina or the thickness of the bronchial wall is of no avail since inflammatory exudate can cause great changes in the diameter and mural cross-section.

The normal lining of the respiratory tract is formed by stratified columnar mucus-producing ciliated epithelium, which is affixed to the basement membrane. The epithelium decreases in height corresponding with the calibre of the bronchus and gradually becomes bilaminar and finally simple columnar epithelium in the bronchioli. This lining is comprised of three different types of cells, namely the superficial

cylindrical mucus-producing ciliated cells, the more deeply situated intermediary cells, and the basal cells as the germinative layer. In the basal cell layer an occasional mitosis is encountered. We view it as erroneous to assume the existence of mucus-producing cells apart from the ciliated cells. We are of the opinion that mucus production is a potential property of the ciliated cells and that they are thus capable of assuming the "goblet" form. The number of mucus-producing cells declines proportionally with the calibre of the passage. A bronchiole has only a few mucus-producing cells; toward the periphery the mucus production disappears entirely and there appear high cylindrical cells devoid of cilia. The respiratory bronchioli are ultimately lined mainly with cubical epithelium (derived either from the intermediary cells, from the basal cells, or from the alveolar cells) among which occasional cylindrical ciliated cells are to be found. The variations in epithelial lining are extreme in these peripheral areas. Clara (1936 and 1937) has reported a fine study of this subject. The mucus production in these regions is nihil, although Clara has reported cells with "secretory" granules as far peripherally as the alveolar ducts. Also, von Hayek (1953) indicates the existence of such cells and considers the granules to be mucus since they become bright red with the Schiff reagent. We have also encountered these cells, but hold that the granules concerned consist not of mucin but of a mucoid which is capable of a positive Schiff reaction. They are also stainable with phloxin and furthermore were not found in all cases but rather predominately in asthmatic patients, in whom a formidable eosinophilia was found in the bronchial wall.

There is little known about the degenerative and regenerative processes in general of the ciliated respiratory tract epithelium. Winternitz (1919 and 1920) and Brandt (1926) describe the epithelial regenerations in man and in animals after inhalation of war-gases. Experimental studies after mechanical lesions of the nasal mucosa (lamb) are reported by Boling (1935) and of the tracheal mucosa (rat) by Condon (1941) and Wilhelm (1953). It is the latter, especially, who describes the regeneration accurately with respect to time elapsed. In all these studies mention is made of a temporary squamous reaction of the regenerating epithelium which is sometimes described as "metaplasia". Vitamin A deficiency also causes "metaplastic" changes in the bronchial epithelium in the rat (Passey, Leese, and Knox (1936)). However, most of the experimental studies with animals in which the epithelial pictures are described accurately with respect to duration of illness, are found in influenza pathology.

"Metaplasia" of the bronchial epithelium in man occurs frequently and is encountered in the large as well as in the small bronchi and bronchioli. It is described with chronic inflammations (Hers (1951)), bronchiectases (Siegmund (1922), Krompecher (1924), Brandt (1926), Robinson (1933 and 1939), Engel (1950), Whitwell (1952), and Mulder and Hers (1954)), and tuberculosis (Kitamura (1907)) as well as with acute respiratory tract infections, influenza, measles, and pertussis. In the latter it is especially the respiratory bronchioli which show "metaplastic" epithelial changes. Foreign body aspirations can also cause "metaplasia" of the bronchial epithelium, which can or must be preceded by an ulceration of the wall. Mulder and Hers (1954) point out the fact that "metaplasia" is not predominantly encountered at the carinas as has been reported in the literature (von Möllendorff (1940), Bauer (1949), von Hayek (1953)).

Various histological types are distinguished:

1) True "metaplasia" to squamous epithelium consisting of a superficial cell layer with keratinisation, stratum granulosum, and papillae and lacking a basement membrane.
2) "Metaplasia" with flattened surface cells and pyknotic nuclei in which no keratinisation appears, loss of cilia and mucus production, and maintenance of basement membrane without papilla formation.
3) "Metaplasia" in which islands of squamous cells without keratinisation are encountered to the depth of the basal cell layer amid normal ciliated epithelium. These islands may, on their borders, still be covered with normal ciliated epithelium. Here also the basement membrane is still present.

Nothing is known with certainty of the existence of "metaplasia" in man; Teutschlaender (1919) sees every metaplasia as a stage in regeneration of the bronchial epithelium. Lubarsch (1918), Wätjen (1921), and Brandt (1926), on the contrary, see in each form of "metaplasia" a dedifferentiation as a consequence, respectively, of stagnation of the secretion, dessication, or irritation with chemical substances, *e.g.*, tar products and tobacco.

Chapter II

METHODS AND MATERIALS

Postmortem Technique

Autopsy of confirmed or suspected cases of fatal influenza was performed as quickly as possible to facilitate bacteriological and virological investigation and to preclude sloughing off through postmortem autolysis of the respiratory tract epithelium. Prior to opening the thorax the abdominal organs were removed. Contamination of the lungs with blood and intestinal flora was prevented by proximal and distal clamping of all the great vessels and the digestive tract before transection. Lungs, trachea, and heart were then easy to remove *en bloc*. The lungs were cut (with a sterile knife) in four slabs as nearly perpendicular to the bronchi as possible.

A bacteriological examination was done on exudate expressed from the cross-sections from the lungs and bronchi or bronchioli. A culture of heart blood was included in the autopsy of each patient. A section of tracheal cylinder bordering on the bifurcation and about $1\frac{1}{2}$ c.m. in length was excised sterile for virological, bacteriological, and cytological examination. Small pieces of lung tissue were cut from each pulmonary lobe for virological investigation. The trachea and bronchi were not sectioned but rather fixed *in toto* in 10 % formalin. Berblinger (1918), Askanazy (1919), Wätjen (1921), and Straub (1948) point out that only in this fashion can artefacts of the respiratory tract epithelium be avoided.

Bacteriological Technique

In cases where therapeutic penicillin had been administered, penicillinase was added to the culture media. All specimens were cultured on blood agar, Levinthal agar, and in human plasma broth. Mouse-inoculations were performed only in two of the twenty cases examined.

All the isolated strains of *Staphylococcus aureus* were coagulase positive. The strains were phage-typed by the Central Public Health Laboratory, Colindale Avenue, London through the kind help of Dr. R. E. O. Williams. Isolated strains of Pneumococci were typed with type-specific sera from the Statens Serum Institute, Copenhagen (Dr. E.

Lund). Strains of non-encapsulated *H. influenzae* were identified by subculture in proteose broth (Difco) with haematin and cozymase (extracted from yeast). None of the colonies of the isolated strains showed diffraction of strong artificial light on transparent Levinthal agar and none showed slide agglutination with any of the type-specific antisera a-f (kindly sent to us by Miss Margaret Pittman, National Institutes of Health, Washington). All isolated strains of pathogens were freeze-dried to facilitate studies after subsidence of the influenza epidemics under study.

Virological Technique

Cylindrical portions of trachea and pieces of lung tissue were preserved at — 130° C. The rings of trachea were cut open after thawing and the mucosa scraped off with a sharp spoon. Weighed pieces (1 gm.) of lung tissue were finely ground with sterile sand, then emulsified with 10 ml. phosphate buffer (*p*H 7.0) to which had been added penicillin (500 U/ml.), streptomycin (500 U/ml.), and sulfamethylpyrimidine (200 mg per 100 ml.). Ten thirteenday chick embryos were inoculated with the emulsion in the amnion (technique following Taylor and Chialvo (1942)). After three days incubation at 35° C the amniotic fluid was harvested and examined for influenza virus. Whenever no virus was thus encountered at the first passage, two to three amnion passages were done. All isolated strains were serologically classified with antisera from ferrets against strains from the influenza A and B group (van der Veen and Mulder (1950), Brans (1952)). All the strains isolated belonged to the A group and underwent a complete antigenic analysis in the Virus laboratory of the Department of Internal Medicine, Leiden.

Serological Technique

Blood was obtained during life and (or) by heart puncture during the autopsy. The blood serum was titrated (after treating with enzymes of crude filtrate of Vibrio cholera cultures in order to destroy non-specific inhibitors) for antibodies against strains of influenza virus as determined by Hirst (technique according to van der Veen and Mulder (1950)) and with the complement fixation test, using plain egg-fluids as antigens.

Cytological Technique

A sterile wire-loop was passed over the tracheal mucosa and the material removed suspended in three or four drops of physiologic saline on an object glass. A few drops of methylene blue were added to the suspension and the preparation surrounded with vaseline to prevent evaporation.

The wet methylene blue preparation thus created provides an excellent cytological picture of the tracheal epithelium. Other preparations were made by streaking the material out on an object glass, then either drying and later staining by the May-Grünwald-Giemsa method or fixing directly while wet in a mixture of equal parts of alcohol (96%)-aether and afterwards colouring with haematoxylin-eosin according to the method of McDonald and Woolner (1947).

Histological Technique

After several days fixation each slice of lung tissue was split into two thinner slices as nearly perpendicular to the bronchi as possible. Thereafter, to exclude lesion of the bronchial epithelium small samples of lung tissue were excised from these slices with a very sharp knife for microscopical investigation. The excised samples were imbedded either in paraffin or in celloidin. Imbedding in celloidin consumed in total five weeks, during which time a very gradual dehydration was accomplished. In this manner, sloughing off of the diseased bronchial and tracheal epithelium and exudates through shriveling of the tissue was circumvented. It was necessary to decalcify the pieces of trachea from elderly patients since the cartilaginous rings sometimes contained large amounts of calcium and occasionally were even ossified. The decalcification was achieved electrolytically by the methods of Richman, Gelfand, and Hill (1947), by dissolving in formic acid, or with nitric acid. Occasionally a combination of the three methods was necessary to attain complete decalcification.

A thorough examination of larynx and trachea was executed by transecting both throughout their entire length into rings three to four mm. thick. In the majority of the cases every second trachea ring was examined, in certain cases all rings. The number of investigated trachea rings comprised about sixteen, the number of lung tissue, bronchial, and bronchiolar samples approximately fifty per case. Further, one piece of lung tissue or bronchus from each case, depending upon the microscopic picture, was examined in serial section; in most of the series each tenth slice was investigated; in a few cases an entire series was scrutinized. As a general rule, from each imbedded tissue block, serial sections were stained consecutively by several mucus staining methods — muco carmine, periodic acid-Schiff reaction (Hotchkiss (1948), McManus (1952)), and toluidine blue. Other serial sections were stained by haematoxylin-eosin, phloxin-tartrazine (according to Lendrum (1947)), the bi-colour method for D.N.A. and polysaccharides (acid

hydrolysis-thionine SO_2-P.A.S.) according to van Duijn *et al.* (1954) and the methylgreen-pyronin method for R.N.A. according to Trevan (1951). Pretreatment of the sections were done with a ribonuclease-extract according to Gomori (1952). Elastin and connective tissue were coloured according to the method of Weigert and Van Gieson. Silver impregnation of the slices were performed according to Bodian (1936 and 1937). Bacterial stainings in the tissue slices were done with Löffler's methylene blue and the Gram-Weigert staining method according to MacCallum (1919). The rectangular paraffin blocks were sliced in turn from each of the four sides of the presenting face. In all four consecutive slices the bacteria occupied the same relative position in the tissue. Care was taken that the same bronchus or bronchiole was examined both histologically and bacteriologically.

Documentation

All bacteriological and virological examinations were systematically described as to composition of the culture media and the anatomical localization of the specimen. Each lung was photographed after fixation *in toto* and in cross-section, whereby the lung slices were laid open from cranial to caudal end. The trachea was also photographed and thereafter the tissue blocks were cut out and sketched into their corresponding locations (Plate I, II, and III). By transposing these excised and photographically indicated regions to a trachea-bronchus schema, the microscopical examination was anatomically localized. The branches of the bronchial tree have been indicated by Anglo-Saxon nomenclature, currently employed in The Netherlands.

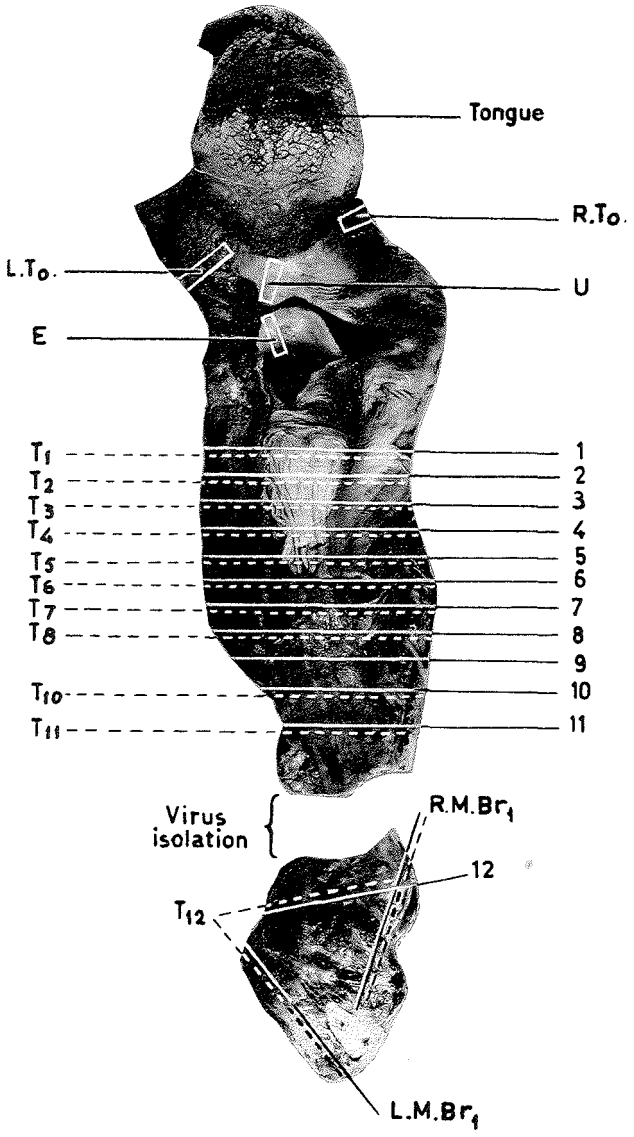


Plate I. Trachea and larynx (Case No. 5, F. 69) with histologically investigated niveaux designated. Arc points out the direction in which the histological sections of the trachea were sliced. R.To. = right tonsil; L.To. = left tonsil; U = uvula; E = epiglottis; R.M.Br. = right main bronchus; L.M.Br. = left main bronchus.

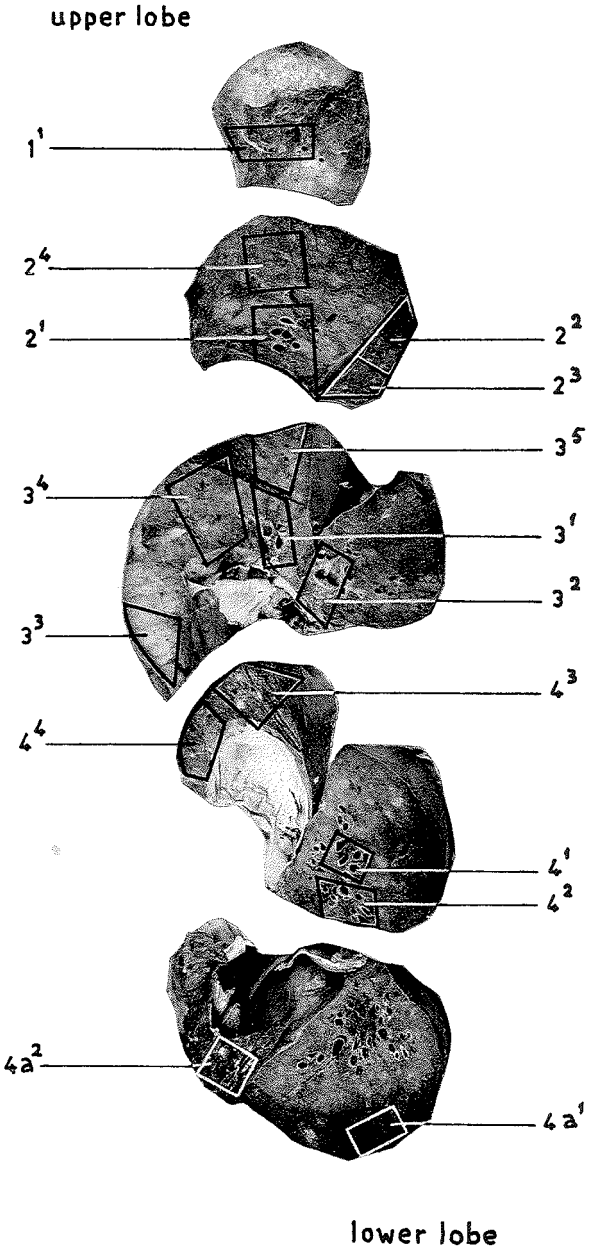


Plate II. Transverse sections from left lung (Case No. 5, F. 69) in which are designated the bronchi and bronchioli to be investigated histologically.

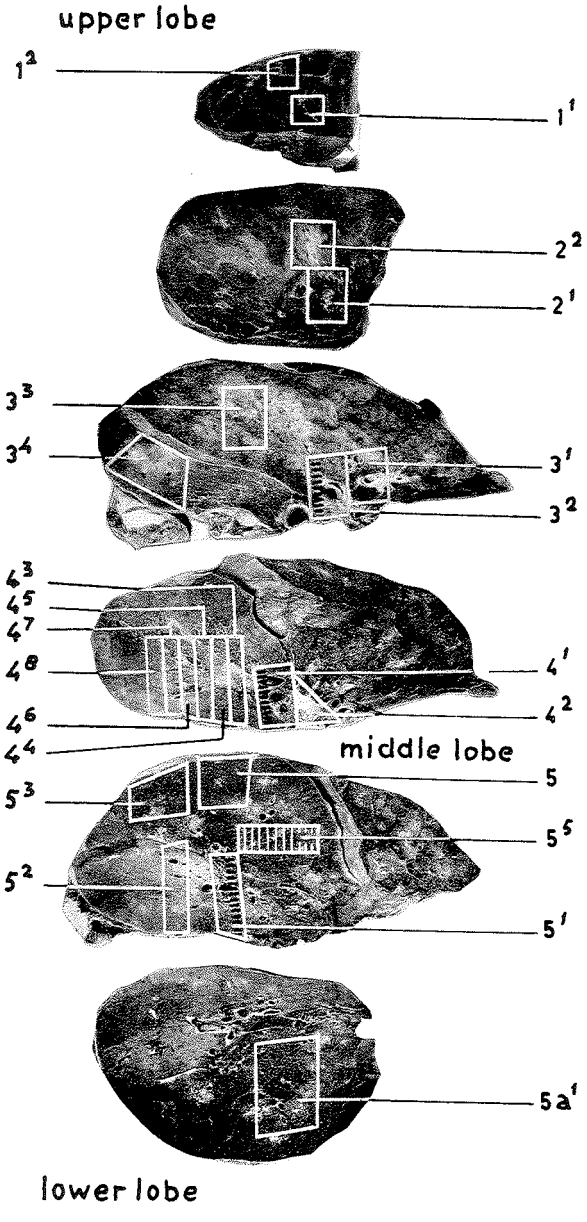


Plate III. Transverse sections from right lung (Case No. 5, F. 69) with histologically investigated niveaux of the bronchial tree specified. Arc points out the direction in which the histological sections were sliced.

Chapter III

HISTOPATHOLOGY OF ACUTE CASES OF BRONCHO-BRONCHIOLITIS AND CASES OF PNEUMONIA NOT ASSOCIATED WITH INFLUENZA-VIRUS BUT SHOWING BACTERIAL INFECTION OF TRACHEA, BRONCHI, AND (OR) LUNGS OTHER THAN *STAPHYLOCOCCUS AUREUS*

Four cases were available for investigation.

No. 1, Case History (63/1949, Leiden): Patient C. J. v. D., woman, 71 years of age

Clinical Diagnosis: Myocardial infarction(?); capillary bronchitis(?); influenza(?)

Duration of Illness: 7 days

Clinical Observation: several hours

Laboratory Data: Bacteriological examination of the sputum: Non-encapsulated *H. influenzae;* number of leucocytes in the peripheral blood: 24,900; eosinophile leucocytes: 0

Antibacterial Treatment: 800.000 U. penicillin totally

Autopsy: (11/1949, Leiden) 1½ hours after death, 13th January, 1949

Postmortem Diagnosis: slight acute tracheitis; muco-purulent bronchitis and bronchiolitis with peribronchitis in right upper, middle, and lower lobes; vesicular emphysema bilaterally; fresh ischaemic necrosis and old fibrous scars in the posterior wall of the left ventricle; hypertrophy of the left and right ventricle with steatosis of the cardiac muscle fibres; lipomatosis of the heart; acute splenitis; extensive arteriosclerosis; no adrenal haemorrhages

Bacteriology and Virology: Blood culture P.M.: sterile. From the trachea and the bronchi of both left and right lungs non-encapsulated *H. influenzae* was cultivated in pure culture. It was impossible to isolate influenza virus from the tracheal mucosa or lungs in chick embryos. Results of the Hirst test were as follows. The blood serum possessed no antibodies against influenza virus A -PR_8 (1934, U.S.A.), FM_1 (1947, U.S.A.) or B-Bon (1943, Australia), all titres being lower than 12, and titred only 160 against influenza C-1233 (1947, U.S.A.). All three were in conformity with the complement fixation test.

No. 2, Case History (138/1951, Leiden): Patient E. S. v. d. B., woman, 52 years of age

Clinical Diagnosis: Capillary bronchitis; bronchial asthma; bilateral bronchiectases; fibrosis of the left lung; cor pulmonale

Duration of Illness: 13 days

Clinical Observation: several hours

Laboratory Data: Bacteriological examination of the sputum: Non-encapsulated *H. influenzae;* number of leucocytes in the peripheral blood: 19,700; eosinophile leucocytes: 0

Antibacterial Treatment: 800.000 U. penicillin totally

Autopsy: (33/1951, Leiden) 5 hours after death, 24th January, 1951

Postmortem Diagnosis: Slight acute tracheitis; muco-purulent bronchitis and bronchiolitis in both right and left lungs; emphysema of the right lung; fibrosis of the left lung; fibrous pleuritis bilaterally; small cylindrical bronchiectases bilaterally; hypertrophy and dilatation of the right ventricle; no adrenal haemorrhages

Bacteriology and Virology: Blood culture P.M.: sterile. Non-encapsulated *H. influenzae* and Pneumococcus type 21 were cultivated from trachea as well as bronchi of both left and right lungs. No influenza virus was isolable in chick embryos from tracheal mucosa or lungs. The blood serum contained no antibodies against influenza virus A-PR_8 (1934, U.S.A.), FM_1 (1947, U.S.A.) or B-Bon (1943, Australia) all titres being lower than 12 in conformity with the complement fixation test.

No. 3, Case History (1457/1951, Leiden): Patient J. B. B., man, 57 years of age

Clinical Diagnosis: Lobar pneumonia of right upper lobe; capillary bronchitis; bronchial asthma; emphysema; cor pulmonale

Duration of Illness: 14 days

Clinical Observation: 1 day

Laboratory Data: Bacteriological examination of the sputum: Non-encapsulated *H. influenzae;* number of leucocytes in the peripheral blood: 13,300; eosinophile leucocytes: 0

Antibacterial Treatment: 4.000.000 U. penicillin totally

Autopsy: (284/1951, Leiden) 3 hours after death, 10th November, 1951

Postmortem Diagnosis: Squamous cell carcinoma with liquefaction and post-stenotic atelectasis in the right upper lobe; acute muco-purulent tracheitis; muco-purulent bronchitis and bronchiolitis with peribronchitis throughout both lungs; vesicular emphysema bilaterally; fibrous pleuritis of the right upper lobe; hypertrophy and dilatation of the right ventricle; acute splenitis; no adrenal haemorrhages

Bacteriology and Virology: Blood culture P.M.: sterile. Non-encapsulated *H. influenzae* and Pneumococcus type 11 were cultivated from trachea as well as bronchi of both left and right lungs. No influenza virus was isolable in chick embryos from tracheal mucosa or lungs. The blood serum contained no antibodies against influenza virus A-PR_8 (1934, U.S.A.), FM_1 (1947, U.S.A.) or B-Bon (1943, Australia), all titres being lower than 12 in conformity with the complement fixation test.

No. 4, Case History (206/1949, Leiden): Patient G. v. d. H., woman, 76 years of age

Clinical Diagnosis: Lobar pneumonia and confluent areas of bronchopneumonia in the left lung.

Duration of Illness: 8 days

Clinical Observation: several hours

Laboratory Data: Bacteriological examination of the sputum: Pneumococcus type 3; number of leucocytes in the peripheral blood: 18,000; eosinophile leucocytes: 0

Antibacterial Treatment: 400.000 U. penicillin totally

Autopsy: (32/1949, Leiden): 1½ hours after death, 10th February, 1949

Postmortem Diagnosis: no tracheitis; slight acute bronchitis left; purulent bronchitis right; lobar fibrinous pneumonia of the left upper lobe; atelectasis of the left lower lobe; vesicular emphysema of the right upper lobe; lipomatosis of the heart; dilatation of the right ventricle; kyphoscoliosis of the spinal column at the levels of Th. III and Th. IV; no adrenal haemorrhages

Bacteriology and Virology: Blood culture P.M.: Pneumococcus type 3. Pneumococcus type 3 was cultivated in pure culture from trachea and bronchi as well as from the lung tissue of the left upper lobe. No influenza virus was isolable in chick embryos from tracheal mucosa or lungs. The blood serum contained no antibodies against influenza virus A-PR_8 (1934, U.S.A.), FM_1 (1947, U.S.A.) or B-Bon (1943, Australia), all titres being lower than 12 in conformity with the complement fixation test.

Histopathology of the four above-described cases

The Cases Number 1, 2, and 3 all showed in histological sections the familiar picture of muco-purulent tracheo-bronchitis, bronchiolitis and peribronchiolitis characteristic of acute *H. influenzae* infection (Hers and Mulder (1953)). The epithelium was fully intact throughout the entire length of the respiratory tract despite the often very dense leucocytic infiltration of the epithelium and tunica propria. There was no extreme hyperaemia in the tunica propria (Fig. 1, 2, and 3). The lumina of the bronchi were filled with a leucocytic mucous exudate. In addition to the leucocytic infiltration many lymphocytes were found in the tunica propria and peribronchial tissues, frequently situated in follicles with well-defined germinative centers. The interstitial lung tissue was drastically fibrosed in Case No. 2, predominantly in the left lung. The mucosa of the bronchiectases occurring here also had normal ciliated epithelium. In none of these cases pneumonic infiltrations were found. In Case No. 4 the epithelium of the air passages was completely intact. The leucocytic infiltration of the epithelium and tunica propria in the trachea and main bronchi was relatively weaker than in Cases No. 1, 2, and 3 but very strong in the bronchi and bronchioli. The exudate in the lumina was serofibrinous leucocytic. The entire left upper lobe showed a typical fibrino-cellular pneumonia. The remaining lung lobes exhibited only a slight leucocytic bronchitis and bronchiolitis.

Bacterial stainings of the tissue slides revealed *H. influenzae* lying among the epithelial cells (Fig. 4*a* and *b*). Size, morphology and sometimes bipolar staining help to identify *H. influenzae* in the sections with reasonable certainty. They were found basally between the ciliated cells in the basement membrane and also deep to the latter in the tunica propria

and mucous glands (Fig. 5*a* and *b*). In the tunica propria they were lying sometimes adjacent to the blood capillaries and in the neighbourhood of mast cells. With Löffler's methylene blue the bacteria are stained often distinctly bipolar, a relatively rare staining phenomenon according to MacCallum. They occured singly as well as in small groups of about ten.

The pneumococci of Case No. 2 were also encountered in the ciliated epithelium and occurred only rarely in the tunica propria. The pneumococci of Case No. 4 were seen predominantly in the lung tissue and to a lesser degree between the epithelial cells and in the tunica propria of the bronchi. Encapsulation was often illustrated beautifully.

The bacteria and pneumococci were demonstrated at every level of the air passages, but were most clearly evident in the small bronchi and in bronchioli, in correspondence with the leucocytic, partly lymphocytic infiltrate.

Discussion

In the four cases concerned we have to do with bacterial inflammations of the respiratory passages and lungs not associated with influenza virus. They can, thus, possibly serve as primary bacterial inflammations, although the existence of another unknown virus as precursor is always feasible. The epithelium, despite the culturally and histologically demonstrated bacterial invasion (non-encapsulated *H. influenzae* and pneumococci), is fully intact in every case and at each niveau of the air passages. In the older literature deliberate emphasis is nowhere laid upon the presence of intact epithelium with *H. influenzae* infection. Pfeiffer (1893) sketched intact epithelium wherein lay *H. influenzae*, but failed further to describe the fact (Fig. 6). Hübschmann (1914, 1917, 1918, and 1919), who—as had Pfeiffer—considered *H. influenzae* the instigator of influenza, described desquamation of normal epithelium. Hübschmann, Olsen (1919), Levinthal (1921), and others spoke of a "catarrhal bronchitis". We regard this desquamation, however, as an artefact caused by postmortem autolysis. MacCallum (1921) also finds normal ciliated epithelium in pure Haemophilus bronchiolitis and points to the fact that the epithelium is damaged in influenza. The histopathology of pneumococcal pneumonia is, in contrast, much better known. The epithelium of the bronchi and bronchioles in this disease has been described by many writers as normal (Lauche (1928)).

Chapter IV

HISTOPATHOLOGY OF CASES OF RESPIRATORY DISEASES ASSOCIATED WITH INFLUENZA VIRUS COMPLICATED BY BACTERIAL INFECTION OF TRACHEA, BRONCHI AND LUNGS OTHER THAN *STAPHYLOCOCCUS AUREUS*

Five cases were available for examination.

No. 5, Case History (Psychiatric Institute "Endegeest" at Oegstgeest): Patient W. G. C. den H.-S., woman, 69 years of age

Clinical Diagnosis: Schizophrenia; influenza-pneumonia(?); infectious toxic shock

Duration of Illness: 3 days

Clinical Observation: 3 days

Laboratory Data: Number of leucocytes in the peripheral blood: 3,400; eosinophile leucocytes: 0; no bacteriological examination of the sputum; no antibacterial therapy

Autopsy: (37/1949, Leiden) 2 hours after death, 11th February, 1949

Postmortem Diagnosis: Hyperaemia of the submucosa of the trachea and bronchi without suppurative or fibrinous inflammation; muco-purulent bronchiolitis in both lower lobes; extensive haemorrhagic-oedematous focal pneumonic processes, partly confluent, and partial atelectasis in left and right lower lobes and right middle lobe; haemorrhagic peribronchial pneumonic foci in both upper lobes; sero-fibrino-haemorrhagic pleuritis right; emphysema of both lower lobes; dilatation of the right ventricle; brown atrophy of the remaining organs; no adrenal haemorrhage

Bacteriology and Virology: Blood culture P.M.: sterile. From the trachea, bronchi, and bronchioli of both lungs non-encapsulated *H. influenzae* and Pneumococcus type 3 were cultured. From the trachea and all lung lobes influenza virus A was isolated in chicken embryos (concentration 10^5 M.L.D.$_{50}$ per gram of lung tissue (right upper lobe and left lower lobe)). The blood serum contained no antibodies against influenza virus A-PR$_8$ (1934, U.S.A.), FM$_1$ (1947, U.S.A.) or B-Bon (1943, Australia), all titres being lower than 12, in conformity with the complement fixation test.

Histopathology:

The tonsils manifested a normal epithelial covering. In the crypts local ulcerative inflammations were found. The lymphadenoid tissue possessed many large lymph follicles with large germinative centres in which were encountered nuclear and cellular debris and occasional polymorphonu-

clear leucocytes. The reticular structure was largely disintegrated and replaced by connective tissue.

The uvula and posterior pharyngeal wall were coated with a non-cornified, squamous epithelium without epithelial defects. The loose connective tissue was slightly oedematous and showed no hyperaemia. Leucocytic infiltrates were not present. The number of lymphocytes was very small. No abnormalities were found in the capillaries.

The larynx, with the true and false vocal cords, was respectively coated with a completely intact, ciliated and squamous epithelium. Subepithelially, dense lymphocytic infiltrates and hyperaemia were encountered. Oedema was not found in this tissue, and leucocytic infiltrates were lacking.

The epithelium of the trachea, the bifurcation, the main bronchi, and the lobar and segmental bronchi all showed extensive superficial degenerative changes beginning at the vocal cords without ulcerative or fibrino-necrotizing inflammations (Fig. 7). The basal cell layer of the epithelium was intact at every investigated niveau.

The degenerative changes were limited exclusively to the mucus-producing ciliated cells and the intermediary cells. These degenerations were highly variable and very divergent in character. The initial degenerative changes consisted of a swelling of cells and nuclei. The longitudinal axis of the nuclei lay diagonally or even transversely in relation to that of the cells, giving rise to a very irregular structure. The mucus production was sharply decreased or entirely lacking. The cells became rounded and sometimes lay separated from each other, and cilia were further difficult to find (Fig. 8 and 9). The severely swollen nuclei were quite round and showed hyperchromatosis of the nuclear membrane. Sometimes, through cell membrane disintegration of the individual mucus-producing ciliated cells, a superficial syncytial structure was seen.

This degeneration led to desquamation of the superficial cell layer of the epithelium, whereby locally the intermediary cells also became involved (Fig. 10). Thus arose the typical pictures whereby the coating of the trachea and bronchi at many places consisted of the single layer of basal cells alone or accompanied by a layer of intermediary cells (Fig. 11). If only the basal cell layer was present, the cells were frequently flattened out. Where the layer of intermediary cells was still present, however, the latter were usually round. They formed thereby a nodular surface in contrast to the tense surfaces of the bared surviving basal cells. Mitoses were very seldom observed in the superficial degenerative layer but were very numerous in the remaining fragments of the basal

and intermediary cell layers. Remarkably, the various grades of the degenerative changes appeared focally in alternating sequence on the cartilagineous part as well as on the membranaceous part. Sometimes these changes stretched out over an extensive area; on other occasions they were strictly focal so that, not only from niveau to niveau but even at a single niveau, varied stages could be distinguished amidst normal mucus-producing ciliated epithelium. The transition from normal epithelium to superficial and minor degenerative changes could be very abrupt although gradually degenerative transitions were also observed. On several occasions small fields of disordered structure and vacuolization were encountered in normal ciliated epithelium in which there existed no hint of desquamation (Fig. 12). Gradual transformations were encountered where the lesion already had acquired a certain extent and the critical depth of desquamation, the basal cell layer, had locally been reached. On the borders of such an area all stadia of beginning oedema and swelling of cells and nuclei were distinguishable.

The tunica propria in the proximal parts of the respiratory tract sometimes showed a strong hyperaemia without haemorrhages. The infiltration was predominantly lymphocytic, polymorphonuclear leucocytes being only sparsely present. In places the cellular infiltration of the tunica propria was within normal range, but elsewhere definite oedema of the tunica propria appeared focally, mainly subepithelially between basement membrane and epithelium. The basement membrane acquired hereby a swollen and irregular aspect. The oedema, hyperaemia, and lymphocytic infiltration in the tunica propria increased peripherally as did the number of leucocytes, although dense leucocytic infiltrations were lacking. The mucous glands displayed a variable picture. Both cell types, muciferous and serous, were found but often demonstrated degenerative changes whereby loss of metachromasia or greater than normal metachromasia of the mucus was present. In the former case the cells showed a swelling of cells and nuclei and partial desquamation; in the latter case they showed signs of shrinkage with loss of cell borders and pyknosis of the nuclei, and the mucus in the glands was a homogeneous compact mass. The drainage passages of the mucous glands were of normal width, however the epithelial cells of these passages frequently showed the same degenerative changes as have been described for the tracheal epithelium.

The small bronchi and bronchioli also showed superficial degenerative changes of the epithelium (swelling of cell and nuclei, loss of mucus production and cilia). Here, too, the basal cell layer was not broken

at any investigated level (Fig. 13 and 14). The thin tunica propria showed strong hyperaemia and oedema. Haemorrhages were not found. The mucous glands displayed only minimal changes. The bronchioli were always greatly dilated whereby in some instances the folds of mucosa were fully distended. The focal character of the changes in the epithelium was remarkable at these points; serial sections revealed that many of the small bronchi and bronchioli showed absolutely no alteration, while at distal niveaux these changes were again clearly evident (Fig. 15). The epithelial alterations were completely independent of the lung changes. They appeared in completely normal, well-aerated lung tissue (Fig. 16) as well as in strongly leucocytic foci of bacteriogenic bronchopneumonia and haemorrhagic oedematous lung regions (Fig's 17, 18, and 19). The only difference was that in the latter an extreme leucocytic infiltration of tunica propria and epithelium was observed. No leucocytic infiltrate was to be found in normal well-aerated areas, and the bronchial lumina contained no exudate. In the strongly leucocytic inflammatory foci, nevertheless, the bronchi were filled with a muco-leucocytic exudate.

The respiratory bronchioli exhibited no epithelial degenerative changes. The high cylindrical and cuboidal epithelium, which showed neither mucus production nor cilia, were entirely intact. Fibrinoid necrosis of the epithelium was nowhere encountered. Hyperaemia and peri-bronchiolar cellular infiltration were of variable degree and associated with the presence of hyperaemia and oedema in the lung tissue or leucocytic foci of bacterial inflammation. A marked dilatation of the bronchioli was invariably encountered.

The lung tissue was extremely variable, in keeping with the macroscopic aspect. Especially in the upper lobes there appeared great areas in which the alveoli showed no peculiarities, although even here degenerative alterations of the epithelium of bronchi and bronchioli were observed. In the caudal lung lobes numerous sero-leucocytic broncho-pneumonic foci were present. Neither necrosis of the alveolar septa nor abscesses were encountered. Collateral and often in gradual transition to these foci were extensive oedematous lung areas with a striking hyperaemia of their capillaries and local haemorrhages. The oedema fluid was mainly serous; although locally a little fibrin was found. The capillary walls were locally swollen, but hyaline thrombi or necrosis of the alveolar septa were lacking. A cellular infiltration was not seen here, only a few lymphocytes being present.

Bacterial stainings of the tissue blocks revealed that *H. influenzae*, lying between the degenerated epithelial cells of the bronchi and

bronchioli, were present mainly against the basement membrane and in the tunica propria (Fig. 20). Pneumococci were found in the lung foci whereas *H. influenzae* were only sparsely to be seen. The bipolar staining of *H. influenzae* was clearly apparent with methylene blue.

No. 6, Case History (314/1953, Leiden): Patient M. v. d. Gr.-D., woman, 62 years of age

Clinical Diagnosis: Capillary bronchitis; influenza pneumonia(?); infectious toxic shock

Duration of Illness: 4 days

Clinical Observation: 1 day

Laboratory Data: Number of leucocytes in the peripheral blood: 8,000; eosinophile leucocytes: 0; no sputum

Antibacterial Treatment: 4×10^6 U. penicillin and $1\frac{1}{2}$ gram streptomycin in total

Autopsy: (45/1953, Leiden) 2 hours after death, 25th February, 1953

Postmortem Diagnosis: Muco-purulent tracheo-bronchitis and bronchiolitis with bronchopneumonia in the lower lobes; emphysema of both upper lobes; dilatation of left and right ventricles; scoliosis of the vertebral column; no adrenal haemorrhages

Bacteriology and Virology: Blood culture P.M.: sterile. Non-encapsulated *H. influenzae* and Pneumococcus type 15 were cultured from the trachea and bronchi of both lower lobes. Influenza virus A was isolated in chicken-embryos from the trachea and all lung lobes. The blood serum contained no antibodies against influenza virus A-PR_8 (1934, U.S.A.), FM_1 (1947, U.S.A.) or B-Bon (1943, Australia), all titres being lower than 12, in conformity with the complement fixation test.

Histopathology: Pharynx and larynx were not removed, therefore examination of the tonsils, uvula, and pharyngeal and laryngeal walls could not be performed.

The trachea, bifurcation, main bronchi, and lobar and segmental bronchi all portrayed the same histological picture of the epithelium as Case No. 5. Here, too, focal degeneration and necrobiosis of the superficial cell layer of ciliated epithelium had eliminated mucus production and cilia (Fig's 21, 22, and 23). A swelling of the nuclei and cells was invariably present. The cytoplasm and interstitial spaces of the epithelial cells in the methylgreen-pyronin method according to Trevan (1951) were more strongly pyroninophilic than in the control cases (Fig. 24). This pyroninophilia probably was caused by higher quantities of R.N.A. in the virus infected cells since it could be completely abolished by pretreatment of the sections with a ribonuclease-extract according to Gomori (1952). In the swollen nuclei with hyperchromatic nuclear membranes, numerous disks sometimes appeared which were barely coloured by haematoxylin. They were very weakly stained by eosin and

phloxin and were not pyroninophilic (Fig. 25). In the bi-colour method for D.N.A. and polysaccharides these disks were not coloured blue and therefore did not contain D.N.A. They probably may be considered nuclear degeneration products, possibly containing basic proteins. The basal cell layer, although often the only epithelial lining, was everywhere intact, yet here too the nuclei were often severely swollen (Fig. 26 and 27).

The tunica propria, in contrast to Case No. 5, revealed rather large focal haemorrhages in addition to hyperaemia and oedema. The oedema in the tunica propria was further developed than that in Case No. 5 and was present in particular under and above the basement membrane, so that the epithelium was locally upraised as a bulla. The oedema fluid contained no fibrin and was, just as the haemorrhages and hyperaemia, only locally present. The epithelial degenerations were not more strongly developed at sites of oedema, hyperaemia, or haemorrhages in the tunica propria than where these were lacking (Fig. 28). The leucocytic infiltration of epithelium and tunica propria consequent on secondary bacterial infection with Haemophilus and Pneumococcus type 15 was slight in the proximal parts of the respiratory tract but increased gradually in density peripherally, especially in the lower lobes, so that leucocytes were also found in the submucosa. The mucous glands and their excretory ducts often revealed the same epithelial changes as in the superficial epithelium of the trachea and bronchi and corresponded with those of Case No. 5.

The small bronchi and bronchioli were partly covered with a normal ciliated epithelium and partly showed the same superficial degenerative epithelial changes which often reached the basal cell layer (Fig. 29 and 30). The latter was found, however, at every niveau examined (Fig. 31). The thin tunica propria also failed to reveal any difference from Case No. 5. Hyperaemia as well as oedema was found therein, but these were not more severe or extensive. In the lower lobes dense leucocytic infiltration of the epithelium and tunica propria appeared locally in the bronchi and bronchioli with normal as well as in those with degenerative epithelial linings (Fig. 32 and 33). Furthermore, there were bronchioli, especially situated in the upper lobes, which displayed no leucocytic inflammation of epithelium or tunica propria, but nevertheless had unmistakable epithelial degenerations.

The respiratory bronchioli were lined by normal epithelium. Peribronchiolarly lay dense lymphocytic infiltrates. The lumina in the bronchopneumonic foci were filled with leucocytes, which also were found in the epithelium.

The lung tissue was emphysematous in the upper lobes; in the remaining lobes bronchopneumonic foci occurred in which the alveoli were filled with leucocytes. The alveolar septa revealed no abnormalities. There was no noteworthy hyperaemia of the lung capillaries or extravasation of erythrocytes. Locally, however, oedema and hyperaemia appeared collaterally about these foci. Neither necrosis of the alveolar septa nor abscesses were encountered.

Bacterial Stainings revealed *H. influenzae* in the leucocytic infiltrated epithelium of the bronchi and bronchioli at the same sites as in Case No. 5. Pneumococci were not identified with certainty (Fig. 34).

No. 7, Case History (241/1949, Leiden): Patient J. F.-O., woman, 64 years of age

Clinical Diagnosis: Diabetes mellitus; influenza pneumonia; infectious toxic shock

Duration of Illness: 5½ days

Clinical Observation: 1 day

Laboratory Data: Number of leucocytes in the peripheral blood: 2,300; eosinophile leucocytes: 0; no sputum

Antibacterial Treatment: 800.000 U. penicillin in total

Autopsy: (43/1949, Leiden) 2 hours after death, 17th February, 1949

Postmortem Diagnosis: No tracheitis; hyperaemia of the submucosa of the trachea and bronchi; haemorrhagic-oedematous foci of partially confluent pneumonias in both lower lobes and right middle lobe; peribronchial haemorrhagic foci in both upper lobes; dilation of the right ventricle; extensive arteriosclerosis; no adrenal haemorrhage

Bacteriology and Virology: Blood culture P.M.: sterile. The cultures from trachea and both lungs showed no growth despite penicillinase. In the Gram preparation from the lungs and bronchi, however, several Gram positive coccal-chains were to be seen. A mouse infected with lung emulsion remained alive. No influenza virus could be isolated from the tracheal mucosa. Influenza virus A was isolated in chicken embryos from specimens of both lower lobes (concentration 10^1 M.L.D.$_{50}$ per gram of lung tissue (right lower lobe)). The blood serum contained no antibodies against influenza virus A-PR$_8$ (1934, U.S.A.), FM$_1$ (1947, U.S.A.) or B-Bon (1943, Australia), all titres being lower than 12, in conformity with the complement fixation test.

Histopathology:

The tonsils were strongly fibrotic, with a normal unbroken epithelial covering.

Uvula and Pharyngeal wall showed no distinguishing features. The stratified squamous epithelium was intact, and there were no leucocytic or lymphocytic infiltrates in the submucosa.

The larynx and vocal cords were respectively coated with normal ciliated and squamous epithelium. In the submucosa were found strong

local lymphocytic infiltrates. There was no convincing hyperaemia or oedema.

The trachea, the bifurcation, the main bronchi, and the lobar and segmental bronchi in general showed the same epithelial degenerative changes as in Cases No. 5 and 6, but the focal aspect was much more manifest and the lesions were more widely separated, so that multiple niveaux were found to be covered with completely normal epithelium. Degenerative changes were observed down to the basal cell layer, but at no niveau the latter was broken. Remarkably, a considerable regeneration of the epithelium from the basal cell layer had already occurred in this case. Thus an undifferentiated epithelium arose of about trilaminar thickness, occasionally even higher. The number of mitoses was also greater than in Cases No. 5 and 6, and the mitoses did not limit themselves to the basal cell layer but were also evinced in the more superficial layers. The surface of this undifferentiated epithelium sometimes displayed thin flat cells and small pyknotic nuclei and at other places swollen, superficially necrobiotic cells. A cuticular margin with cilia was not encountered. In the midst of this undifferentiated epithelium, however, a few cells could be demonstrated with intracellular mucus production. A leucocytic or lymphocytic infiltration of the epithelium was not found. In contrast to the sparse foci of altered epithelium in the tracheal bifurcation and main bronchi, these were numerous in the lobar and segmental bronchi. Regenerative as well as degenerative changes appeared alternately, so that there arose a very polymorphic overall picture (Fig. 35). The basal cell layer, however, was here also intact and the cellular infiltration of the epithelium very slight.

The tunica propria showed a hyperaemia and oedema which were more pronounced than in Cases No. 5 and 6, also appearing subepithelially and predominantly between basement membrane and epithelium. The basement membrane was very irregularly thickened, especially in the lobar and segmental bronchi. Extravasation of erythrocytes was seen, but this was very slight. The cellular infiltration consisted mainly of lymphocytes, sometimes in follicles, and a few polymorphonuclear leucocytes. The exudative changes in the tunica propria were most strongly developed peripherally. The mucous glands exhibited only minor degenerative changes. The cells of the ducts corresponded with the degenerative changes of the tracheal epithelium, and already regenerative pictures were also encountered here.

The small bronchi and bronchioli were also coated focally with a degenerated epithelium. Regeneration to undifferentiated epithelium was

seen here in much lesser degree. The basal cell layer was not interrupted although there was a moderately strong cellular infiltration with lymphocytes and leucocytes in the epithelium. The lumina of the bronchioli were usually filled with a sero-leucocytic exudate. The tunica propria was oedematous and hyperaemic and densely strewn with leucocytes and lymphocytes. Numerous haemorrhages were evinced. The mucous glands present showed meagre mucus production and were similarly infiltrated with cells. In the pneumonic-free regions similar epithelial alterations were found, but leucocytic infiltration of epithelium and tunica propria was then lacking. Sometimes the bronchioli were severely dilated.

The respiratory bronchioli were also strongly dilated. The epithelium, which showed neither cilia nor mucus production, was intact. The lumen was filled with a sero-leucocytic exudate or a haemorrhagic oedema-fluid poor in fibrin and cells.

The lung tissue: Bronchopneumonic leucocytic inflammatory foci appeared alternately with oedema and haemorrhages. Suppuration and necrosis of alveolar septa was not encountered. The capillaries were distended and filled with erythrocytes. Hyalinoid thromboses of the capillaries were not encountered.

Bacterial stainings: Micro-organisms could neither be demonstrated with certainty in the large or small bronchi or bronchioli nor in the lung tissue. Only locally were a few Gram-positive diplococci encountered in the lung tissue.

No. 8, Case History (207/1949, Leiden): Patient C. v. d. H., man, 71 years of age
Clinical Diagnosis: Influenza pneumonia; lung emboli(?); myocardial infarction(?)
Duration of Illness: 9 days
Clinical Observation: 4 days
Laboratory Data: Number of leucocytes in the peripheral blood: 15,400; eosinophile leucocytes: 0; Pneumococcus type 19 was cultured from the sputum
Antibacterial Treatment: 800.000 U. penicillin per day
Autopsy: (39/1949, Leiden) 1½ hours after death, 14th February, 1949
Postmortem Diagnosis: Slight hyperaemia of the trachea; muco-purulent bronchitis and bronchiolitis in both lower lobes and right middle lobe; occasional bronchopneumonic foci in right lower and middle lobes; subpleural vesicular emphysema in both upper lobes; slate-coloured induration of both apices with calcium deposits; fibrous pleuritis with obliteration of both pleural cavities; fresh posterior wall myocardial infarction; lipomatosis of the heart; dilation of the right ventricle; fibrous perihepatitis and perisplenitis; no adrenal haemorrhages
Bacteriology and Virology: Blood culture P.M.: sterile; cultures from trachea and bronchi showed no growth despite penicillinase. No micro-organisms were seen in the Gram preparations of the exudate from the lungs and the bronchi,

nor could influenza virus be isolated from the trachea or lungs in chicken embryos. From the fifth until the ninth day of illness the blood serum showed a sharp rise in antibodies (haemagglutination inhibition titres against the strain PR_8 (1934, U.S.A.): 96/768 and against the strain A-Hess (1949, Netherlands): $<$ 12/512.

Histopathology: Pharynx and larynx were not removed, so that examination of tonsils and pharyngeal and laryngeal walls was impossible.

The trachea and bronchial tree displayed large areas of normal epithelium proximally. The cellular infiltration of this epithelium was very meagre. The tunica propria showed no oedema here, and there was only slight hyperaemia. Polymorphonuclear leucocytes were only sparsely encountered although lymphocytes were present in fairly large numbers. More distally (the bifurcation, main bronchi, and lobar and segmental bronchi) the epithelium was locally replaced by an undifferentiated epithelium three or four layers in height. These areas were especially extensive in the bronchi (Fig. 36). The most superficial cell layer was partly flattened out as a layer of flat cells with pyknotic triangular nuclei or consisted of swollen necrotic cells. Degenerative changes such as those in Cases No. 5, 6, and 7 were not encountered. Many cells which already displayed an intracellular mucus production were locally encountered in the midst of the undifferentiated epithelium. Cilia and cuticular margin, however, were completely lacking. The mucous glands were atrophic and produced only meagre mucus. The excretory ducts were normal or possessed a coating of undifferentiated epithelium two or three layers high. In the epithelium leucocytes were encountered only sparsely. In addition to slight oedema and hyperaemia, the tunica propria here displayed a strong accumulation of lymphocytes, often forming follicles.

The small bronchi and bronchioli: These showed throughout the whole lung a predominantly normal epithelial picture and normal tunica propria, the latter being strewn with many lymphocytes. A three-layered undifferentiated epithelium occurred focally in the bronchi and bronchioli, especially those of the right middle and lower lobes. The surface of this epithelium showed signs of necrobiosis and mucus-secreting cells were encounterd only rarely. A superficial layer of flat cells with pyknotic nuclei was entirely lacking. In this epithelium sparse lymphocytes and leucocytes were met, and the tunica propria showed fairly strong hyperaemia as well as oedema. Haemorrhages in the tunica propria were not seen. The cellular infiltration was rather marked and

consisted of lymphocytes and leucocytes. The mucous glands made an atrophic impression, and the lumina of the bronchi and bronchioli were partially filled with muco-leucocytic exudate.

The respiratory bronchioli showed no peculiarities. The epithelium was intact, and peribronchiolarly neither oedema nor hyperaemia appeared. The lumina of the bronchioli were filled with a sero-haemorrhagic, leucocytic exudate only in the pneumonic foci of the right middle and right lower lobes. The epithelium and interstitia here were similarly infiltrated with leucocytes.

The lung tissue manifested an overwhelming emphysema and old tuberculous processes in the apices. In the right middle and lower lobes there occurred several bronchopneumonic foci with a sero-leucocytic, locally haemorrhagic exudate. Necrosis was not encountered, and the capillaries of the alveolar septa showed no peculiarities except a moderate hyperaemia.

Bacterial stainings in the lung tissue, bronchi, and bronchioli showed no micro-organisms. Only in the main bronchi a few Gram positive diplococci were encountered in the superficial layer of the undifferentiated epithelium.

No. 9, Case History (141/1949, Leiden): Patient J. S. d. B., woman, 54 years of age

Clinical Diagnosis: Influenza pneumonia(?); haemorrhages from oesophageal varices due to hepatic cirrhosis(?)

Duration of Illness: 15 days

Clinical Observation: 1 day

Laboratory Data: Number of leucocytes in the peripheral blood: 14,700; eosinophile leucocytes: 0; no sputum

Antibacterial Treatment: 800.000 U. penicillin total

Autopsy: (20/1949, Leiden) 1 hour after death, 29th January, 1949

Postmortem Diagnosis: Anaemia of the organs; no clear-cut tracheitis or bronchitis with peribronchitis; sharply circumscribed focal pneumonia in the left lower lobe; emphysema of both upper lobes; Laennec cirrhosis of the liver and fibroadenia of the spleen; strong dilatation of the venous plexus of the oesophagus and of the veins of the round ligament of the liver; extensive clots in the stomach and blackened blood in the intestine as a consequence of a ruptured oesophageal varix.

Bacteriology and Virology: Blood culture P.M.: sterile. In the Gram preparations of trachea, lung focus, and bronchi Gram-positive cocci were seen in short chains. The cultures, however, showed no growth despite penicillinase. A mouse survived an injected emulsion from the lung focus. No influenza virus was isolable in chicken embryos from trachea or lungs. The blood serum showed a very high antibody titre in the haemagglutination inhibition test according to Hirst against

influenza virus A-PR_8 (1934, U.S.A.) of 4096 and against the strain A-Heer (1949, Netherlands) of 2304. The complement fixation test against A-PR_8 (1934, U.S.A.) titred 4120 (final dilution of serum).

Histopathology: The tonsils were small and fibrotic with large lymph follicles which occassionally contained reaction centres. The epithelial coating showed no peculiarities.

The uvula and the pharyngeal wall showed a normal squamous epithelium. The submucosa had a normal aspect.

The larynx and the vocal cords were likewise without pathological changes. The epithelium was intact and the submucosa showed neither hyperaemia nor oedema.

The trachea and the bronchial tree contained over their entire length nearly normal epithelium without infiltration of leucocytes. Only very locally, *e.g.*, at the level of the tracheal bifurcation, in the main bronchi, in the lobar bronchi and a few segmental bronchi of both lower lobes, undifferentiated epithelium to sometimes six layered height was found in limited fields (Fig. 37).

Intracellular mucus production was encountered in several places amid this undifferentiated epithelium. The surface manifested only very meagre necrotic changes or a single layer of flat cells with pyknotic nuclei. The forming of a cuticular margin and small cilia were regularly observable in this epithelium. The still disordered structure deviated markedly from normal ciliated epithelium, and leucocytes and lymphocytes were constantly encountered therein (Fig. 38). The small bronchi and bronchioli in the pneumonic focus of the left lower lobe as well as those of the uninvolved lobes were covered with normal ciliated epithelium. A cellular infiltration of this epithelium with leucocytes was present. In general the tunica propria manifested few peculiarities, hyperaemia and oedema occurring slightly or not at all. It was everywhere infiltrated with lymphocytes, but infiltrated with leucocytes only in the pneumonic focus of the left lower lobe. The mucous glands and their ducts showed no peculiarities.

The lung tissue revealed no peculiarities and was well-aerated except in the left lower lobe. Neither hyperaemia nor oedema was encountered. Interstitial lymphocytic infiltrates were lacking, and the respiratory bronchioli showed a normal epithelial lining. A lobar pneumonia in lytic stage was encountered in the left lower lobe. The alveoli were filled with a fibrinous leucocytic exudate which already was partially liquefied. Considerable amounts of lipids were encountered in the alveolar cells and leucocytes. Necrosis of the alveolar septa or respiratory

bronchioli was not observed. The capillaries in the septa showed no peculiarities.
Bacterial stainings of the lung focus and the bronchi demonstrated no micro-organisms.

Discussion:
The bacterial inflammations of the air passages in these five cases are not caused by *Staphylococcus aureus* and are all associated with an influenza virus A infection.

In two cases, No's. 5 and 6, the bacterial flora consists of non-encapsulated *H. influenzae* combined with Pneumococcus type 3 and type 15 respectively. As in Cases No. 1, 2, and 3 in *Chapter III*, they are found mainly in the epithelium and tunica propria of the respiratory tract. A bacterial infection with Pneumococcus type 19 has been established in Case No. 8. In both other cases, No's. 7 and 9, probably as a result of the antibiotic therapy employed, no bacteria could be found either clinically or postmortem. In accordance with these findings and due to the time interval (lysis) no bacteria are found in the histologic slides from Cases No's. 8 and 9 only a few in Case No. 7.

Histopathologically the epithelium manifests superficial necrobiosis which can extend to the basal cell layer, whereby the latter, however, remains intact. A fibrino-necrotizing inflammation was nowhere encountered. The superficial epithelium lesions are most probably of viral and not bacterial origin since the groups concerned here are bacterial groups which in both the control cases and reports from the literature cause no epithelial lesions *(Chapter III)*.

The necrobiosis of the superficial cell layers of epithelium appears to be focal and occurs on the pars membranacea as well as on the pars cartilaginea. The epithelial lesions are found focally to the level of the intralobular bronchioli, having spread along the bronchial tree to all lobes. They occur independently of bacterial inflammatory foci, oedema, or hyperaemia, thus may also appear in normal lung tissue where every vascular reaction (oedema, hyperaemia, or haemorrhage) of the tunica propria is lacking.

Regeneration of the epithelium occurs from the basal cell layer and may give rise to an undifferentiated epithelium within four to six days. It may be said with assurance that this regeneration invariably begins within five days of illness (Case No. 5) since at that time many mitoses may already be found in the basal cell layer. From about the ninth day of illness (Case No. 7) the undifferentiated epithelium predominates

over the degenerative changes, reaching a maximal thickness on the fifteenth day (Case No. 9) and already showing small cilia and mucus-producing cells locally. The bronchopneumonic foci in Cases No. 5, 6, 7, and 8 reveal no so-called specific marks of identification pathognomonic of influenza virus infection, while Case No. 9 is typical of pneumococcal pneumonia in which the fibrinocellular exudate has already reached the stage of lysis.

The hyaline thrombi in the lung capillaries described by MacCallum (1919), Winternitz (1920), and others as characteristic of influenza were not found, nor were fibrinoid necroses of the epithelium lining the terminal bronchioli or large extravasations of erythrocytes. The localisation of the hyperaemia and oedema in these cases must therefore most probably also be explained as collateral to bacterial inflammation.

Chapter V

HISTOPATHOLOGY OF CASES OF RESPIRATORY DISEASES ASSOCIATED WITH INFLUENZA VIRUS AND SHOWING BACTERIAL INFECTIONS OF TRACHEA, BRONCHI, AND LUNGS CAUSED BY COAGULASE-POSITIVE *STAPHYLOCOCCUS AUREUS*

Seven cases were available for examination. The trachea and lungs of two of these (No's. 10 and 15) were sent us from Groningen by Dr C. van Oven and Dr Th. G. van Rijssel (see Table III).

Clinical Data

In all cases the clinical diagnosis of influenzal-staphylococcal pneumonia and infectious toxic shock was made before death. The leucocytes in the peripheral blood fluctuated between 1,600 and 11,000 per mm^3, the number of eosinophile leucocytes was 0 in all cases. The *Staphylococcus aureus* strains acquired at autopsy from lungs and trachea appeared to be identical in six of the seven cases with those from the sputa and were very sensitive to penicillin (0.04 U. per ml.). In one case, however, this was not observed, and the following data were found.

No. 16, Case History (155/1951, Leiden) (see chart): Influenza virus A was isolated in chicken embryos from the sputum of this patient on the fourth day of illness. The *Staphylococcus aureus* strain from this sputum proved very sensitive to penicillin (0.04 U. per ml.) and quite sensitive to chloromycetin (2.5 gamma per ml.). The infection and the infectious toxic shock were treated with twelve million U. penicillin per twenty-four hours and ACTH. On the eleventh day of illness a rightsided pleural exudate developed from which a colony of *Staphylococcus aureus* was isolated. This strain was also very sensitive to penicillin (0.04 U. per ml.), was quite sensitive to chloromycetin (2 gamma per ml.), and belonged to the same phage type as that from the sputum, namely the 3 A/3 B/3 C/51 group. There developed a severe mycotic infection of pharynx and larynx with Monilia albicans. The patient sank gradually into shock and succumbed on the fifteenth day of illness. The *Staphylococcus aureus* strain cultured from trachea and lungs was completely insensitive to penicillin ($>$ 1000 U. per ml.) and chloromycetin ($>$ 100 gamma per ml.) and belonged to the phage group 29/52. In this case, thus, there seemed to exist a double infection — of which one *Staphylococcus aureus* strain was sensitive and the other insensitive to penicillin and chloromycetin. A hospital cross-infection has to be taken into consideration. Influenza virus could no longer be isolated from trachea or lungs. The blood serum showed a strong increase in antibodies in the haemagglutination inhibition test according to Hirst ($<$ 12/512) and in the complement fixation test ($<$ 40/2560) against the strain A-FM_1 (1947, U.S.A.).

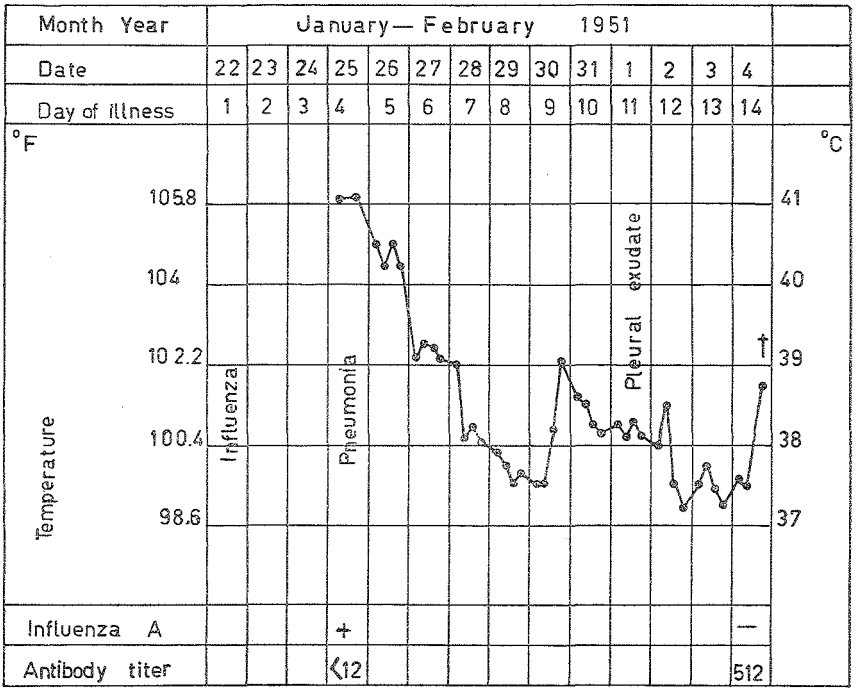
TABLE III

SURVEY OF 7 CASES OF FATAL INFLUENZA-STAPHYLOCOCCAL PNEUMONIA FROM 2 TO 14 DAYS DURATION

Case No.	Clin. Hist. and Post-Mortem No.	Sex and Age (in years)	Date of autopsy	Duration of illness (in days)	Clinical observation	Influenza virus isolation from trachea and lungs	Antibodies against influenza-strains (A group) and B (Lee) and B (Bon)	Blood culture	Staphylococcal phage type of the strains isolated from trachea bronchi and lungs [1]
10	9324 (Groningen)	M. 15	14-2-'49	2	1 hour	positive (A)	absent	sterile	29/52 (Group I)
11	27/1951 7/1951	F. 19	5-1-'51	2	12 hours	positive (A)	absent	sterile	29/52 (Group I)
12	114/1947 26/1947	M. 27	5-2-'47	3	6 hours	positive (A)	absent	3 colonies per 10 ml. blood	29/52 (Group I)
13	135/1949 17/1949	F. 40	27-1-'49	3	15 hours	positive (A)	absent	sterile	29/52 (Group I)
14	178/1949 29/1949	F. 60	7-2-'49	5	2 hours	positive (A)	absent	sterile	3 A (Group II)
15	9330 (Groningen)	F. 15	16-2-'49	5	5 hours	positive (A)	absent	sterile	29/52 (Group I)
16	155/1951	F. 49	4-2-'51	14	11 days	negative	present to high titre	sterile	29/52 (Group I)

[1] Dr R. E. O. Williams (1953), Central Public Health Laboratory Colindale, London.

Case Nº16 (E.H.B.— B.)



Temperature chart of Case No. 16: Influenza virus isolation on the fourth day of illness. An attempted isolation of influenza virus on the fourteenth day was unsuccessful. Strong increase of antibody titre against influenza-virus A.

Postmortem Diagnosis of the 7 Cases: Hyperaemia and oedema of the trachea and bronchi was invariably present in all cases. The granulated aspect of the mucosa described in 1837 by Nonat and due to the fibrino-purulent inflammation was clearly visible in six of seven cases. In one case (No. 16) muco-purulent rather than fibrino-purulent tracheitis and bronchitis were encountered. The small bronchi and bronchioli showed suppurative inflammation in all cases. The lungs exhibited haemorrhagic oedema with numerous large and small bronchial and peribronchial abscesses. In conjunction with atelectatic and emphysematous areas, these abnormalities gave the lungs the typical map-like aspect which is already well known during the pandemic of 1918. Large quantities of haemorrhagic purulent fluid flowed from the cut surface. The abnormalities were most extensive in the lower and middle lobes. In the upper lobes hyperaemia and haemorrhage predominated over the oedema and pus formation. Precisely here the bronchogenic and interstitial extension was clearly visible. In all cases a more or less extensive sero-haemorrhagic pleuritis was encountered. In Case No. 12 the bronchopneumonic changes were mainly encountered in the right lung; in No. 14 a suppurative mediastinitis was found.

The abnormalities in the lung of Case No. 16 were mainly formed by extensive abscess formation; haemorrhagic-oedematous areas were only sparsely present

in this case. In all cases the adrenals showed more or less extensive haemorrhage in cortex and medulla. The remaining organs were without obvious abnormalities.

Histopathology:

Case No. 10 and 11; duration of illness: two days

The tonsils were available for histological investigation in only one case (No. 10). The epithelial covering, consisting of non-cornified squamous epithelium, was intact. In the crypts, a local ulcerative inflammation was found. The lymphadenoid tissue showed a moderate hyperaemia, and polymorphonuclear leucocytes were present diffusely. The lymph follicles were large and exhibited germinative centers containing cellular and nuclear debris and a few leucocytes.

Uvula and pharyngeal wall were in neither case available for histological investigation.

Larynx and vocal cords were available for examination in Case No. 11. The epithelium showed no pathological changes, but the submucosa contained a moderately strong leucocytic infiltration as well as hyperaemia and oedema. The lymphocytic accumulations present frequently exhibited germinative centra with cellular and nuclear debris and a few leucocytes.

The trachea, bifurcation, and the main, lobar and segmental bronchi showed, throughout their respective lengths, extensive degenerative changes of the epithelium, which were distributed focally and appeared on the pars membranacea as well as on the pars cartilaginea. These epithelial degenerations were exactly the same as described in Cases No. 5 and 6 (*Chapter IV*). Here, also, all stadia of necrobiosis (swelling of cell and nucleus, loss of mucus production and cilia) were observed locally in conjunction with normal ciliated epithelium (Fig. 39 and 40). They extended into the excretory ducts of the mucous glands.

The diseased areas, however, were much more extensive and occurred more frequently. Sometimes an initial degeneration, to the depth of the basal cell layer and five to ten cells broad, interrupted the normal epithelium. For considerable intervals only the basal cell layer remained as covering for the tunica propria. In contrast with Cases No. 5 and 6 the basement membrane was also destroyed over distances of more or less corresponding extent. The necrobiosis of the basal cell layer was always very clearly evident (Fig. 41). The cells were severely swollen or small and shriveled with pyknotic nuclei. Within and between these necrotic cells innumerable Staphylococci were encountered focally (Fig. 42 and 43). The pyknotic cells were often entirely filled with

Staphylococcus colonies. Sometimes the basal cell layer between these necrotized areas devoid of epithelium showed signs of early regeneration, to wit, low epithelium two or three layers in height in which mitotic figures were evident (Fig. 44). The focal character of the degenerative alterations was especially clear in the upper trachea. At lower niveaux this was obscured by the very extensive, thick fibrino-purulent exudate which had been formed on the epithelial defects of the basal cell layer or on the already regenerating epithelial layer. Always, nevertheless, small fields of basal cells were encountered, especially about the excretory ducts of the mucous glands. It was noteworthy that leucocytic infiltration was encountered neither in the superficial epithelial degenerations nor in normal epithelium.

The basement membrane was present wherever an epithelial protection remained but lacking over places where the basal cell layer was necrotized. The membrane was irregular and thickened, however, especially where superficial epithelial degeneration was encountered. The tunica propria was extremely oedematous in both cases and showed strong hyperaemia with many small haemorrhages. Oedema fluid, rich in fibrin and containing some leucocytes and erythrocytes, was encountered particularly beneath and atop the basement membrane, so that the epithelium was locally upraised. Diffusely in the tunica propria, between the mucous glands as well as between the smooth muscle fibres, lymphocytes, plasma cells, and some polymorphonuclear neutrophile leucocytes were seen. Wherever the epithelium was lacking or the basal cell layer was destroyed by Staphylococci, marked leucocytic infiltrates were found. These infiltrates were sometimes of great extent, but at other times only small and corresponding in size with that of the epithelial defect. The exudate was extruded onto the surface wherever the basal cell layer and the basement membrane were necrotized and destroyed, so that typical explosive pictures arose, and the epithelium was pushed to both sides (Fig. 45). This situation prompted the surmise that the exudate plugs in the lumen of the air passages arise through pressure of the inflammatory infiltrate in the tunica propria and the resultant defects of the surface (Fig. 46). The exudate plugs were a conglomerate of oedema fluid, leucocytes, a few histiocytes, and remnants of the basement membrane; on more peripheral niveaux, however, they consisted mainly of a thick fibrin mass and numerous necrotic leucocytes. The exudate in the tunica propria showed a tendency to suppuration which grew progressively stronger with increasing depth of the level. The lumina of the lobar and segmental bronchi were substantially narrowed by the

exudate in the tunica propria and the fibrino-purulent, plug-forming debris. The peribronchial tissue was also strongly oedematous and infiltrated with leucocytes. The cartilage was often surrounded by a mantle of leucocytes.

The small bronchi and bronchioli exhibited the greatest destruction. Especially in the lower lobes, where there were extensive parenchymal changes, they showed complete suppuration of their walls. Mucous glands, smooth muscle fibres, and epithelial rests were all absent. The peribronchial lung tissue was also affected, culminating in typical bronchogenic lung abscesses. In the upper lobes (above all in Case No. 11) these abscesses were present in much lesser degree, and the focal epithelial degenerations were still clearly detectable independent of whether the affected bronchioli were situated in normal lung tissue or in haemorrhagic oedematous lung areas (Fig. 47 and 48). Normal ciliated epithelium often occurred over considerable distances. Serial investigation, however, revealed that small superficial epithelial degenerations, sometimes to the basal cell layer, or even necrobiotic areas from five to ten cells in breadth were encountered in peripheral niveaux (Fig. 49 and 50). The bronchioli, especially in the peripheral lung parts, were nearly always dilated with resultant distension of the mucosal folds.

In Case No. 10 numerous bronchogenic abscesses occurred in the upper lobes in which the small bronchi and bronchioli were so extensively involved that they were barely recognizable. The epithelium and tunica propria consisted here of a necrotic mass in which were found dense infiltrates of leucocytes which for the greater part were similarly necrotic.

The respiratory bronchioli were either involved by the abscess-forming process, in which case only a suppurative necrosis of epithelium and tunica propria was encountered, or showed a normal epithelium without degenerative changes, independently of whether they were situated in haemorrhagic-oedematous lung areas or normal lung tissue. Leucocytes were invariably encountered peribronchiolarly. It was noted that even in the lung segments with the most severe changes in the bronchi, bronchioli, and lung tissue considerable normal respiratory bronchioli were still encountered. A dilatation of the respiratory bronchioli was always present.

The lung tissue showed the familiar picture of haemorrhagic-oedematous bronchopneumonia with local abscesses, alternating with emphysema and atelectasis. The capillaries were strongly hyperaemic, and the alveoli were filled with fibrin-poor serous fluid in which numerous

erythrocytes often induced locally the picture of haemorrhage. The alveolar walls and capillaries showed no peculiarities. An occasional hyaline thrombus in a capillary was locally encountered, but these lay in the neighbourhood of abscesses. The alveolar cells were swollen and often desquamated. The cellular infiltrations of these areas were only sparsely present. Large quantities of Staphylococci were observable here without the expected leucocytic reaction. In addition to the haemorrhagic-oedematous areas there were extensive lung abscesses which always were situated peribronchially and contained no structures, but only necrosis and degenerated leucocytes. The extension of the haemorrhagic-oedematous as well as the suppurative changes was furthermore also interstitial, whereby the interlobular septa were either very strongly oedematous and haemorrhagic or severely swollen by leucocytic inflammation. Often the walls of the pulmonary and bronchial arteries were also markedly infiltrated with leucocytes and thrombi were observed in their lumina (pneumonia dissecans).

Bacterial stainings of the tissue sections showed that Staphylococci were found in large numbers on, between, and in the basal cells (Fig. 51), on the denuded tunica propria (Fig. 52), and also on the surface of the exudate plugs from the large and small air passages (Fig. 53). They sometimes lay in small colonies upon the cilia of the normal ciliated epithelium but never lay between or in these cells. They did not occur in the tunica propria and were not encountered in capillaries or lymph spaces. They were observed in large amounts in the alveoli and especially in the haemorrhagic-oedematous areas.

Cases No. 12 and 13; duration of illness: three days

Case No. 12 was published by Mulder and Verdonk (1949). Their publication included an extensive description of the epithelial degenerations and regenerations. Since the localization along the bronchial tree was only briefly treated by them, this case has been investigated anew.

The tonsils were examined in both cases and showed no peculiarities. The non-cornified squamous epithelium was intact. The uvula and pharyngeal walls were available for study only in Case No. 13 and here showed no singularities in epithelium or submucosa.

The larynx and trachea: The epithelium of the true and false vocal cords was intact and showed no abnormalities. The epithelium and the tunica propria of the trachea directly below the cords generally revealed in both cases the same changes as were just reported for Cases No. 10 and 11. Also in these cases typical epithelial degenerations were observed

ranging from superficial to basal cell layer involvement, even including necrobiosis of the latter by Staphylococci (Fig. 54). Another characteristic was explosive protrusion of the exudate in the tunica propria through the basement membrane and basal cell layer (Fig. 55). In the exudate plugs, consisting mainly of fibrin, necrotic leucocytes and rests of basal cells were encountered. In accordance with the epithelial degenerations the exudate props were focal and especially numerous and extensive at lower niveaux, so that they became confluent above the epithelial remnants (Fig. 56). In Case No. 13 the latter condition was more strongly developed than in Case No. 12. In contrast to Cases No. 10 and 11 the regeneration originating from the basal cell layer was still further advanced. In many places an undifferentiated epithelium of three to four layers was encountered. As in the superficial epithelial degenerations leucocytic infiltration of this regenerating epithelium was slight, and many mitoses were observed. The surface was nodulated by swelling of the cells and nuclei or flattened through shrinkage with characteristic pyknosis of the nuclei of the cells in the uppermost layer.

The tunica propria showed the same picture as in Cases No. 10 and 11. Here also strong hyperaemia, haemorrhage, and oedema had upraised the epithelium into small mounds. Leucocytic infiltrates were only encountered where the epithelium was missing or the basal cell layer interspersed with Staphylococci. Lymphocytes and plasma cells were seen at places where the epithelial covering was still extant. The production of the mucous glands was often depressed with increased or decreased metachromasia of the mucus. The epithelium of the excretory ducts, as had that of the tracheal surface, showed both degenerative and regenerative changes. The excretory ducts of the mucous glands were often occluded by props of mucus and/or exudate. The fibrino-purulent inflammation changed at lower levels to a suppurative inflammation. Due to this suppurative inflammation the destruction of epithelium and tunica propria was very extensive and that of mucous glands and smooth muscle fibres total. Small fields of basal cells or areas with regenerating epithelium to three layers in height were still to be found especially in the vicinity of the excretory ducts of mucous glands.

The tracheal bifurcation, the main bronchi, and the lobar and segmental bronchi: The tracheal bifurcation and the main bronchi of Case No. 13 no longer showed superficial epithelial degenerations, but locally small fields were encountered with a single layer of cells or epithelial regenerations to three or four layers. Extensive areas appeared in which fibrino-purulent inflammation of the mucosa gaining in intensity at lower

niveaux, predominated. The mucosa of the lobar and segmental bronchi was entirely transformed into a thick fibrino-purulent mass due to which the lumina of the bronchi were stenosed severely. No epithelial rests were seen here but only sparse mucous glands and smooth muscle fibres. Mulder and Verdonk (1949), in a report concerning Case No. 12, stated that the right half of the tracheal bifurcation and the right main bronchus were affected, but not the left half of the bifurcation or the left main bronchus, both of which were lined with a completely normal epithelium.

A comparative study revealed that the alterations in the right half of the bifurcation and the right main bronchus were of the same nature as have been described in Cases No. 10, 11, and 13. All stadia of superficial epithelial degenerations to the depth of the basal cell layer, penetration of the basement membrane and the basal cell layer with formation of exudate plugs in the lumen, and epithelial regenerations to an undifferentiated epithelium of four layers were found both contiguous to and in the midst of normal ciliated epithelium. The intensity of the fibrino-purulent inflammation was less than in Case No. 13, although here also, the condition gained in severity proportionally to the depth of the bronchial level. The lobar and segmental bronchi of the right lung showed in turn a progressive fibrino-purulent inflammation of the mucosa, in which small fields of normal epithelium appeared alternately with superficial epithelial degenerations and regenerations.

The left main bronchus and its lobar branches displayed normal epithelium in contrast to the segmental bronchi, especially of the left lower lobe, the epithelium of which exhibited short stretches with clear signs of degeneration. In some places the covering consisted only of the basal cell layer with a beginning regeneration of two to three layers, while small areas were also found with exudate plugs (Fig. 57). In contrast to levels nearer the hilus, the tunica propria here was hyperaemic and oedematous. Leucocytic infiltrates appeared wherever the basal cell layer was penetrated.

The small bronchi, bronchioli, respiratory bronchioli, and lung tissue did not vary in any respect from the changes described for Cases No. 10 and 11. Here also the greatest destruction was found in the caudal lung parts, whereby it was noteworthy that the respiratory bronchioli often escaped the destruction. Neither in the apico-dorsal segments of the upper lobes of Case No. 13 nor in the left upper lobe or apical, latero-basal, or antero-basal segments of the left lower lobe of Case No. 12 were haemorrhagic purulent bronchopneumonias present. The

epithelium of the small bronchi and bronchioli in these segments was intact over considerable lengths. Locally, however, superficial epithelial degenerations to the depth of the basement membrane were encountered amid the normal epithelium of the small bronchi and bronchioli. In these areas the tunica propria often showed both hyperaemia and oedema without leucocytic infiltration.

Bacterial stainings revealed no new vantage point, and the data were in both cases identical with those of Cases No. 10 and 11.

Cases No. 14 and 15; duration of illness: five days

The tonsils, uvula, pharyngeal wall, and glottis of Case No. 15 were not available for histological examination. The epithelium and the tunica propria of the trachea and the main, lobar, and segmental bronchi in both cases exhibited approximately the same alterations as the aforementioned cases. Typical superficial epithelial degenerations, however, were only sparsely present. The basal cell layer, as the only epithelial covering of the respiratory passages, was only locally discernible. Regeneration of the epithelium to a thickness of four to five layers were nevertheless numerous (Fig. 58) although differentiation to mucus-producing ciliated cells was lacking, only undifferentiated epithelium being observed. In this epithelium many mitoses were visible while polymorphonuclear leucocytes were rarely if ever encountered. The fibrinous character of the exudate plugs had more or less disappeared, and there prevailed a purulent inflammation. In the tunica propria a leucocytic inflammation, locally very purulent, predominated over the hyperaemia and oedema. This gained in strength peripherally and large areas, especially in the segmental bronchi, were completely denuded of epithelium. A diffuse purulent inflammation, which had caused a marked stenosis of the lumen and protruded outside the cartilaginous pieces into the peribronchial tissue, was encountered here.

The small bronchi and bronchioli were all involved in this necrotic process to such an extent that the epithelium was lacking or so strongly infiltrated with leucocytes as to become unsuitable for epithelial studies.

Besides hyperaemia and oedema, the lung tissue in all lung lobes showed extensive bronchogenic abscesses involving bronchial and pulmonary arteries and interlobular septa, so that several places exhibited the picture of beginning pneumonia dissecans.

Bacterial stainings of the tissue sections revealed nothing of importance. The localisations of the Staphylococci were the same as in the previously described cases.

Case No. 16; duration of illness: fourteen days

This case progressed clinically and bacteriologically otherwise than the afore-mentioned cases: the air passages and lungs also varied strikingly both macroscopically and histologically.

The tonsils and pharyngeal wall were lined by a normal, non-cornified squamous epithelium. The submucosa revealed neither hyperaemia nor oedema, and leucocytic infiltrates were also absent.

The trachea, bifurcation, and main bronchi were lined throughout their entire length with epithelium; at no level was there a fibrino-purulent inflammation. The epithelium consisted partly of normal ciliated epithelium and in part of regenerative foci of undifferentiated epithelium four to six layers in height. Unlike the preceding cases, the epithelial lining was nowhere reduced to the basal cell layer alone or even to a bilaminar low epithelium. In general the structure of the undifferentiated epithelium was entirely in keeping with that already described for earlier cases. The regeneration was farther advanced, however, so that locally in the midst of the epithelium a few cells produced mucus, and on the surface occasional cells already flaunted small cilia (Fig. 59). The epithelial surface was here nodular due to bulging of the cells. Mitoses were encountered, but occurred much less frequently than in the already described cases of five days duration. On the other hand, this epithelium showed a strong tendency towards "metaplasia", due to which the cells and nuclei of the upper most three layers were flattened over large expanses and epithelium similar to squamous epithelium was observed (Fig. 60 and 61). The surface of the epithelium was here tense and flat, whereby the entire epithelial picture acquired a very polymorphic character which is also ascribable to the irregular nuclear position and variable sizes of the cells and nuclei. In contrast to the earlier described cases, it was noted that the undifferentiated epithelium — in whatever stage encountered — was always strongly infiltrated with polymorphonuclear neutrophile leucocytes. In the normal epithelium, on the other hand, these were present in much lesser degree.

The tunica propria showed moderate hyperaemia without haemorrhages or oedema although the cellular infiltration, which consisted of polymorphonuclear neutrophile leucocytes, lymphocytes, and scattered plasma cells, was extreme. The leucocytes were by far the most numerous and diffusely spread. The mucous glands were small, often with atrophic ducts. For the most part the epithelium of the excretory ducts showed no peculiarities, but locally the same alterations were encountered as

in the surface epithelium. The mucus production was often slight, and the mucus exhibited an increased metachromasia due to which the excretory ducts sometimes contained dense rolled-up threads. The smooth muscle fibres were locally severely swollen and infiltrated with leucocytes.

The lobar and segmental bronchi, in contrast, still showed clearly a fibrino-purulent inflammation, which peripherally underwent a gradual transition to suppuration. The original focal character was emphasized by the intervening regenerative epithelial fields. These regenerations were less advanced. Neither beginning mucus production nor superficial cells with cilia were encountered. The epithelium varied in height from two to four layers and in places consisted only of the basal cell layer (Fig. 62). Mitoses were frequently seen. Normal ciliated epithelium was only sporadically present and early epithelial degenerations were not observed.

The leucocytic infiltration of epithelium and tunica propria was very dense so that the tunica propria was drastically swollen. Hyperaemia and oedema occurred but were less pronounced than in the previous cases. Besides leucocytic infiltration there were numerous lymphocytes and plasma cells and connective tissue, which gave the inflammation a chronic character. The destruction of mucous glands and smooth muscle fibres was very extensive, and only rests of these were found. As the lower levels were approached, the proliferative and suppurative character increased until finally whole areas were denuded of epithelium. The bronchial lumina were filled with a mainly leucocytic exudate, in which no mucus could be demonstrated and fibrin only locally. The pathological changes were furthest developed in the right middle and both lower lobes. The lobar and segmental bronchi of the upper lobes showed more advanced epithelial regenerations with local "metaplasia" of the superficial layers, although here, too, the proliferative purulent character came to predominate in the peripheral segments, and regions without epithelial lining were present.

The small bronchi and bronchioli were partly involved in the purulent inflammatory process whereby the epithelium and tunica propria were scarcely discernible and partly covered by an undifferentiated epithelium of two or three layers. The cells and nuclei of these layers were swollen and the uppermost layer sometimes flattened. Mitoses were encountered in this epithelium, but mucus production and cilia were lacking. The tunica propria here showed more the picture of a chronic than of a purulent inflammation. Connective tissue proliferation had assumed

predominance, and hyperaemia and oedema were not encountered. Smooth muscles, elastic fibres, and mucous glands were seen only as remnants (Fig. 63). The epithelial regenerations occurred to the level of the bronchioli. Many bronchioli were encountered, however, in which the epithelium was normal and the tunica propria showed only a slight cellular infiltration consisting of lymphocytes and a few leucocytes (Fig. 64).

The respiratory bronchioli were either entirely taken up in the necrotic purulent inflammation or quite intact and without lesions.

The lung tissue failed to show the picture of acute staphylococcal pneumonia accompanying influenza. Hyperaemia, haemorrhage, and oedema were not found, but very large bronchogenic abscesses in which large vessels and lobular septa were involved did occur. The abscesses were always encapsulated by a dense connective tissue wall, and the remainder of the lung tissue exhibited atelectasis and interstitial fibrosis with profuse lymphocytic infiltrates, interspersed with more or less emphysematous regions. The atelectatic regions encompassed the entire lobules including the bronchioli as well as respiratory bronchioli.

Bacterial stainings revealed Staphylococci intra- and extracellularly in the uppermost layers of the regenerative, sometimes "metaplastically" transformed epithelium of the trachea and bronchi. They were furthermore encountered on the tunica propria denuded of overlying epithelium and in the abscesses, but never in normal ciliated epithelium, capillaries, lymph spaces, of free in the lung tissue. Monilia albicans was encountered locally on the regenerative epithelium, especially where "metaplastic" changes also appeared.

Discussion:

The seven cases described above are typical of influenza A complicated by secondary staphylococcal tracheo-bronchitis and bronchiolitis with purulent bronchopneumonia. For histopathological examination cases were available with durations of illness of two, three, five and fourteen days.

As in the cases in *Chapter IV* there are changes in the ciliated epithelium which can be described as degenerative and regenerative. In these cases it is also evident that the mucus-producing cells of the ciliated epithelium necrotize in a few days and are sloughed off. The basal cell layer remains intact, however, except for foci of fibrino-purulent inflammation.

Here, also, mitoses are encountered after three days in the basal cell

layer. Sometimes mixed pictures arise, in which the superficial cells show necrosis and the basal cell layer has already generated a second or third layer above itself. The regeneration to multilaminar undifferentiated epithelium is very pronounced in the cases of five and fourteen days duration. In one case (No. 16, duration fourteen days) a squamoid epithelium is extensively present in trachea, bronchi, and bronchioli. The fibrino-purulent inflammation can appear very restrictedly in the mucosa so that mushroom-shaped exudate plugs bulge out into the lumen. The cause of the explosive extrusion of these exudate plugs, which consist first of serous exudate and later of fibrin, is probably the high pressure consequent on hyperaemia and oedema in the tunica propria and the focal necrobiosis of the epithelium and destruction of the basement membrane.

One may ask whether the necrosis of the ciliated epithelium in the cases here described is caused by staphylococcal toxin. A great difficulty in deciding this question is that primary *Staphylococcus aureus* infection of the bronchial epithelium is very rare (Gibson and Belcher (1951)), so that one has no control cases available. Counter arguments, however, are the following:

1. The epithelial degenerations in the respiratory tracts of Cases No. 10 through 15 are of the same nature as those of Cases No. 5 through 9 in *Chapter IV*, in which no *Staphylococcus aureus* infection had been present.
2. In Cases No. 10 through 15 they were also encountered in the epithelium of the bronchi, where no secondary bacterial inflammation of *Staphylococcus aureus* aetiology was present.
3. An effect induced at greater distances by staphylococcal toxin is here an improbable aetiology since fibrino-purulent inflammatory areas are elsewhere encountered which border on normal epithelium.
4. It is also difficult to imagine that staphylococcal toxin working at a distance might cause the mucus-producing ciliated epithelium to necrotize while the basal cells remain intact as germinative layer.
5. Furthermore, such epithelial lesions are never encountered with a staphylococcal sepsis, although the Staphylococci in such cases are found in great numbers in the arterioles and capillaries of the lung tissue and cause haematogenic Staphylococcus abscesses directly involving both bronchi and bronchioli. Nor are there inflammatory changes in the mucosa of the bronchi or bronchioli slightly distant from the haematogenic staphylococcal foci.
6. The Staphylococci are furthermore found in large numbers only in

the basal cells wherever these are denuded of the protective mucus-producing ciliated cell layer. One can never find them in the normal intact epithelium.

7. The regeneration of the epithelium in the respiratory tract ultimately succeeds despite the continued existence of a secondary *Staphylococcus aureus* inflammation (Case No. 16). This pleads for an aetiology other than staphylococcal toxin in the primary epithelial lesion.

In our opinion these arguments confirm the theory that epithelial lesions in the above-named seven cases are to be considered a consequence of the primary influenza virus infection.

The bronchopneumonias of Cases No. 10 through 15 show the familiar haemorrhagic-purulent aspect, accepted as characteristic of influenza with secondary Staphylococcus infection. A grave purulent inflammation accompanied by formation of thrombi is often seen in the bronchial and pulmonary arteries. The interstitial extension of the inflammation is so very obvious that an initial pneumonia dissecans is feasible. "Fibrinoid" necrosis of the alveolar septa and terminal bronchioli, even as "hyalin" thrombi of the lung capillaries, are present only in slight degree. They always lie in the neighbourhood of large lung abscesses and Staphylococcus colonies, *i.e.*, never in regions free of bacterial inflammation. On this basis these findings must be considered more likely bacterio-toxic than viro-toxic.

Case No. 16 is important because of its duration. It proves clearly that after a prolonged illness the diagnosis cannot be established on the macroscopical aspect of the lung tissue alone. In this case no further haemorrhages are encountered, the proliferative purulent character predominating. Microscopically, however, the epithelial regeneration in the respiratory tract is clearly seen.

Finally, it is essential to remark that in all cases (No. 10 through 16) more or less extensive adrenocortical haemorrhages are encountered. The absence of similar haemorrhages in Case 5 through 9 in *Chapter IV* render it likely that the adrenal haemorrhages are of bacterio-toxic and not of viro-toxic nature.

Chapter VI

RAPID TENTATIVE POSTMORTEM DIAGNOSIS OF INFLUENZA THROUGH CYTOLOGICAL PREPARATIONS OF THE TRACHEAL EPITHELIUM

The epithelial degenerations and regenerations in the trachea and bronchi accompanying influenza are so typical that the value of cytological smears as an tentative diagnostic aid during autopsy is self-evident (Hers and Mulder (1951)). In Cases No's 1 to 16 inclusive we have employed this technique. The various degenerations of the ciliated cells, such as loss of cilia and mucus production, transverse nuclear position, hyperchromatosis of the nuclear wall, and protoplasmic changes are easily recognizable in the cytological smears and correspond with those in the histological preparations. Epithelial regenerations of the basal cell layer, including mitoses, are also to be found. Experience has taught that the cell structures can best be studied in the wet methylene blue (or toluidine blue) preparations. The dried smears stained according to May-Grünwald-Giemsa or Woolner and McDonald, retain fewer details. The advantage of the dried preparations is that they are permanent. Figures 65*a* and *b*, 66*a* and *b*, and 67*a* and *b* are examples of cytological preparations corresponding with the histological sections from the trachea in Cases No. 1 (control case of primary bacterial infection with non-encapsulated *H. influenzae*) and No's. 5 and 14 (cases of influenza virus A infection secondarily invaded, respectively, by non-encapsulated *H. influenzae* and *Staphylococcus aureus*.)

From these figures it is evident that with this method one can make a tentative postmortem diagnosis of influenza virus infection. Obviously, cytological examination of the tracheal and bronchial epithelium in cases such as those described above can never prove with certainty the existence of an influenza virus infection. It can, however, quickly inform the pathologist and virologist of the categorical possibility of infection with an epitheliotropic virus in the air passages (see *Chapter X*, Measles) and is valuable as an indication for the further need of extensive virological examination of the bronchial tree and lungs, serological investigation of the blood serum, and accurate histological study of the epithelium lining

the respiratory tract. On one occasion a very probable diagnosis of influenzal-staphylococcal tracheo-bronchitis was missed through neglect of this cytological diagnostic technique of the tracheal epithelium.

No. 17, Case History (101/1949, Leiden): Patient A. J. F.-v. d. L., woman, 49 years of age

Clinical Diagnosis: Status asthmaticus; shock

Duration of Illness: 1 day

Clinical Observation: 1 hour

Laboratory Data: Number of leucocytes in the peripheral blood: 25,400; eosinophile leucocytes: 0

Autopsy: (14/1949, Leiden) 1 hour after death, 21st January, 1949 (during the influenza epidemic 1948/1949)

Postmortem Diagnosis: Slight hyperaemia of the trachea and bronchi; extensive vesicular emphysema of both lungs; all bronchi and bronchioli were filled with a tenuous transparent, sometimes turbid mucus. The remaining organs showed no abnormalities.

Bacteriology: Blood culture P.M.: Sterile. *Staphylococcus aureus* was cultured from the mucus of trachea and bronchi. No cytologic examination of the tracheal epithelium was performed. Virologic investigation of trachea and lungs and serologic examination of the blood serum were neglected because the diagnosis influenza was not suspected.

Histopathology: The epithelium of the trachea and bronchi to about the level of the interlobular bronchioli showed focal epithelial degenerations such as were described in Cases No's. 5, 6 and 10 to 15 inclusive. The basal cell layer and the basement membrane were destroyed by the *Staphylococcus aureus* infection, and exudate plugs, sometimes fibrinous, were found protruding into the lumina. Beginning regeneration was present locally to bi-layered depth. The tunica propria showed strong hyperaemia with haemorrhages, oedema, and leucocytic infiltrates. Several small bronchi and all bronchioli were still free, however, of any inflammation. Pneumonia was not present, in fact, only in one small region hyperaemia of the capillaries and slight oedema of the alveolar septa were found. Interstitial inflammation of the interlobular septa was nowhere encountered. Eosinophilic cells in the bronchial walls were numerous.

Discussion:

A method is described for early postmortem establishment of a tentative diagnosis of influenza by means of cytological smears of tracheal epithelium. The value of this technique, whereby ciliated epithelium in degenerative and regenerative stages is easily recognized, is discussed with reference to 16 cases.

Chapter VII

THE CYTOLOGY OF THE SPUTUM OF PATIENTS SUFFERING FROM INFLUENZA

During the influenza pandemic of 1918 Citron (1918) reported that the sputum of influenza patients contained much desquamated epithelium. Van Bruggen and co-workers (1947) described a similar fact, but neither reported specific alterations except that the size of the sloughed epithelial cells corresponded with those of the trachea and bronchi. In these publications a sharp distinction is drawn between the sputum of uncomplicated influenza and that of bacterially complicated cases. Influenza patients who are not secondarily infected with bacteria expectorate meagre white mucous sputum, but upon secondary infection of the bronchi muco-purulent or purulent sputum is coughed up. The precise character of the sputum is to a certain degree dependent upon the nature of the secondarily infective micro-organism.

We have examined cytologically six sputa from uncomplicated and eight from bacterially complicated cases of influenza with positive virus-isolation. In the patients with uncomplicated influenza (three men, aged resp. 21, 49, and 40 years of age, and three women, aged resp. 22, 41 and 20 years of age) influenza virus A (epidemics 1949 and 1953) was isolated from the sputa of five cases and influenza virus B (epidemic 1950) from that of the sixth. The influenza virus from the sputa of the eight bacterial complicated cases belonged to the A group (epidemic 1949). None of the patients was suffering from chronic bronchitis.

We employed as controls the sputa of fifty patients suffering from acute or chronic aerobic bacterial infections of the respiratory tract (but free of influenza virus as determined by isolation attempts and negative serology), of one hundred-fifty cases of chronic aerobic bacterial infections, and of ten cases of chronic anaerobic bacterial infections. The latter two groups were complicated by bronchiectases, whereby concurrent influenza virus infection was highly improbable since influenza was not prevalent during this period.

Sputa of patients suffering from a bronchus or lung carcinoma are nowhere included in this investigation.

The number of cytological preparations per case was usually ten, partly stained with haematoxylin and eosin according to Woolner and McDonald, partly with May-Grünwald-Giemsa. Wet methylene blue preparations were made in a few cases. In some cases the sputum was fixed in formalin and mounted in paraffin for microtomy.

The cytology of the control cases, as earlier indicated by Kaufmann (1904), revealed that freshly expectorated sputum in acute and chronic bronchitis caused by aerobic micro-organisms contains very few ciliated cells, none of which show degenerative changes. Recent publications, Mulder (1938, 1940 and 1952) and Mulder *et al.* (1952), prove that non-encapsulated *H. influenzae* is the most prevalent and important bacterial pathogen concerned in respiratory tract infections. The cytological examination of sputa is also in complete accord with the fact that non-encapsulated *H. influenzae* leaves the ciliated epithelium of the trachea and bronchi intact even in chronic cases of long duration (Hers and Mulder (1953)). Only in a status asthmaticus where a meagre, tenuous, strongly eosinophilic, transparent sputum is produced and bacterial flora may be absent, can reasonably abundant ciliated cells be found. But in such cases the cells show no degenerative changes.

In the cases of chronic anaerobic infected bronchitis accompanied by bronchiectases, sloughed-off ciliated epithelium cells were regularly encountered in the sputum. They are present in larger numbers than with the aerobic infections, nevertheless show no degenerative abnormalities. Occasionally a multinuclear ciliated cell or a clump of ciliated cells can be discerned. It is important, however, that in these cases clusters of stratified squamous cells are sometimes found which frequently show cornification. They are clearly distinguishable from pharyngeal epithelium and have a compact protoplasm with small pyknotic or round chromatin-rich nucleus. Peripherally in the protoplasm, keratin or prekeratin are often encountered. These findings in the sputa of sufferers from chronic anaerobic respiratory tract infections accompanied by bronchiectasis concur with recent histological investigations of these ectatic bronchi and bronchioli whereby extensive focal metaplastic changes of the ciliated epithelium into cornified stratified squamous epithelium are described (Mulder and Hers (1954)).

The cytology of the sputa from influenza cases shows a quite different picture. In the mucous sputum of uncomplicated cases very few leucocytes are encountered. Ciliated cells are present in considerable numbers,

and all show clear degenerative changes. The cells have lost their elongated form and are more or less rounded off through swelling of the protoplasm, whereby rests of cilia are discernible. The mucus production has largely disappeared (Fig. 68). Sometimes the cilia are lacking, and the form is more or less retained except that protoplasmic outcroppings appear on the surface. The nuclei are strongly swollen and rounded off. Several cells are discernible as undifferentiated epithelial cells often in clusters in which mitoses betray regeneration. Differentiation of these cells from alveolar cells is not always easy although the absence of intracellular dust-pigments and the structure of the protoplasm, especially if situated in groups, helps in the cytological diagnosis. The difference from pharyngeal epithelium, on the other hand, is very clear.

The sputa of the complicated cases of influenza are much more difficult to evaluate, especially when the secondary bacterial instigator belongs to the pyogenic group of micro-organisms. In these cases the ciliated cells are very necrotic and often scarcely recognizable as such. The undifferentiated epithelium, however, is present in enormous quantities, often concentrated in small fields. Depending on the day of illness, it is apparent (see preceding chapters) that this cellular morphology predominated in the sputa between the fifth and tenth days. Erythrocytes are almost invariably present and fibrin occasionally. In sputum sections parts of the fibrin plugs may be found whenever a necrotic purulent tracheitis has arisen through *Staphylococcus aureus* infections.

Discussion:

As a consequence and with the guidance of cytological investigation of the tracheal epithelium, the degenerations and regenerations of the ciliated epithelium in sputa of influenza patients were more accurately described, sputa from two hundred and ten cases of acute and chronic aerobic and anaerobic bacterial infections of the bronchi serving as controls. From this investigation it is evident that the sputa in influenza exhibit typical epithelial degenerations and regenerations and are thereby sharply differentiable from acute and chronic bacterial infections and status asthmaticus.

Chapter VIII

HISTOPATHOLOGY OF BRONCHIECTATIC LUNG LOBES SURGICALLY REMOVED FROM PATIENTS HAVING SUFFERED RECENTLY FROM INFLUENZA VIRUS INFECTION

The alterations prevailing in the bronchi and bronchioli as a consequence of an influenza undergone several months earlier and complicated by bacterial bronchitis and/or bronchopneumonia were described by Hübschmann (1917), Bossert (1919), and McCallum (1919 and 1921) as an obliterative bronchiolitis. An accurate account of the structure of the epithelium and mucosa is lacking in their writings.

It is apparent from the foregoing that eventual permanent alterations are not solely dependent upon the virulence of the secondary bacterial invader but that the duration of the infection may also affect regeneration of the damaged epithelium and the mucosa. One difficulty, however, is that after prolonged duration simple bacterial bronchitis or pneumonia unassociated with influenza virus is quite certainly capable of inducing irreversible changes in the mucosa while sparing the epithelium. One may thus ask whether a histopathological examination concerned with permanent post-influenzal changes is of any value, especially since only the lungs of patients from whom an accurate virological and serological anamnesis and follow up exists and death was caused by an intercurrent disease without pulmonary complication, are admissible to this study. It is evident that these requisites are hardly feasible at autopsy but can be fulfilled as an adjunct to thoracic surgery.

We have therefore conducted a search for permanent post-influenzal changes in the epithelium and mucosa in the bronchi and bronchioli from two patients subsequently subjected to lobectomy.

No. 18, Case History (3/1949, Leiden): Patient D. H. v. K., woman, 36 years of age

Clinical Diagnosis: Cylindrical and sacciform bronchiectases in the right lower and middle lobe; chronic muco-purulent bronchitis

Case History: The patient had suffered since her 25th year from chronic muco-purulent bronchitis. There were cylindrical bronchiectases in the right lower and middle lobes. A practically pure culture of non-encapsulated *H. influenzae* was

repeatedly obtained from the muco-purulent sputum and multiple small haemoptyses were observed.

In January, 1949 she was admitted for lobectomy of the right lower and middle lobes. Increased resistance against antibiotics of the *H. influenzae* prevented its complete elimination from the sputum (Mulder *et al.* (1952)). During clinical observation she acquired influenza (February, 1949), and influenza virus A was isolated from the sputum in a concentration of 10^6 per ml. The muco-purulent sputum became much more copious during the influenza. The blood serum exhibited a sharp rise in antibodies against influenza virus of the A group (versus A-Heer (1949, Netherlands): $<$ 12/10240 in the haemagglutination inhibition test). After the influenza an attempt was made to drive the *H. influenzae* from the bronchial tree with aureomycin. Failure was due to violent gastro-intestinal side-effects. The amount of sputum was finally reduced by sixteen million Units of penicillin per day, without complete elimination of *H. influenzae* from the sputum. Two months (57 days) after the intercurrent influenza virus infection lobectomy of the right lower and middle lobes was performed by Prof. Dr W. F. Suermondt. Non-encapsulated *H. influenzae* was cultured postoperatively from the muco-pus in the bronchi. Influenza virus could not be isolated from the bronchial mucosa or the lung tissue of the excised lung lobes.

Macroscopical Pathology: (1391/1949, Leiden) Cylindrical, partly sacciform bronchiectases in the right lower lobe; cylindrical bronchiectases in the middle lobe; peribronchial fibrosis; muco-purulent exudate in the bronchial lumina; peripheral to the ectatic bronchi lobular atelectasis appearing alternately with emphysema.

Histopathology: The bronchiectases were partly filled with a muco-leucocytic exudate in which no fibrin was encountered. They were lined by a normal ciliated epithelium which exhibited an irregular structure locally with only small cilia and decreased mucus production (Fig. 69). Regenerative undifferentiated epithelium or stratified squamoid epithelium was not encountered. At no niveau was there a total lack of epithelium. Polymorphonuclear neutrophile leucocytes were present in reasonably large numbers in the epithelium and increased peripherally although the ciliated cells exhibited no signs of desquamation.

The basement membrane was irregularly thickened locally and the tunica propria fibrotic and poor in capillaries, especially where the bronchi were strongly dilated. At such points elastic fibres were sparse and the smooth muscle fibres atrophic, whereby the tunica propria had become too thin over large areas. A dense lymphocytic infiltrate interspersed with plasma cells and polymorphonuclear neutrophiles was encountered in the tunica propria. The cellular infiltrate was not uniform, but increased in density near the periphery where, especially in the small bronchi, the leucocytes dominated. The mucous glands and their excretory ducts were of normal structure except for a few atrophic ones. The mucus production was normal.

Some of the bronchioli distal to the bronchiectases were not distended. Beyond the distal border of several bronchiectases, the mucosa of which often exhibited polyposis, bronchiolar regions denuded of epithelium to the extent of about 150 μ. were encountered. These were always to one side of the bronchiole, where granulation tissue densely infiltrated with leucocytes bulged out the tunica propria and sometimes nearly occluded the lumen. The leucocytic inflammation of the fibrotic tunica propria was very strong as was that of the epithelium directly bordering on these areas. The ciliated cells were normal here, and neither desquamation nor superficial necrobiosis was encountered. Remarkably, the bronchioli at peripheral niveaux exhibited a normal tunica propria and epithelium, almost no leucocytes but rather lymphocytes being encountered in the former. The lumina were narrow and the bronchioli often fully collapsed so that the opposed epithelium lay in mutual contact. The respiratory bronchioli revealed a normal structure with dense peribronchiolar lymphocytic infiltrates. They were either collapsed and visible as narrow slits or of normal width.

The lung tissue was lobularly atelectatic where the septa contained excessive connective tissue and were infiltrated densely with lymphocytes (sometimes in "follicles"). These areas concurred topographically with the occluded bronchioli and were adjacent to well-aerated alveoli which were sometimes emphysematously distended. However, pneumonic infiltrates were lacking.

Bacterial stainings of the tissue slices revealed *H. Influenzae* in large numbers in the epithelium and tunica propria, especially of the small bronchioli as well as in the granulation tissue. They lay extracellularly in the epithelium but intracellularly in the leucocytes.

Discussion: The interpretation of the lesions in the bronchi and bronchioli is difficult in this case because a combination exists here of chronic muco-purulent bronchitis caused by non-encapsulated *H. influenzae* with an influenza virus infection three months earlier. In another paper (Hers and Mulder (1953)) we have demonstrated that the ciliated epithelium of the respiratory tract remains intact even in long-standing infections with *H. influenzae* regardless of the inflammatory exudate.

It is therefore possible that the incomplete restoration and more or less disordered structure of the epithelium locally is due to the prolonged effect of the bacterial infections induced by the influenza virus infection suffered three months earlier.

The granulation tissue in the more distal bronchiectases and collapsed

rather than distended bronchioli cannot be attributed to the aforementioned influenza virus infection. Haemorrhages from the region of the bronchial artery, which can occur as a consequence of the severe connective tissue proliferation of the bronchiectatic bronchial wall, are the most probable cause for this granulation tissue, especially since multiple small haemorrhages were observed in this patient (Hers, to be published). It is apparent that such a granulation tissue can eventually lead to an obliterative bronchiolitis.

No. 19, Case History (237/1949, Leiden): Patient M. C. H. S.-S., woman, 29 years of age

Clinical Diagnosis: Cylindrical bronchiectases established after influenzal-staphylococcal tracheo-bronchitis and bronchiolitis with bronchopneumonia.

Case History: In February, 1949, the patient was admitted on the third day of illness with a fulminant tracheo-bronchitis and bronchiolitis with bronchopneumonia bilaterally in all lobes except the left upper. The sputum contained a coagulase-positive *Staphylococcus aureus*, phage type 29/52 (Group I), in pure culture and influenza virus A was isolated from the sputum. The blood serum in the acute stage was unfortunately lost, however, there was a significantly sharp decline of the antibody titre versus influenza virus strains of the A group in corresponding haemagglutination-inhibition tests of the convalescent serum from the ninth day of illness and also from serum taken eight months later (versus A-PR_8: 1610 $<$ 12 and versus A-FM_1: 2560/36).

Prior to the influenza virus infection the patient had always been well and had never shown symptoms of bronchitis. She recovered with the help of penicillin in large doses (10 million units daily). Nine months after the influenza there arose in the left lower lobe a focal pneumonia (*Staphylococcus aureus* in the sputum). New focal pneumonias in the left lower lobe due to Pneumococcus types 6 and 16 arose twenty and twenty-six months respectively, after the influenza. All recurrences were treated in the initial stage with penicillin and healed readily. *H. influenzae* was never encountered in Gram smears of the sputum or in cultures. Bronchography seventeen months after the influenza virus infection revealed rosary-like deformities of the segmental bronchi in the entire right lung and left upper lobe and cylindrical bronchiectases in all subsegments of the left lower lobe (Plate IV).

Two years and three months after the influenza lobectomy of the left lower lobe was performed by Prof. Dr A. G. Brom. Bacteriological cultures from the bronchi remained sterile.

Macroscopic pathology (2975/1951, Leiden): Cylindrical bronchiectases in the left lower lobe; peribronchial fibrosis; lumina free of exudate; peripheral atelectasis locally in otherwise reasonably well-aerated lung tissue. Fibrous focus in the apical segment.

Histopathology: The lumina of the bronchi were dilated down to and including the bronchioli. They were lined by a quite normal ciliated

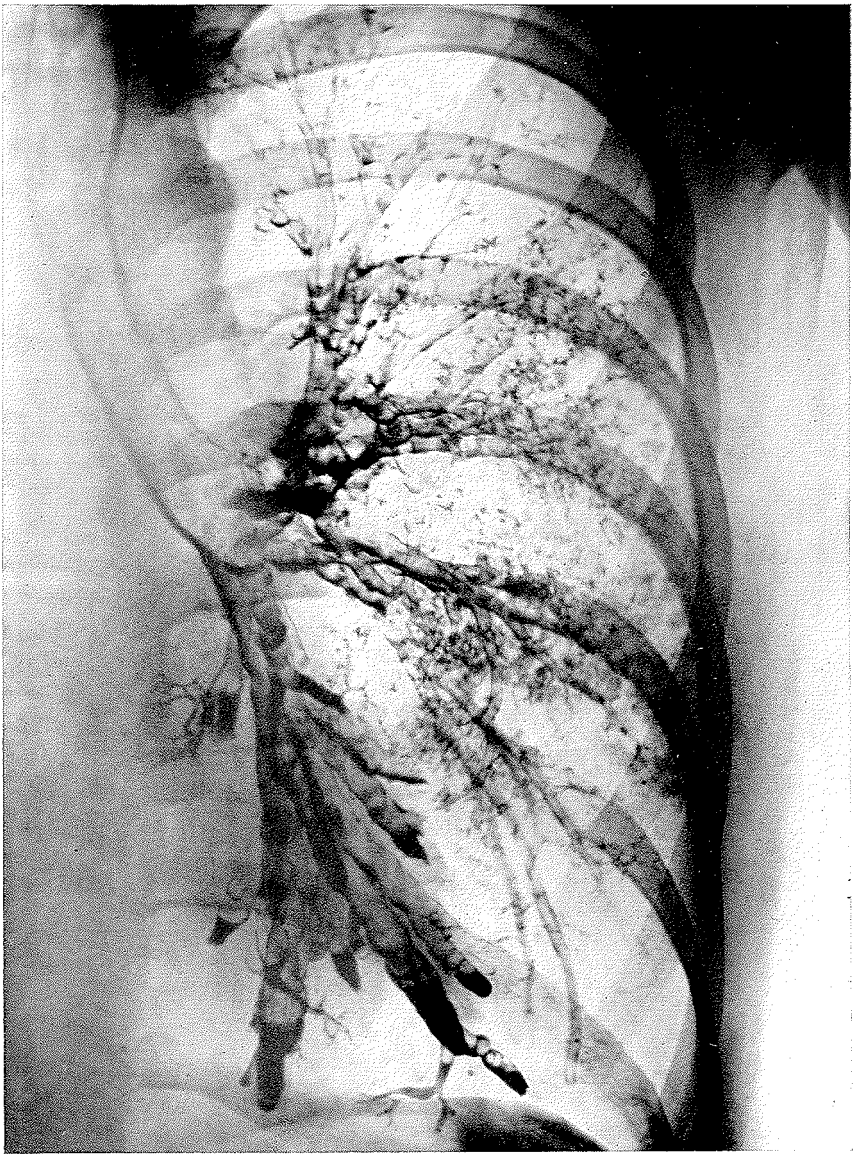


Plate IV. Bronchograph of left lung (Case No. 19, F. 29, 17 months after influenza A (1949-strain) infection complicated by *Staphylococcus aureus* tracheo-bronchitis and broncho-pneumonia): Cylindrical bronchiectases especially in the lower lobe.

Chapter IX

HISTOPATHOLOGY OF THE RESPIRATORY TRACT EPITHELIUM IN FATAL CASES OF PANDEMIC INFLUENZA IN 1918

A fortunate circumstance offered the opportunity for postmortem examination of lungs from patients who had died of influenza during the pandemic of 1918 since the Laboratory for Pathological Anatomy of Leiden had retained among its preparations the lungs from twelve such patients. The main bronchi of all cases and the trachea of a single case were available. The fixations partly in glycerine-alcohol, partly in formalin had been very carefully performed so that histological sections made after thirty-two years could still be evaluated. The autopsy reports were retained in eight cases, but the clinical data from only two cases (No's 1 and 4) were complete. No clinical or pathologic anatomical data were available from four cases (see Table IV).

Clinical Data

All cases were attributable to the Autumn epidemic of 1918 and included both men and women. Only two patients (No.'s 4 and 12) were older than thirty years of age. The duration of illness ranged from four to twelve days; in four cases this information was lacking. The co-existence of a bacterial infection was unfortunately in only a few cases detectable from the case histories due to the paucity of evidence. In Cases No. 1, 3, and 9 as evinced by Gram smears the bacterial flora probably consisted of pneumococci and Staphylococci, in No. 2, 4, and 5 of Staphylococci, and in No. 10 of pneumococci. In Case No. 6 no bacteria were found in Gram smears. Reports of accompanying bacterial cultures were lacking. The leucocytic picture had been toxic in all patients. Leucopenia was reported in most cases; leucocytosis in only one case.

One patient (Case No. 1) had been hospitalized continuously from July 1918 suffering from hepatic cirrhosis (Laennec) and ascites. During this clinical observation period, on 18th October 1918, she contracted influenza as the first case at the hospital and succumbed five days later to a pneumonia, according to the Gram preparation probably caused by pneumococcus and Staphylococcus. The remaining cases showed no complicating symptoms other than pneumonia secondary to influenza.

Postmortem Diagnosis: In all cases a haemorrhagic fibrino-necrotizing tracheobronchitis was found, while the lungs revealed a typical map configuration of confluent haemorrhagic-purulent pneumonia. Bronchogenic and peribronchial

Case No.	Postmortem No.	Year and date of autopsy	Sex and age (in years)	Duration of illness (in days)	Bacteriology of sputum Gram stain (no cultures)	Bacteria identified tentatively in histological sections	Number of leucocytes in peripheral blood
1	56/1918	23-10-'18	F 15	5	pneumococcus Staphylococcus	Staphylococcus	4,200
2	61/1918	24-10-'18	M 13	7	Staphylococcus	Staphylococcus	6,500
3	63/1918	27-10-'18	M 9	5	pneumococcus Staphylococcus	Staphylococcus	5,400
4	71/1918	2-11-'18	M 40	12	Staphylococcus	Staphylococcus	10,600
5	74/1918	2-11-'18	F 29	7	Staphylococcus	Staphylococcus	6,000
6	77/1918	3-11-'18	M 12	7	no flora	*H. influenzae* and pneumococcus	....
7	80/1918	.....	....	..	.....	none	....
8	83/1918	.....	....	..	.....	none	....
9	90/1918	14-11-'18	F 10	4	pneumococcus Staphylococcus	Staphylococcus	4,200
10	91/1918	15-11-'18	F 19	8	pneumococcus	none	....
11	101/1918	.....	....	..	.....	none	....
12	114/1918	25-11-'18	M 30	..	.....	Staphylococcus	....

abscesses in the lung tissue were invariably present, and all cases exhibited sero-haematothorax while in a single case empyema was encountered. In one case (No. 12) a pneumonia dissecans was diagnosed.

Histopathology of the twelve above-described cases:
Because the trachea and main bronchi had been cut open and washed out in the majority of cases, epithelial lesions were only focally recognizable. The epithelium was destroyed over large stretches to the depth of the basement membrane. Only at occasional areas were undifferentiated epithelium and rests of fibrino-purulent inflammation encountered. The tunica propria, however, showed, a strong hyperaemia with lymphocytic and focal leucocytic infiltrates.

In the segmental bronchi and bronchioli, however, the epithelium was much better preserved since these had been fixed without opening. Epithelial degenerations and regenerations were observed in all cases (Fig. 72, 73 and 74) and extended to the depth of the bronchioli in the single case of more than eight days duration (Fig. 75 and 76). In the remaining cases purulent and necrotizing bronchitis and bronchiolitis were found. In one case (No. 12) these had led to an extensive interstitial pneumonia, due to which the interlobular septa were severely inflamed, purulent, and necrotic, and the typical picture of pneumonia dissecans had arisen. In all cases bronchioli and small bronchi were found which showed completely normal ciliated epithelium and in which a strong leucocytic inflammation was present.

The lung tissue showed a haemorrhagic-oedematous focally purulent pneumonia, in which accumulations of Staphylococci often appeared. In Cases No. 6, 8, and 11 these were more fibrino-haemorrhagic and in No. 12 there was a local organization of the fibrinous exudate in which a bronchiolitis obliterans was encountered. Fibrinoid necrosis of the respiratory bronchioli or of the capillaries of the alveolar septa in the haemorrhagic oedematous areas was not observed.

Bacterial stainings of the tissue slices were still possible despite the prolonged fixation of the lungs. Pneumococci were not encountered, but Staphylococci were clearly visible. In one case, No. 6, bacteria which were morphologically strongly reminiscent of *H. influenzae*, were seen in the haematoxylin-eosin as well as in the methylene blue slides of the degenerated epithelium although clinically no bacterial flora was reported (Fig. 77).

Discussion:
It appears that epithelial lesions typical of influenza, as described in

Chapter IV and V, are to be found in all cases of 1918 examined. Through postmortem autolysis the epithelial degenerations were less preserved than the epithelial regenerations.

In concurrence with the duration of illness these last are most clearly observable in the late stages, where the involvement in the bronchial tree are traceble into the bronchioli. They are not encountered in stronger degree in the cases from 1918 than in those from the inter-pandemic influenza of 1949, 1951, and 1953. Also, in the influenza pneumonias of 1918 numerous bronchioli are found to have normal ciliated epithelium. Fibrinoid necroses of the respiratory bronchioli and capillaries of the alveolar septa are not found in areas free of leucocytic inflammation.

Chapter X

HISTOPATHOLOGY OF THE RESPIRATORY TRACT IN CASES SHOWING BACTERIAL INFECTIONS OF THE TRACHEA, BRONCHI AND LUNGS, ASSOCIATED WITH MEASLES AND VARICELLA VIRUSES

It is well-known that early in the eruptive stage of either measles- or varicella-bronchitis and bronchopneumonia, "metaplastic" epithelial alterations are encountered in the respiratory tract. It is feasible that measles and varicella viruses are responsible for these epithelial transformations. In the cases so far described, however, it is impossible to exclude a briefly preceding influenza virus infection as a complicating factor in diagnosis especially since bacteriological studies have revealed that the secondary infections are caused by the same bacterial genera and that these completely dominate the clinical picture (Davis (1908), Hecht (1910), MacCallum (1919 and 1921), Wollstein and Spence (1920), Jochmann (1924), Lubsen and Verlinde (1947), Weinstein and Franklin (1949)). According to the above writers the pyogenic cocci are the most important group complicating foudroyant cases of measles and varicella bronchitis and bronchopneumonia. Outstanding among these reports is that of MacCallum (1919), which describes the correlation of a severe measles epidemic to secondary infection with haemolytic Streptococci in an army camp.

In reply to the question whether the described epithelial changes in the respiratory tract in cases of influenza are pathognomonic for this disease, we have investigated the epithelial changes in the respiratory tracts of seven cases of measles bronchitis and bronchopneumonia and one case of laryngo-tracheo-bronchitis and bronchopneumonia due to varicella haemorrhagica (see Table V).

Clinical Data

Five cases (No's. 1 to 5 incl.) of measles bronchitis stemmed from an epidemic in 1946 among repatriates from the Dutch East Indies and their case histories and histological preparations were generously forwarded to us by Prof. Dr. S. van Creveld, Dr. N. Lubsen and Dr. R. van Dam.

Postmortem bacteriologic examinations of the pus in the bronchi of three cases were performed by Prof. Dr A. Ch. Ruys and Prof. Dr J. D. Verlinde; in each

case non-encapsulated *H. influenzae* was obtained in nearly pure culture. In one case, furthermore, a single colony of anhaemolytic Streptococci was encountered. Two cases of measles bronchopneumonia and the single case of varicella haemorrhagica were secondarily infected with *Staphylococcus aureus haemolyticus* and were obtained from the Pediatric Clinic of the University Hospital at Leiden (Prof. Dr. E. Gorter †).

In one case of measles bronchopneumonia (No. 7) and the case of varicella haemorrhagica, we were able to exclude influenza virus infection on virologic and serologic grounds. The remaining cases could not be examined in this respect.

TABLE V

SURVEY OF 7 CASES OF ACUTE LARYNGO-TRACHEO-BRONCHITIS AND BRONCHOPNEUMONIA, ASSOCIATED WITH MEASLES-VIRUS

Case No.	Sex and Age (in years)	Duration of illness (in days)	Bacteriology of trachea and bronchi	Influenza-virus isolation from trachea and lung	Antibodies against influenza virus A and B
1	F 5	4	*Haemophilus Influenzae*	....	....
2	F 8	..	....	....	....
3	F 14	9	....	....	....
4	F 4	10	*Haemophilus Influenzae*	....	....
5	M 3½	12	*Haemophilus Influenzae*	....	....
6	M 6	3	*Staphylococcus aureus*	....	....
7	M 1	5	*Staphylococcus aureus*	negative	absent

ONE CASE OF ACUTE LARYNGO-TRACHEO-BRONCHITIS AND BRONCHOPNEUMONIA, ASSOCIATED WITH VARICELLA-VIRUS

8	F 1½	5	*Staphylococcus aureus*	negative	absent

The table illustrates that all patients were young children in whom the duration of illness ranged from three to ten days.

Postmortem Diagnosis: In the cases from which non-encapsulated *H. influenzae* was cultivated there appeared a slight hyperaemia of the tunica propria of the trachea and bronchi. The lumina of the bronchi and bronchioli were filled with a muco-purulent exudate. The lungs showed bronchopneumonic foci alternating with atelectatic and emphysematous areas, furthermore, in one case (No. 2) an encephalopathy was encountered. Both the cases not accorded a bacteriologic investigation, No's 3 and 4, showed the same picture in the respiratory passages and lungs.

The respiratory passages and lungs of the cases secondarily infected with *Staphylococcus aureus* revealed a completely different picture. The tunica propria of the trachea and bronchi were very hyperaemic and oedematous and always exhibited a partly purulent, partly fibrino-necrotizing tracheitis and bronchitis extending even to the smallest bronchioli. The caudal lung segments of the upper lobes, the entire lower lobes, and the right middle lobe showed extensive confluent bronchopneumonias of a purulent and haemorrhagic oedematous nature. Aerated lung tissue appeared only sparsely between these regions. Peribronchial abscesses were encountered which sometimes extended even to the pleura.

The histopathology of the eight above-reported cases:

Wherever non-encapsulated *H. influenzae* was found as secondary bacterial invader of the respiratory tract, the epithelium either showed degenerative changes due to which mucus production and cilia were lost or a regenerative lining was formed by a two to four layered, undifferentiated epithelium with large swollen nuclei and nucleoli (Fig. 78). The cells and nuclei varied widely in size, giving the structure a disordered appearance. There was a slight local oedema, especially subepithelially. At no niveau investigated, however, was the basal cell layer broken through and nowhere was a fibrino-necrotizing inflammation encountered. These epithelial changes were strongly reminiscent of those in case of influenza virus infection secondarily infected with non-encapsulated *H. influenzae* and pneumococci. Hyperaemia was present in the tunica propria, but haemorrhages were not encountered. A neutrophile leucocytic infiltration of the tunica propria and epithelium was present in moderate degree and increased in density in the small bronchi and bronchioli.

The lung tissue showed local emphysema as well as local atelectasis, and accompanying the latter there appeared a dense lymphocytic interstitial infiltration, especially peribronchiolarly. Hyperaemia of the alveolar capillaries was only locally present. Oedema and haemorrhages in the lung tissue were lacking, and leucocytic bronchopneumonic foci were present only at broad intervals.

The trachea and main bronchi in the cases secondarily infected with *Staphylococcus aureus* showed severe hyperaemia, haemorrhage, and oedema, and locally a focal "fibrino-necrotizing" inflammation was found with fibrino-purulent exudate plugs which extended into the large bronchi. Due to the undifferentiated epithelium, four to six layers deep between these areas, there was a strong resemblance to influenzal-staphylococcal tracheitis (Fig. 79). Epithelium and tunica propria were densely infiltrated with a leucocytic, partially lymphocytic exudate. Also in the smaller bronchioli typical epithelial degenerations and regenerations were encountered. The undifferentiated epithelium was here two to four layers deep. Cells and nuclei were severely swollen and bulged out above the epithelial surface, or the superficial cells were flattened out giving the appearance of "metaplastic" changes. Mucus production or cilia were not observed. Hyperaemia of the tunica propria was always present, and focally there appeared quite severe lymphocytic infiltrates.

The interstices of the lung tissue were severely inflamed and strongly infiltrated with lymphocytes; giant cells were encountered in all cases of measles. The alveoli were filled with a fibrin-poor, erythrocyte-rich oedema fluid in which lay numerous alveolar cells and locally many leucocytes. The capillaries in the alveolar walls were hyperaemic. At other places the bronchi and bronchioli showed a typically purulent leucocytic inflammation with necrotic epithelium and tunica propria. The surrounding lung tissue was typical of focal purulent (pyogenic) pneumonia. No difference was observed histologically between the epithelial alterations in measles and those in varicella haemorrhagica (Fig. 80).

Cytological examination of the tracheal epithelium was performed in three cases (No's 6, 7, and 8) and revealed the same degenerations of the ciliated epithelium as were encountered in the cytological preparations of the tracheal epithelium in the influenza cases. Here, also, there appeared swelling of the cells and nuclei with loss of cilia and mucus production and hyperchromatosis of the nuclear membranes.

Bacterial stainings of tissue slices from the first five cases was not performed since no lung and tissue blocks had been retained. In the last three cases Staphylococci were clearly demonstrable. Just as in the influenzal-staphylococcal pneumonias, they were found in the undifferentiated epithelium of the bronchi as well as in the lung tissue.

Discussion:

It has been demonstrated that in seven cases of measles and one case of varicella haemorrhagica the air passages exhibited the same epithelial

changes as with influenza and that these are thus not pathognomonic for influenza but rather indicative of epitheliotropic virus infections of the respiratory tract in general. The resemblance to influenzal-staphylococcal tracheitis is remarkable in the larger air passages. In two cases (No's 7 and 8) an influenza virus infection can be excluded on virological and serological grounds. It is therefore most likely that these epithelial degenerations are caused by measles or varicella viruses.

Giant cells so characteristic of measles (Kromayer (1889), Hecht (1910), and Milles (1945) are clearly and convincingly present in our cases. Hecht (1910), Jochmann and Moltrecht (1904), Feyrter (1925), and Wätjen (1921) describe another type of giant cells in cases in which the illness had a duration of about fourteen days, which cells appeared in the small bronchi and alveoli and probably were derived from the undifferentiated epithelium. These were not found in our cases during which the undifferentiated epithelium showed a correspondingly incomplete development probably because the duration of illness was considerably shorter.

It is notable that in the older literature emphasis is frequently laid on the "metaplastic" epithelial changes with acute respiratory tract infections. It seems, however, that the reference is always to measles (McKenzie (1907), smallpox (Koch and Wätjen (1918), and Bras (1950)), or influenza, ignoring the fact that all three are epitheliotropic virus infections.

SUMMARY AND CONCLUSION

An extensive study of the epithelium of the respiratory tract in general and with influenza in particular is only possible if autopsy is performed immediately post mortem and under the conditions laid down in *Chapter II.*

Epithelial degeneration and regeneration in relation to the duration of illness in influenza

In the absence of influenza virus bacterial inflammations of the human respiratory tract due to non-encapsulated *H. influenzae* and pneumococci cause no lesions of the ciliated epithelium (*Chapter III*).

In accordance with animal experiments it is evident that in the human respiratory tract influenza virus causes a characteristic lesion of the ciliated epithelium during the second or third day of illness which consists of necrobiosis of the mucus producing ciliated cells (*Chapter IV and V*). The nuclei of the cells infected by influenza virus in their first stages are characterized by lower quantities of D.N.A. and the appearance of slightly acidophilic disks free of D.N.A. and R.N.A. An increased quantity of R.N.A. appears in the cytoplasm as well as in the interstitial spaces.

The necrobiosis is manifested either through swelling of cells and nuclei — with loss of cilia and mucus production and followed by cell dissociation — or through coagulative necrosis with pyknotic nuclei and disappearance of the cell borders which render virtually impossible any detection of surviving mucus production or cilia. Due to desquamation of the superficial necrobiotic cells, the basal cell layer after two to five days of illness may locally constitute the only epithelial lining. Two to three days after the inception of the disease the basal cells are severely swollen; after five days, however, they have become more flattened, often with a second layer of flat cells.

Mitoses in the surviving basal cells betray regeneration beginning on the third and appearing predominantly during the fifth day of illness. The axis of nuclear division is lying perpendicular or parallel to the epithelial surface. Necrobiosis with dissociation and desquamation of the superficial cell layers and the mitoses of the basal cells which

manifest regeneration often appear contiguously, giving rise to a very polymorphic picture. The regeneration is especially prominent after five days of illness, at which time an undifferentiated epithelium is encountered.

The undifferentiated epithelium, consisting of round cells and nuclei without mucus production or cilia, has a disordered structure due to the varied nuclear positions. The epithelium is approximately three to four layers thick on or about the fifth day of illness and reaches a maximal thickness of six to eight layers after about nine to fifteen days. After the ninth day of illness the surface sometimes reveals "pseudo" squamous epithelium, whereby the uppermost cell layer or even the upper three or four cell layers may locally consist of flattened cells with pyknotic nuclei. "True" metaplasia with papilla formation and stratum granulosum is not present, however, as is the case, *e.g.*, where a foreign body has "irritated" the epithelium during a sufficient period. About the ninth day of illness the undifferentiated epithelium predominates, while after fifteen days initiation of mucus production can be found locally and some surface cells possess cilia.

The epithelial degenerations and regenerations are focal and are to be found in the tracheo-bronchial arborization down to but not including the respiratory bronchioli; sometimes without accompanying hyperaemia, oedema, or haemorrhage in the tunica propria or even without leucocytic infiltration of the epithelium or tunica propria.

The regeneration is most prominent in the cases with a long duration of illness, *i.e.*, nine to fifteen days, and is farther advanced in the upper respiratory passages (trachea and main bronchi) than in the lower respiratory tract (bronchi and bronchioli).

During the degenerative phase of the epithelium the basement membrane is locally irregularly swollen and ragged of aspect yet nowhere absent even if overlaid only by the basal cell layer. During regeneration of the epithelium the basement membrane has a reasonably normal aspect, but may retain a spindle form locally.

The influence of secondary bacterial infection on the epithelial degeneration and regeneration in influenza

The epithelial degenerations and regenerations accompanying secondary bacterial infections with non-encapsulated *H. influenzae* and the Pneumococcus group (*Chapter IV*) are of the same character as those with the *Staphylococcus aureus* group (*Chapter V*). In secondary infections with non-encapsulated *H. influenzae* and pneumococci the basal cell layer is

found intact and uninterrupted at every investigated niveau (*Chapter IV*). Due to secondary infection with *Staphylococcus aureus* the basal cell layer is necrotic at many places and entirely lacking over small and occasional larger areas. The Staphylococci are found exclusively in and between these necrobiotic cells and in large colonies wherever the tunica propria is stripped of overlying epithelium (*Chapter V*).

Due to destruction of the basal cell layer and basement membrane and the high pressure probably attendant on the exudate in the tracheal and bronchial walls, eruptive exudate plugs protrude into the lumina of the respiratory passages (*Chapter V*).

Regeneration of undifferentiated epithelium from the surviving remnants of the basal cell layer progresses to an extreme degree despite the continued existence of a *Staphylococcus aureus* infection (*Chapter V*, Case No. 16, duration of illness fourteen days). Regeneration is, however, less extensively developed in Case No. 16 than in Cases No. 8 and 9, respective durations of illness nine and fifteen days (*Chapter IV*), from which secondary infection with *Staphylococcus aureus* was lacking.

The inhibitory influence of persistent bacterial infection on the regeneration explains the fact that the ciliated epithelium is still not entirely restored long after influenza (*Chapter VIII*, Case No. 18). Eventually the ciliated epithelium is completely restored after influenza, even if the bacterial complication during the acute phase had been caused by the *Staphylococcus aureus* group (*Chapter VIII*, Case No. 19).

The swollen and necrotic basement membrane is also restored provided a new epithelial lining is present here. However, a spindle-shaped swelling and oedema are often still easily discernible between epithelium and basement membrane. Permanent alterations after an influenza-*Staphylococcus aureus* tracheo-bronchitis and broncho-pneumonia are ascribable to the destructive bacterial inflammation. They consist of obliteration of the elastic fibres and fibrosis of the tunica propria with hyalinisation and distension of the basement membrane, which can give rise to bronchiectases (*Chapter VIII*, Case No. 19).

It follows from the above that intensive and early antibacterial treatment during the acute phase of the disease is also of eminent importance to restoration of the tunica propria.

The morphologic picture of the bacterial pneumonia accompanying influenza
This is determined by the secondary bacterial invader and varies with the duration of illness. Hyperaemia is often not present in stronger

degree than with the purely bacterial pneumonias in the control cases (*Chapter III*). Haemorrhages and oedema are sometimes absent, so that the influenzal lung infiltrates of these cases are often macroscopically indistinguishable from a simple bacterial pneumonia particularly when the secondary bacterial infection is caused by non-pyogenic cocci.

Except for Case No. 16 the cases in *Chapter V* all show the macro- and microscopical changes typical of influenzal-staphylococcal pneumonia. Interstitial extension in the lung with accompanying hyperaemia, haemorrhage, and oedema are encountered in extreme measure as are vascular inflammation with formation of thrombi. Comparison with the cases of similar duration secondarily infected by non-encapsulated *H. influenzae* and/or pneumococci, in which these changes are largely absent, reveals that they must be due to the powerful staphylococcal toxins rather than the influenza virus. This is equally true of the adrenocortical haemorrhages encountered in the cases with an influenzal-*Staphylococcus aureus* pneumonia. Hyperaemia, haemorrhage, and oedema are largely absent with a duration of illness longer than nine days. This is most noticeable in Case No. 16 (*Staphylococcus aureus* pneumonia fatal on the fourteenth day of illness, *Chapter V*), in which haemorrhages were absent from the lungs both macroscopically and microscopically.

A pure influenza virus pneumonia such as those described in the pandemic of 1918 and in experimental animals could not be demonstrated in the cases virologically and bacteriologically controlled.

Early diagnosis of influenza virus infection by means of cytological smears

The necrobiosis and regenerations of the ciliated epithelium are so typical that they are easily recognizable in cytological smears of the tracheal epithelium (*Chapter VI*). It is precisely because an influenza virus infection cannot always be diagnosed on the basis of the macroscopic aspect of the lungs that this technique is of value, since it necessitates virological examination of the respiratory tract and lungs in case of necrobiosis of ciliated cells can be established cytologically.

The same necrobiotic epithelial alterations are also cytologically recognizable in the sputum of influenza patients (*Chapter VII*). In influenza periods, therefore, cytological examination of the sputum in addition to bacteriological examination in Gram preparations can also be of value as an orientative aid to diagnosis.

Comparative study of the influenzal pneumonia from the epidemics of 1949—1953 and those of the pandemic of 1918

The epithelial degenerations and regenerations in the respiratory passages had been very well preserved in pathological specimens of the cases from 1918 and did not vary significantly from those of the epidemics of 1949 to 1953 *(Chapter IX)*. The epithelial lesions are focal and persist to the level of the lobular bronchioli.

The haemorrhagic oedematous character of the pneumonias is striking but not more severe than those in 1949—1953. The majority of the cases show secondary infection with *Staphylococcus aureus* due to which abscess formation is not uncommon.

The pathognomonic significance of the epithelial lesions found in the respiratory tract

The epithelial alterations described in the respiratory passages attacked by influenza are not pathognomonic of this disease but rather of infection with an epitheliotropic virus in general since the same changes are also encountered in measles and varicella *(Chapter X)*. Here, too, necrobiosis of the superficial cell layer and regeneration to an undifferentiated epithelium are observed. The cytological preparations of the tracheal epithelium in these cases reveal no dissimilarity to those of influenza virus infection.

The significance of the histological changes found as to pathogenesis and permanent changes

An influenza virus infection is recognizable from the attendant focal necrobiosis of the superficial ciliated epithelium of the respiratory tract, whereby the basal cell layer remains intact and hyperaemia, haemorrhage, and oedema are initially absent from the tunica propria.

Since the basal cell layer obviously possesses insufficient defensive capacity, secondary bacterial invaders gain a *porte d'entrée* into the mucosa *via* this superficial necrobiosis, and the situation is thus analogous to a wound infection. The most feared group of secondary bacterial invaders is that of the *Staphylococcus aureus.*

Regeneration of the epithelium initiated from the basal cell layer proceeds until a stage is reached about the tenth to fourteenth day of illness in which the respiratory tract is lined focally with an undifferentiated epithelium. Late influenza death falls roughly within this period (Tyrrell 1952), in which respiratory acidosis may also be encountered. The cause of this is probably the inability to cough up the mucus and exudate from the lower strata of the bronchial arborization.

"True" metaplasia is not encountered as a permanent after-effect of an influenza virus infection. The possibility is tenable in certain cases, however, that this might arise as a consequence of a persistent irritation of the undifferentiated epithelium (bacterial infection or tobacco smoke).

Fibrosis and focal hyalinisation with destruction of the elastic fibres of the tunica propria and lung tissue due to secondary bacterial infection must be viewed as permanent changes of vital significance. The occurrence of bronchiectases is very probably facilitated by this fibrosis and hyalinisation of the bronchial wall *(Chapter VIII)*.

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ILLUSTRATIONS

CHAPTER III

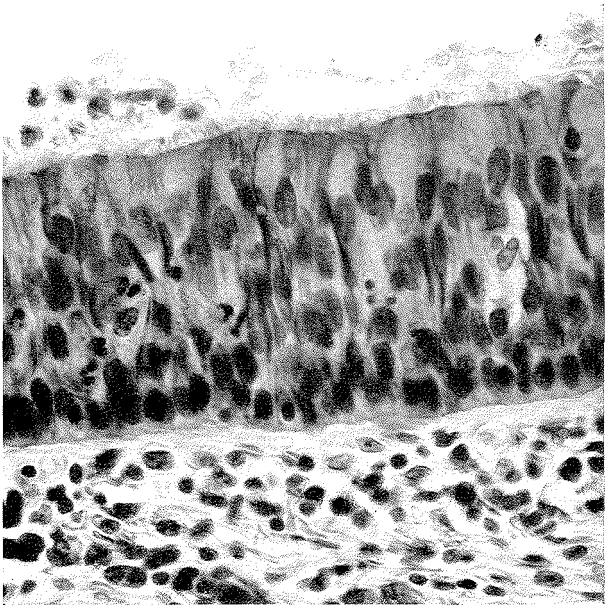


Fig. 1. Left stem-bronchus (Case No. 1, F. 71, duration of illness 7 days): Normal ciliated epithelium; infiltration of epithelium and tunica propria with polymorphonuclear neutrophilic leucocytes. Haematoxylin-eosin staining. (× 600)

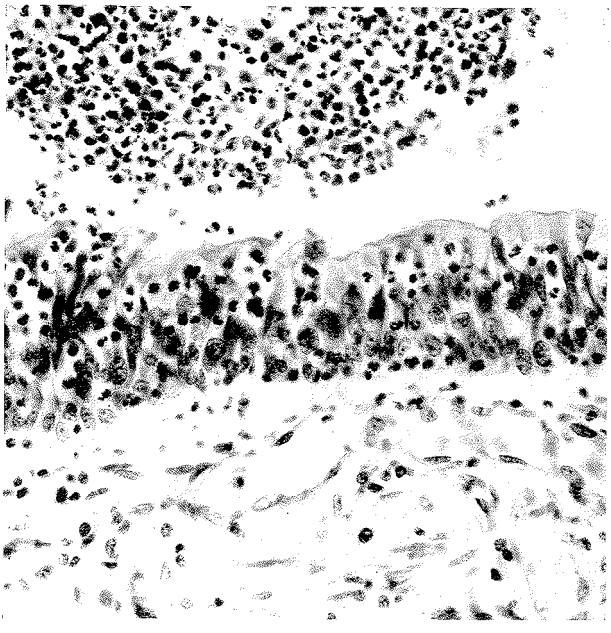


Fig. 2. Bronchus of antero-basal segment of left lower lobe (Case No. 1, F. 71, duration of illness 7 days): Normal ciliated epithelium with strong leucocytic infiltration in epithelium and tunica propria; muco-leucocytic exudate in lumen; hyperaemia of the tunica propria. Haematoxylin-eosin staining. (× 480)

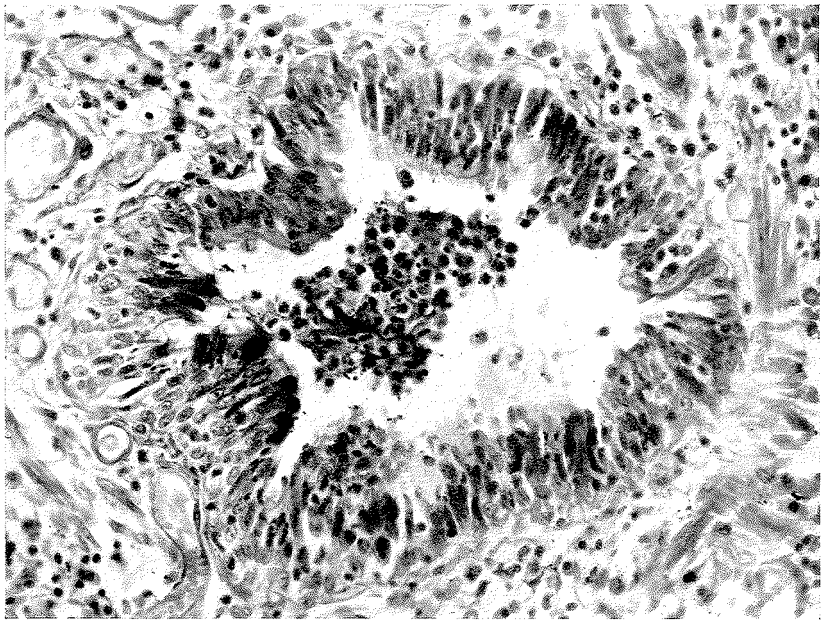


Fig. 3. Bronchiole of right middle lobe (Case No. 1, F. 71, duration of illness 7 days): Normal epithelium with strong leucocytic exudate in epithelium and tunica propria; muco-leucocytic exudate in lumen and normal folds of mucosa; hyperaemia of the tunica propria. Haematoxylin-eosin staining. (× 400)

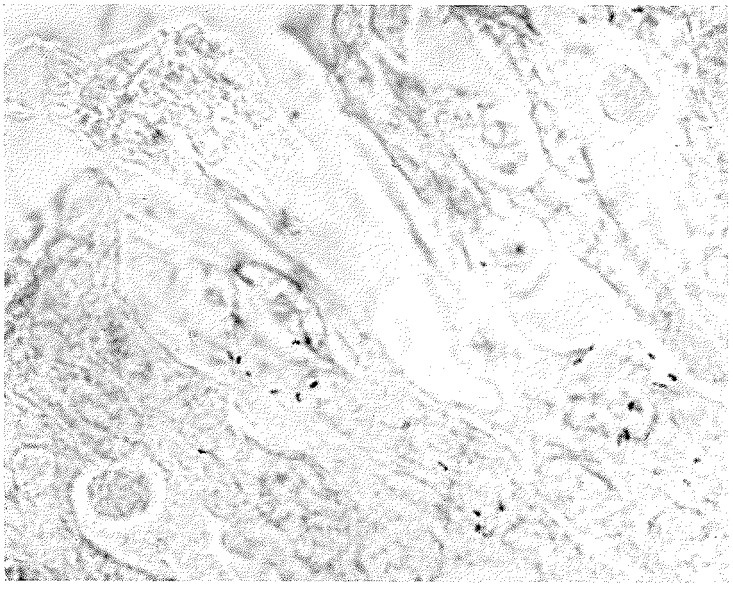


Fig. 4a. *H. influenzae* in clusters lying between the normal ciliated epithelium cells (Case No. 1, F. 71, duration of illness 7 days), corresponding to section of Fig. 1. Clear bipolar staining with methylene blue. (× 1600)

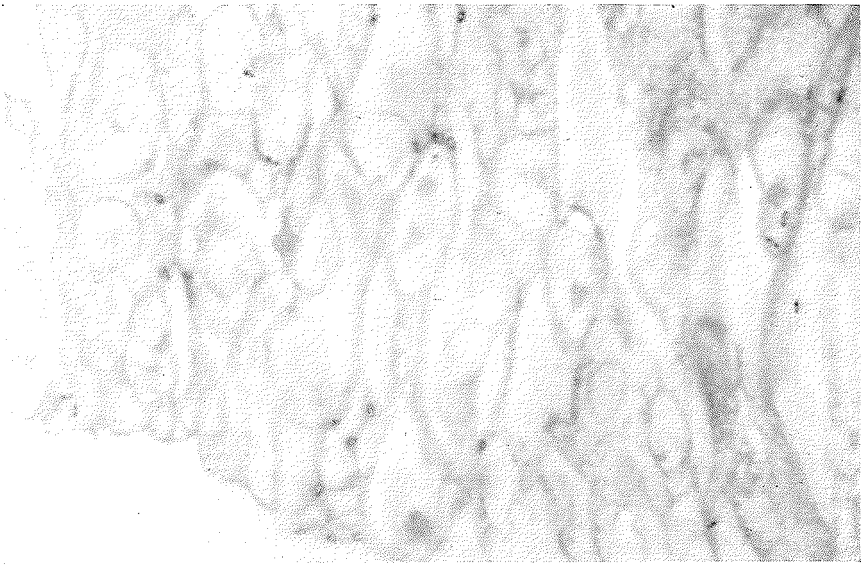


Fig. 4b. *H. influenzae* in clusters lying between the normal ciliated epithelium cells (Case No. 1, F. 71, duration of illness 7 days), corresponding to section of Fig. 1. Clear bipolar staining with methylene blue. (× 1600)

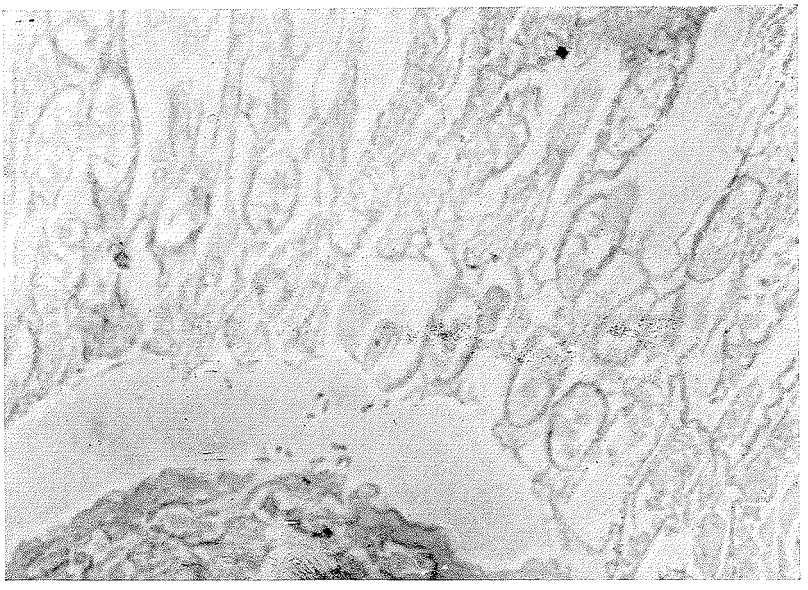


Fig. 5a. *H. influenzae* in clusters in the basement membrane deep to normal ciliated epithelium (Case No. 1, F. 71, duration of illness 7 days), corresponding to section of Fig. 2. Bipolar staining with methylene blue is clearly evident. (× 1600)

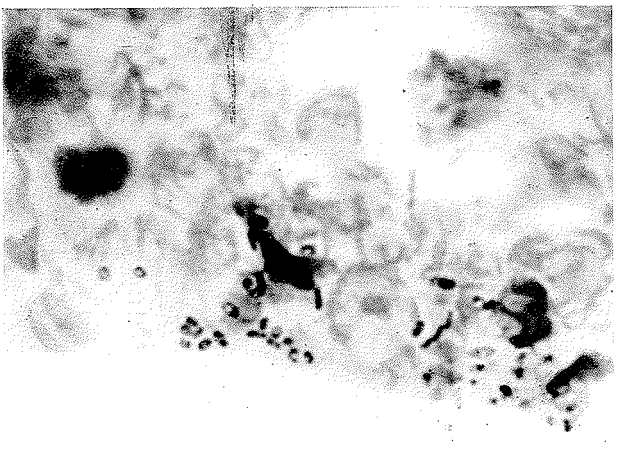


Fig. 5b. *H. influenzae* in clusters in the basement membrane deep to normal ciliated epithelium (Case No. 1, F. 71, duration of illness 7 days), corresponding to section of Fig. 2. Bipolar staining with methylene blue is clearly evident. (× 2000)

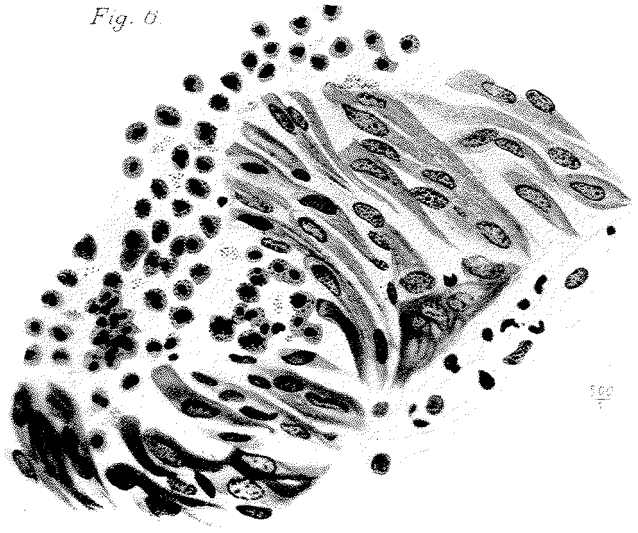


Fig. 6. Reproduction of a drawing from the publication of Pfeiffer in 1893, showing *H. influenzae* within non-necrotizing epithelium.

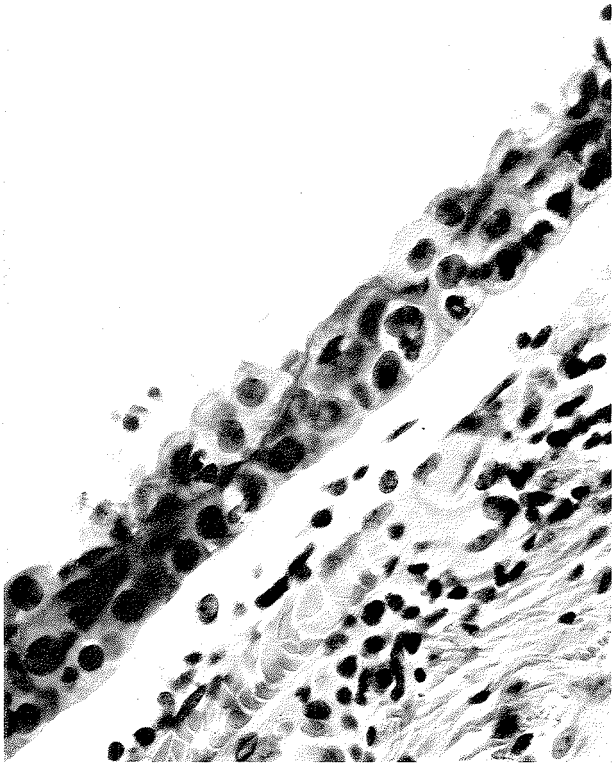


Fig. 7. Trachea (Case No. 5, F. 69, duration of illness 3 days): Absence of mucus- producing ciliated cells locally to the depth of the basal cell layer; swelling of cells and nuclei; no mitoses; occasional leucocytes in the epithelium and tunica propria; no hyperaemia or haemorrhage of the tunica propria. Haematoxylin-eosin staining. (× 600)

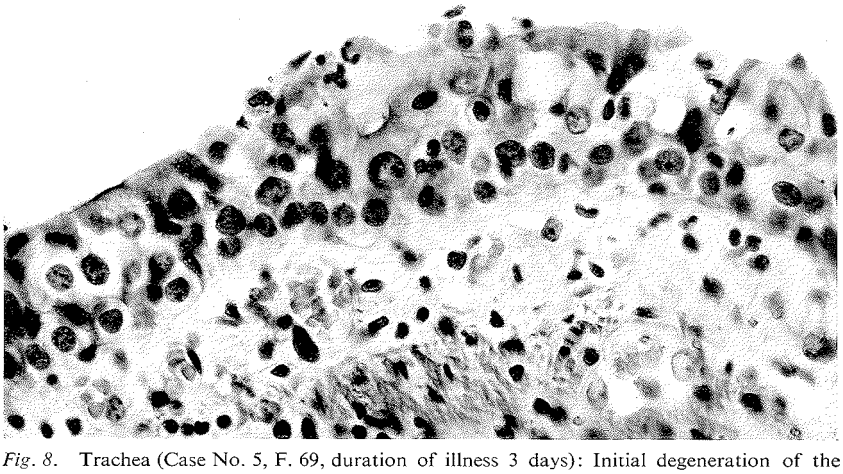


Fig. 8. Trachea (Case No. 5, F. 69, duration of illness 3 days): Initial degeneration of the ciliated epithelium; swelling of cells and nuclei; absence locally of mucus production and cilia; beginning desquamation of the superficial cell layer; occasional leucocytes in the epithelium; hyperaemia in tunica propria; no haemorrhages. Haematoxylin-eosin staining. (× 600)

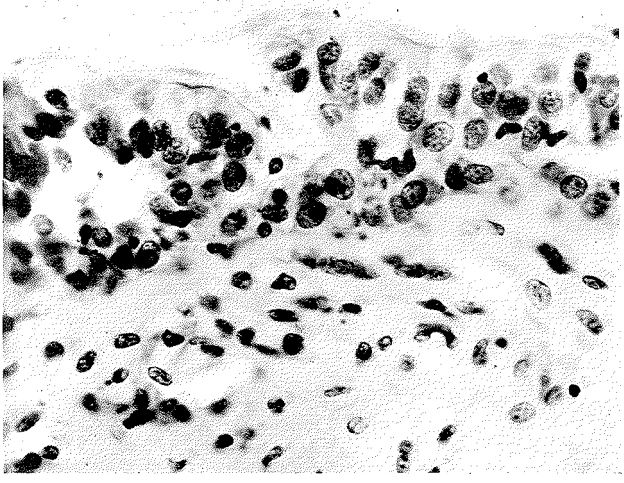


Fig. 9. Trachea (Case No. 5, F. 69, duration of illness 3 days): Initial degeneration of the ciliated epithelium; irregular nuclear position with nuclear swelling; cilia still intact; no mucus production; beginning desquamation; no hyperaemia or haemorrhage in the tunica propria; occasional leucocytes in the epithelium and tunica propria. Haematoxylin-eosin staining. (× 600)

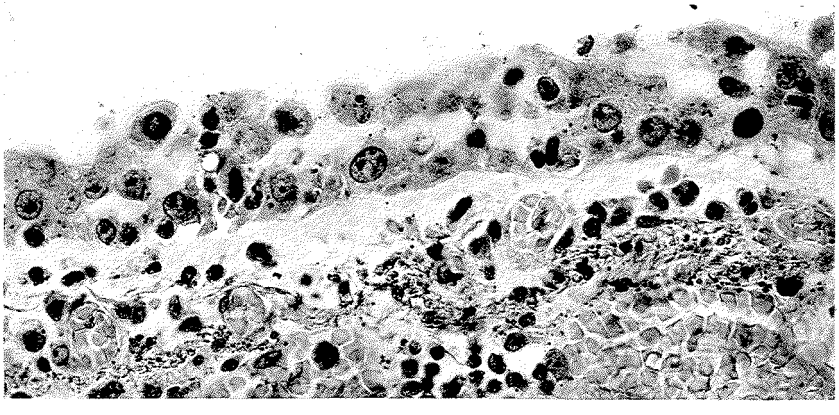


Fig. 10. Right main bronchus (Case No. 5, F. 69, duration of illness 3 days): Dissociation and desquamation of necrobiotic cells of the intermediary cell layer; basal cell layer intact; swelling of some cells and nuclei; occasional leucocytes in the epithelium and tunica propria; strong degree of hyperaemia in the tunica propria with local extravasation of erythrocytes. Haematoxylin-eosin staining. (× 600)

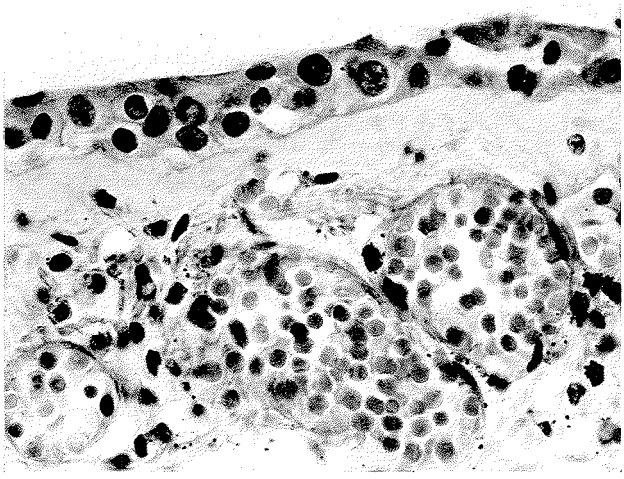


Fig. 11. Bronchus of pectoral segment of left upper lobe (Case No. 5, F. 69, duration of illness 3 days): Epithelial lining by the basal cell layer and occasional cells from the intermediary layer; severe swelling of some cells and nuclei; swelling of the basement membrane; strong hyperaemia of the tunica propria; no haemorrhages or leucocytic infiltration in the epithelium or tunica propria. Haematoxylin-eosin staining. (× 600)

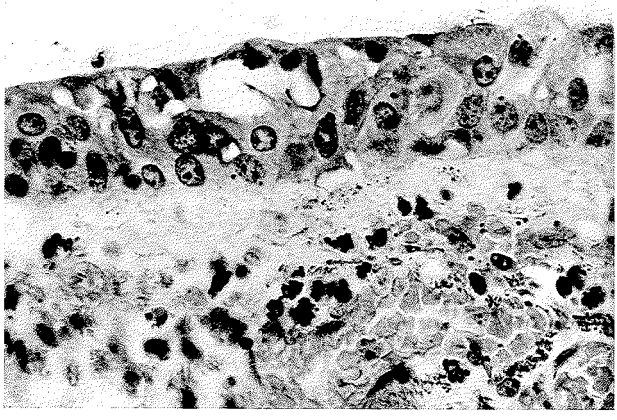


Fig. 12. Bronchus of pectoral segment of right upper lobe (Case No. 5, F. 69, duration of illness 3 days): Beginning degeneration in normal ciliated epithelium while cilia are only locally present; no mucus production; vacuolisation at various sites in the epithelium; irregular nuclear position; swelling of the nuclei in particular; occasional leucocytes; hyperaemia of the tunica propria. Haematoxylin-eosin staining. (× 600)

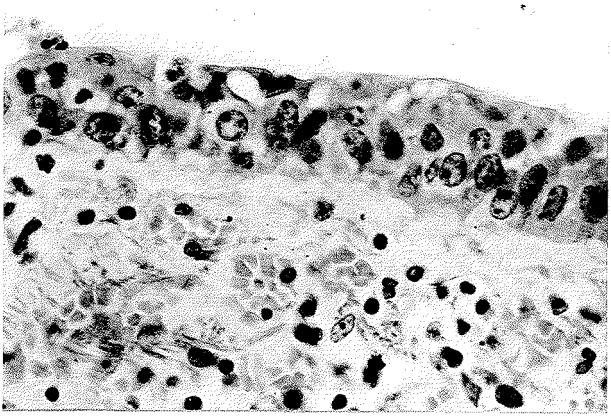


Fig. 13. Small bronchus of apical segment of left upper lobe (Case No. 5, F. 69, duration of illness 3 days): Beginning degeneration of epithelium; cilia not apparent; no mucus production; irregular nuclear position; swelling of the nuclei; vacuolisation of the cells; no leucocytic infiltration in epithelium or tunica propria; slight hyperaemia. Haematoxylin-eosin staining. (× 600)

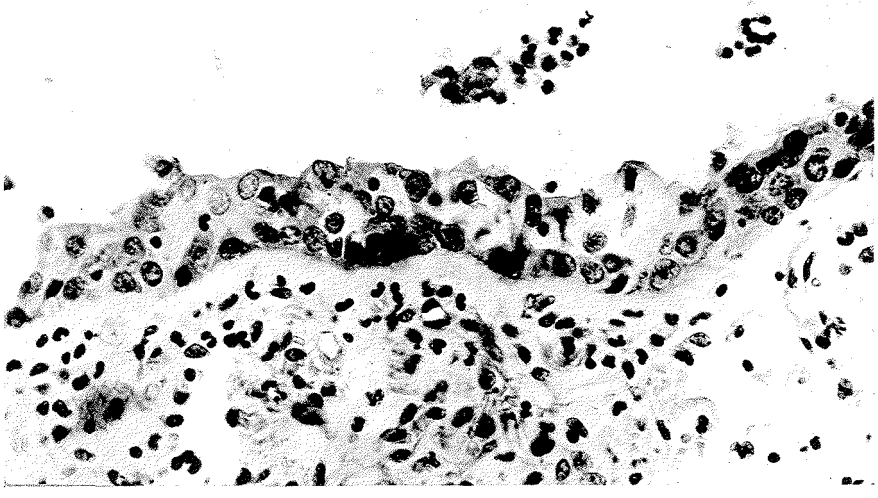


Fig. 14. Small bronchus of antero-basal segment of right lower lobe (Case No. 5, F. 69, duration of illness 3 days): Desquamation of the necrobiotic superficial cells; basal cell layer intact; slight hyperaemia and leucocytic infiltration in the tunica propria. Haematoxylin-eosin staining. (× 600)

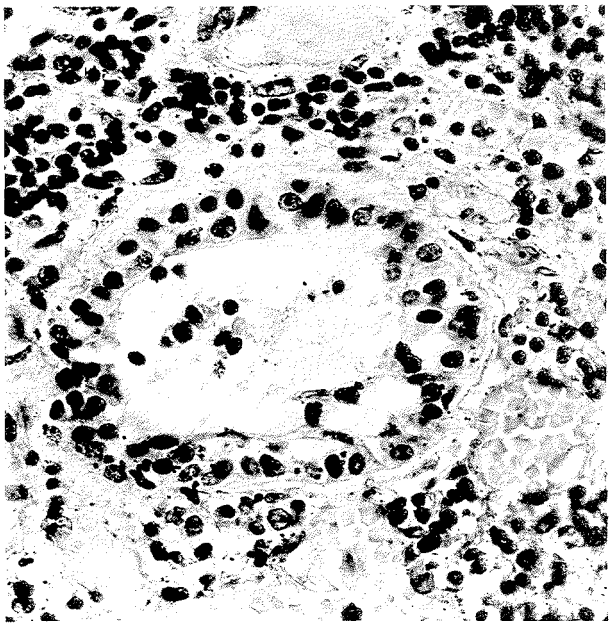


Fig. 15. Distal part of a lobular bronchiole bordering on a respiratory bronchiole (Case No. 5, F. 69, duration of illness 3 days): Beginning degeneration with vacuolisation of the cells; severe peribronchiolar hyperaemia; infiltration with lymphocytes rather than leucocytes. Haematoxylin-eosin staining. (× 600)

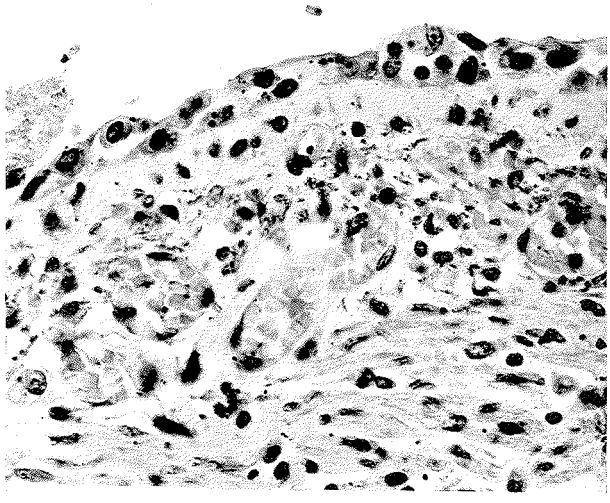


Fig. 16. Lobular bronchiole of dorsal segment of left upper lobe (Case No 5, F. 69, duration of illness 3 days): Only basal cell layer intact; desquamation of the intermediary cells; no leucocytic infiltration; hyperaemia of the tunica propria. Haematoxylin-eosin staining. (× 600)

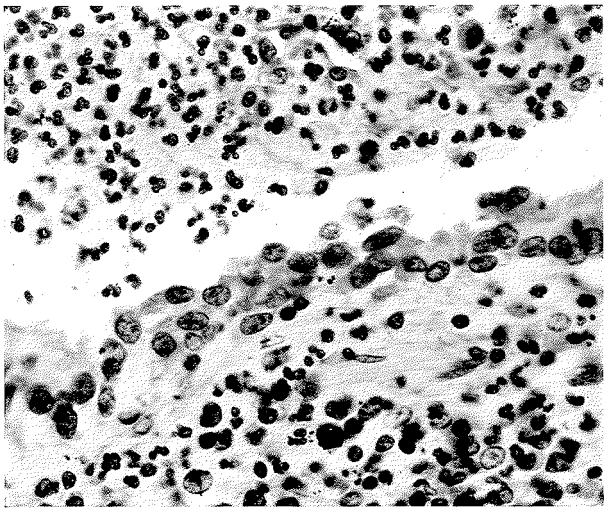


Fig. 17. Lobular bronchiole of antero-basal segment of left lower lobe (Case No. 5, F. 69, duration of illness 3 days): Degeneration of the epithelium to the depth of the basal and intermediary cell layer; no hyperaemia of the tunica propria; dense leucocytic infiltration and leucocytic exudate in bronchial lumen. Haematoxylin-eosin staining. (× 600)

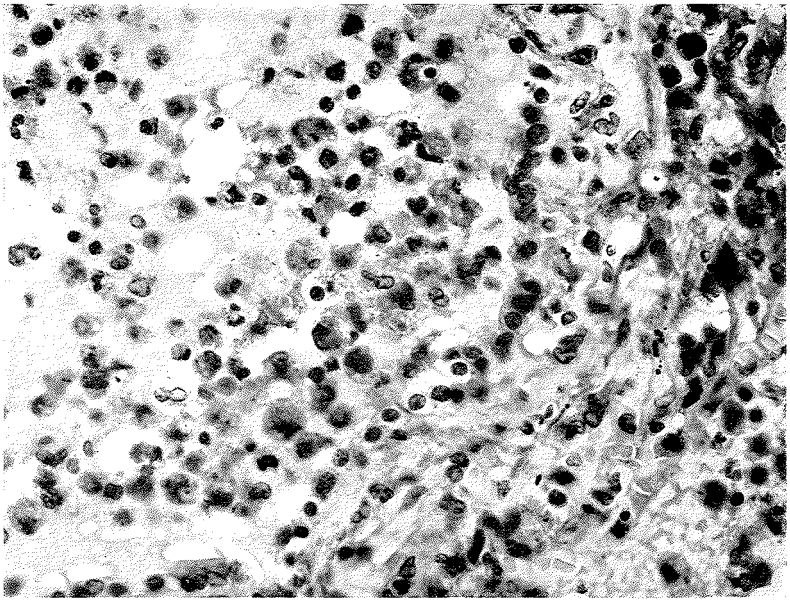


Fig. 18. Lobular bronchiole of postero-basal segment of right lower lobe (Case No. 5, F. 69, duration of illness 3 days): Epithelial lining exclusively by basal cell layer; hyperaemia and haemorrhage of the tunica propria; in the bronchiolar lumen oedema, leucocytes, and necrobiotic epithelial cells between which appear micro-organisms. Haematoxylineosin staining. (× 600)

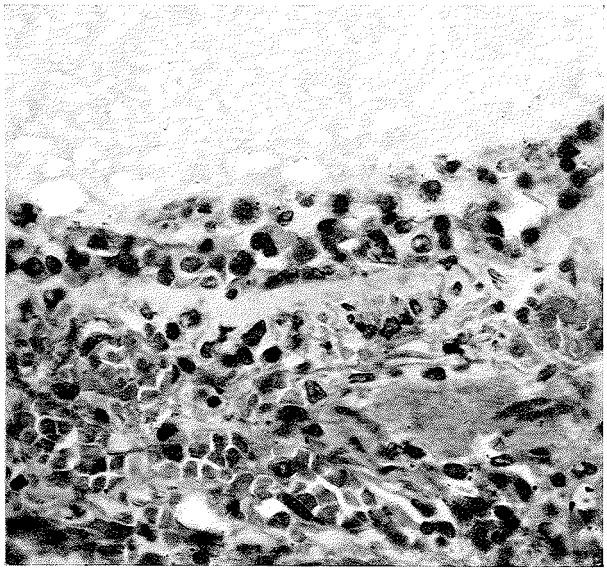


Fig. 19. Lobular bronchiole of the same lung segment (Case No. 5, F. 69, duration of illness 3 days): Desquamation of necrotic superficial epithelial cells to the depth of the basal cell layer; haemorrhage and hyperaemia of the tunica propria; protein-containing exudate in bronchiolar lumen, between which micro-organisms; no leucocytic exudation. Haematoxylin-eosin staining. (× 600)



Fig. 20. Small bronchus of left lower lobe (Case No. 5, F. 69, duration of illness 3 days): *H. influenzae* and a few pneumococci in the degenerated epithelium to the depth of the basement membrane. Methylene blue staining. (× 2000)

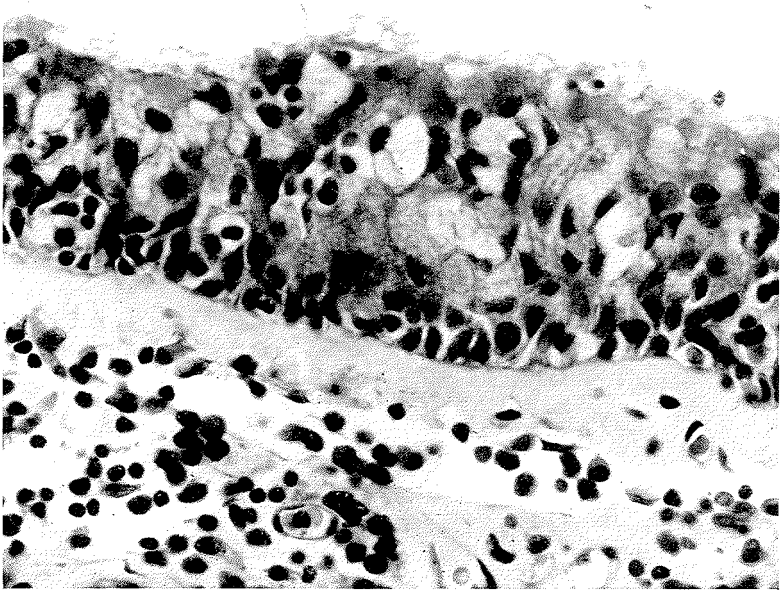


Fig. 21. Trachea (Case No. 6, F. 62, duration of illness 4 days): Superficial necrobiosis of the ciliated epithelium with loss of cilia and mucus production; irregular nuclear position; no hyperaemia, haemorrhage, or leucocytic infiltration of the tunica propria. Haematoxylin-eosin staining. (× 600)

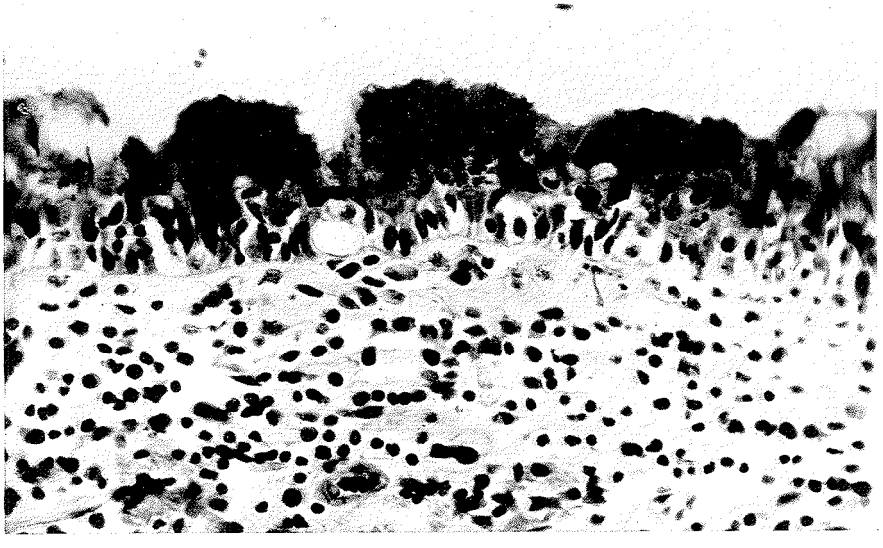


Fig. 22. Trachea (Case No. 6, F. 62, duration of illness 4 days): Superficial coagulative necrosis; loss of cilia and mucus production; pyknosis of the nuclei; no hyperaemia or haemorrhage but rather slight lymphocytic infiltration of the tunica propria. Haematoxylin-eosin staining. (× 500)

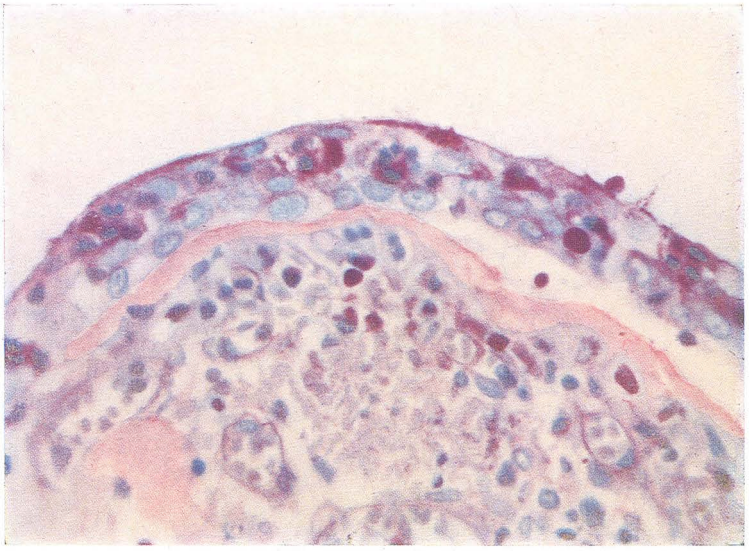


Fig. 23. Trachea (Case No. 6, F. 62, duration of illness 4 days): Superficial necrobiosis; loss of normal mucus production; no goblet cells; irregularly distributed Schiff-positive granules about the cells: irregular nuclear position in which diminished quantities of D.N.A.; no hyperaemia or leucocytic infiltration of the tunica propria; no haemorrhage. Bi-colour staining according to van Duijn. (× 600)

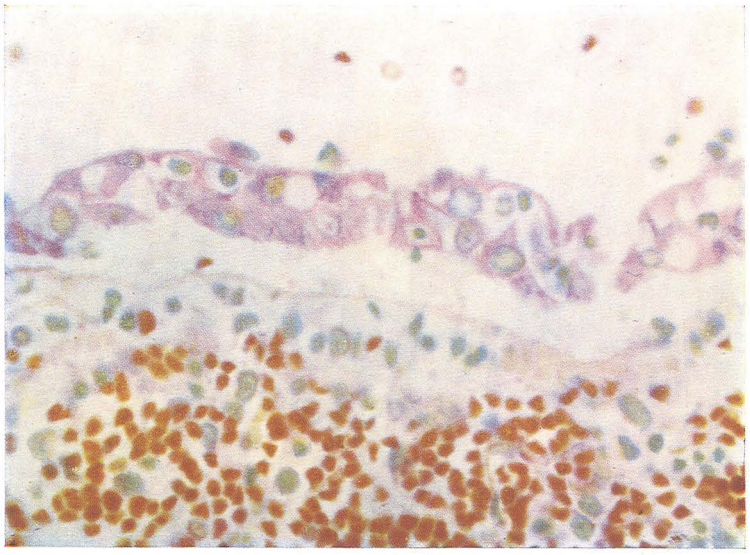


Fig. 24. Trachea (Case No. 6, F. 62, duration of illness 4 days): Superficial necrobiosis; loss of normal mucus production; marked pyroninophilia of the cytoplasm and interstitial spaces of the epithelium cells, due to high concentration of R.N.A.; slight haemorrhage of the tunica propria. Methylgreen-pyronin staining according to Trevan. (× 600)



Fig. 25. Left main bronchus (Case No. 6, F. 62, duration of illness 4 days): Epithelial lining by basal and intermediary cell layers; no bacteria, leucocytic infiltration, hyperaemia, or haemorrhage; hyperchromatosis of the wall of the nucleus of one basal cell; central „inclusion body" with halo in which failed D.N.A. and R.N.A. Phloxin-tartrazine staining. (× 1800)

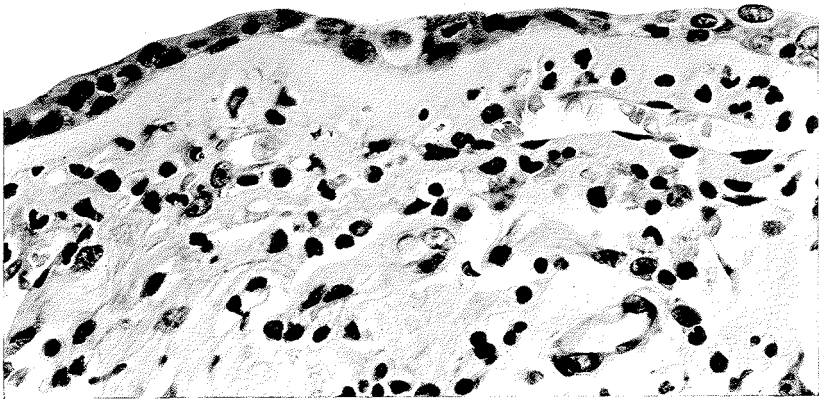


Fig. 26. Bronchus of left lower lobe (Case No. 6, F. 62, duration of illness 4 days): Epithelial lining consists exclusively of basal cell layer and occasional intermediary cells; swelling of basement membrane; no hyperaemia or haemorrhages in the tunica propria; occasional leucocytes. Phloxin-tartrazine staining. (× 600)

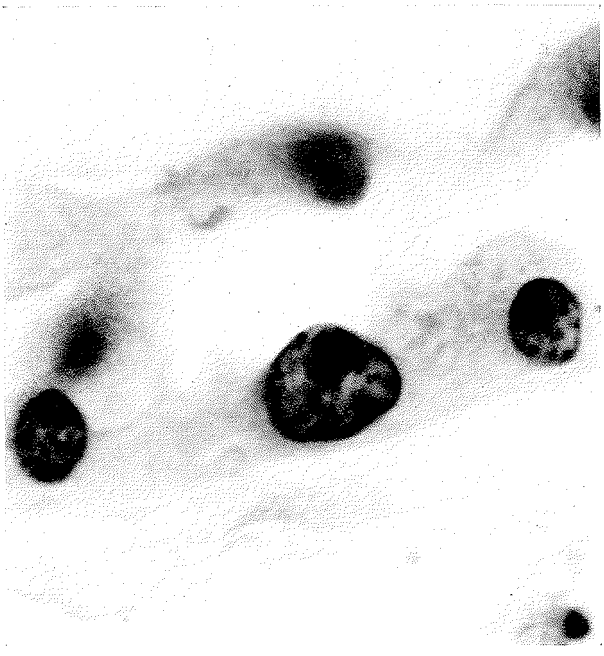


Fig. 27. Small bronchus of right lower lobe (Case No. 6, F. 62, duration of illness 4 days): Epithelial lining by basal cell layer with desquamation of the intermediary cells; polymorphic nuclei and large nucleoli in the basal cells with high concentration of D.N.A. (hyperactivity?); no bacteria, leucocytic infiltration, or hyperaemia. Bi-colour staining according to van Duijn. (× 1800)

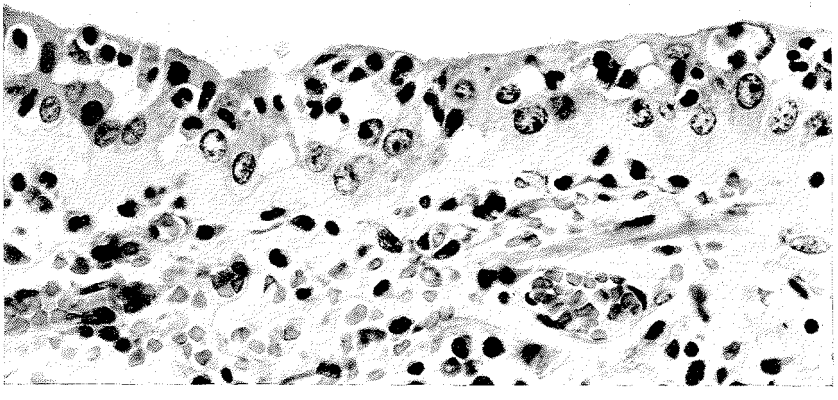


Fig. 28. Bronchus of latero-basal segment of left lower lobe (Case No. 6, F. 62, duration of illness 4 days): Beginning degeneration of the epithelium with vacuolisation; swelling of the nuclei; occasional leucocytes; extravasation of erythrocytes. Phloxin-tartrazine staining. (× 600)

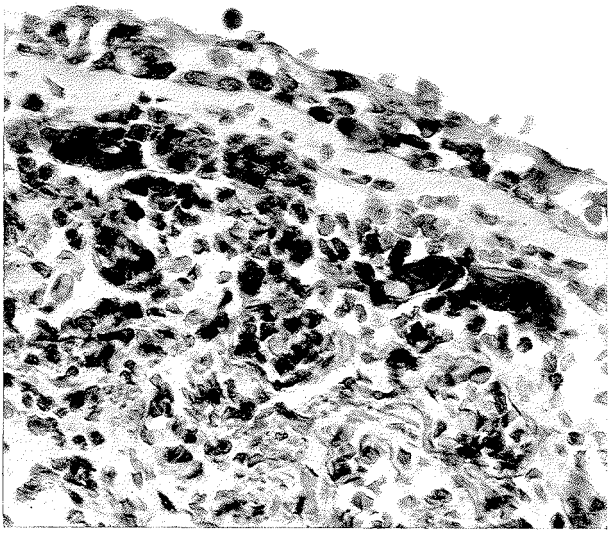


Fig. 29. Small bronchus of latero-basal segment of left lower lobe (Case No. 6, F. 62, duration of illness 4 days): Desquamation of necrobiotic epithelial cells to the depth of the basal cell layer; haemorrhage in tunica propria no leucocytic infiltration. Phloxin-tartrazine staining. (× 600)

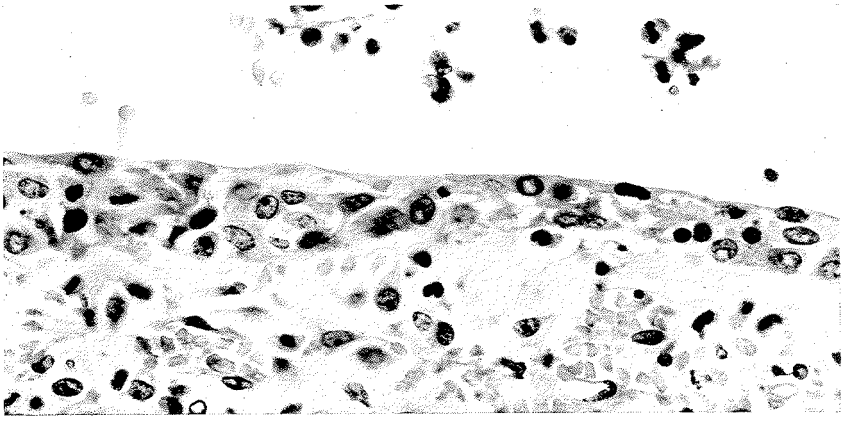


Fig. 30. Small bronchus of postero-basal segment of right lower lobe (Case No. 6, F. 62, duration of illness 4 days): Epithelial lining consists of the basal and intermediary cell layers, the latter cells being flattened out; no haemorrhage or leucocytic infiltration of the tunica propria. Phloxin-tartrazine staining. (× 600)

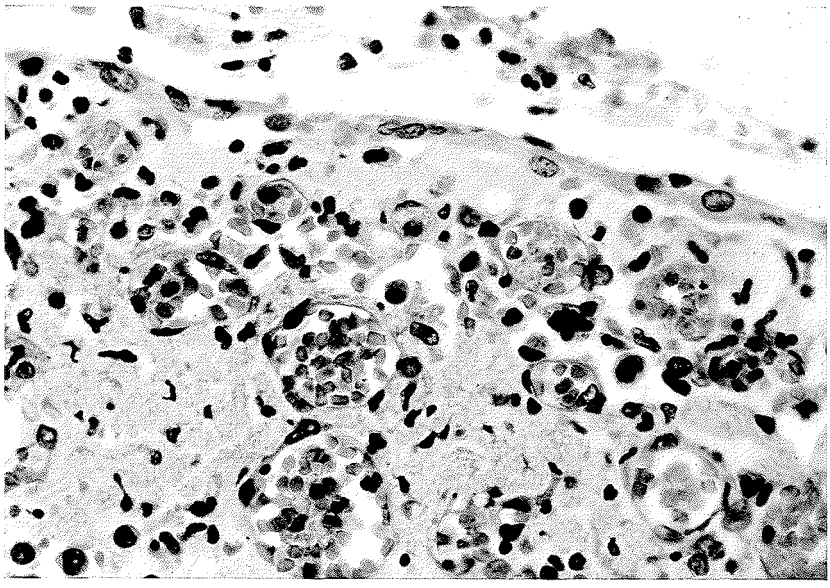


Fig. 31. Lobular bronchiole of right middle lobe (Case No. 6, F. 62, duration of illness 4 days): Epithelial lining consists exclusively of basal cell layer; occasional leucocytes; hyperaemia and extravasation of erythrocytes in the tunica propria. Phloxin-tartrazine staining. (× 600)

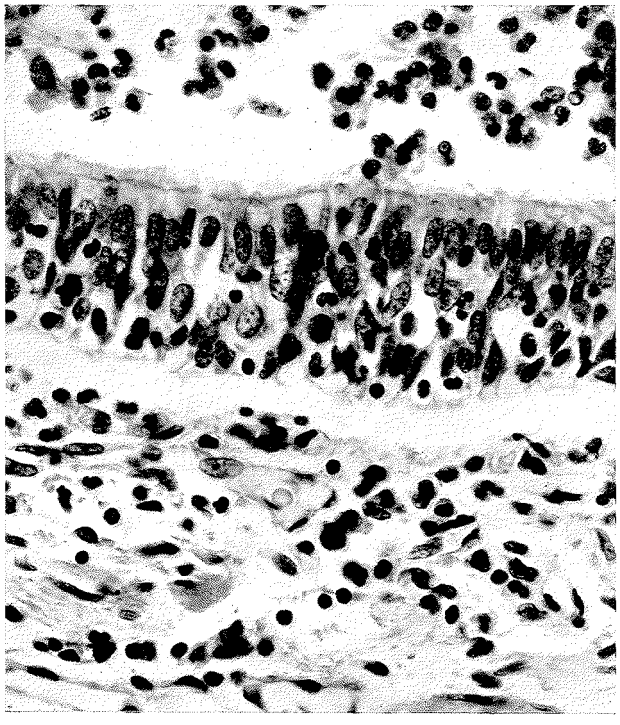


Fig. 32. Small bronchus of latero-basal segment of left lower lobe (Case No. 6, F.62, duration of illness 4 days): Normal ciliated epithelium; infiltration of the epithelium and tunica propria with leucocytes and lymphocytes. Phloxin-tartrazine staining. (× 600)

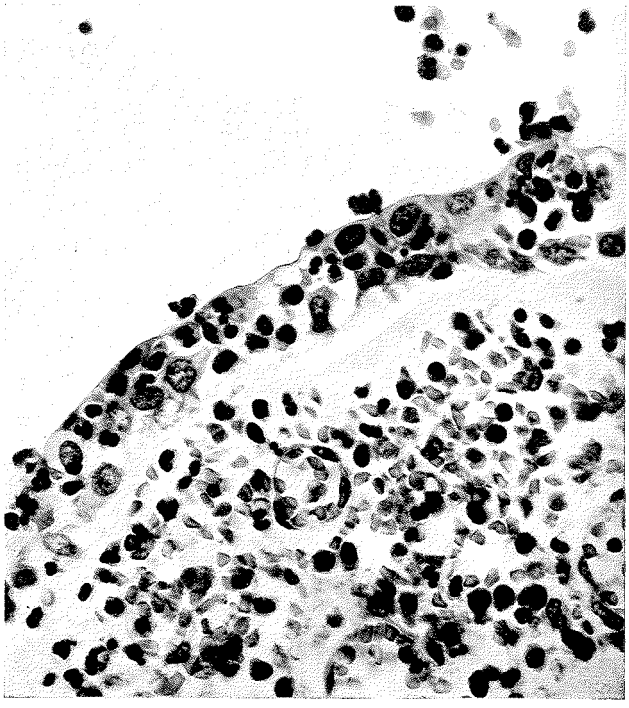


Fig. 33. The same small bronchus of the latero-basal segment of the left lower lobe, but at a more peripheral level (Case No. 6, F. 62, duration of illness 4 days): Degenerated epithelium; vacuolisation and swelling of cells and nuclei; leucocytic infiltration of epithelium and tunica propria; hyperaemia and haemorrhage in tunica propria. Phloxin-tartrazine staining. (× 600)

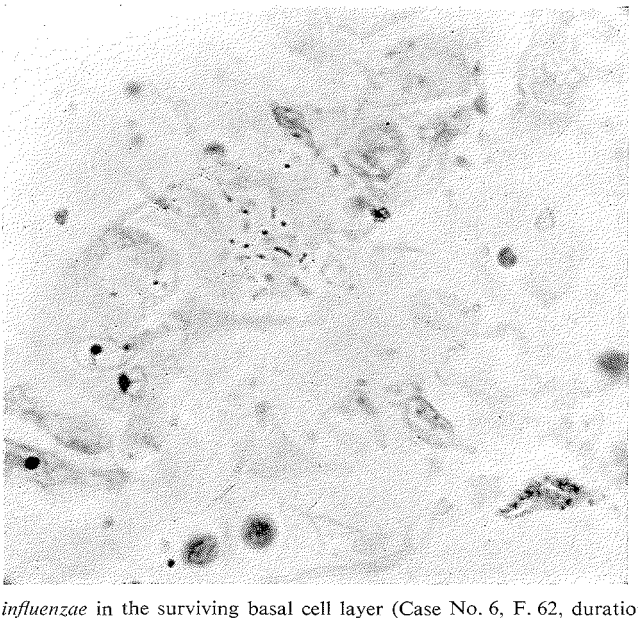


Fig. 34. *H. influenzae* in the surviving basal cell layer (Case No. 6, F. 62, duration of illness 4 days); bipolar staining clearly present. Methylene blue staining. (× 1600)

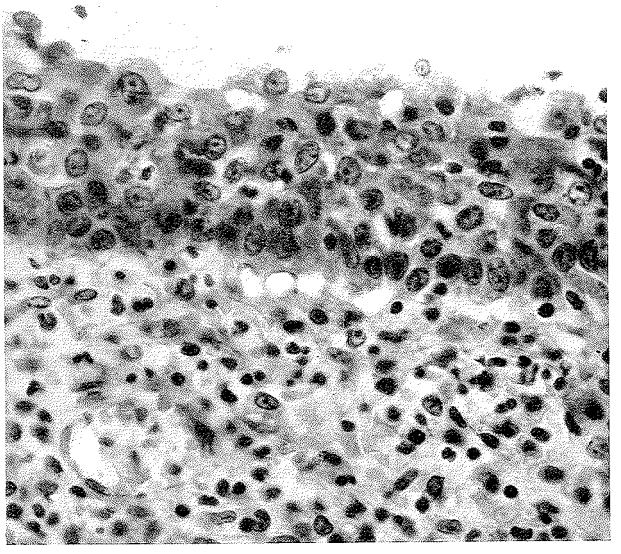


Fig. 35. Bronchus of right lower lobe (Case No. 7, F. 84, duration of illness $5\frac{1}{2}$ days): Regeneration to six layers, the uppermost of which exhibits necrobiosis and one mitosis; hyperaemia and focal haemorrhage of the tunica propria; leucocytes and many lymphocytes in epithelium and tunica propria. Haematoxylin-eosin staining. ($\times$ 600)

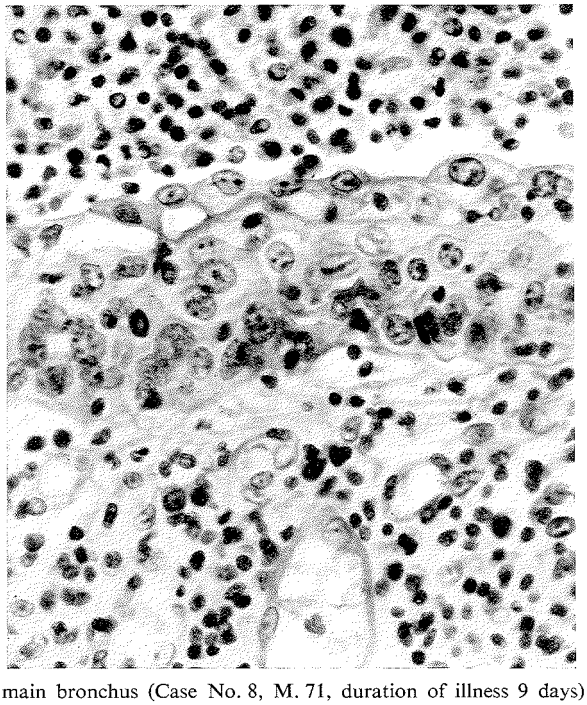


Fig. 36. Left main bronchus (Case No. 8, M. 71, duration of illness 9 days): Regeneration to a four layered undifferentiated epithelium with pseudostratification of the uppermost cell layer; swelling of cells and nuclei; slight hyperaemia of the tunica propria; predominantly lymphocytic infiltration of epithelium and tunica propria. Haematoxylin-eosin staining. ($\times$ 600)

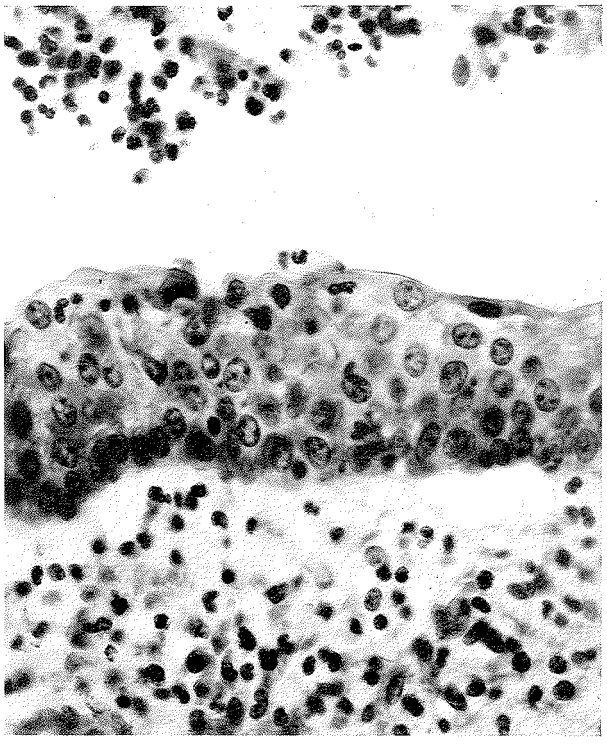


Fig. 37. Bronchus of right lower lobe (Case No. 9, F. 54, duration of illness 15 days): Epithelial lining with a six layered undifferentiated epithelium, the uppermost layer of which is flattened out; lymphocytic and leucocytic infiltration of epithelium and tunica propria; no hyperaemia or oedema. Haematoxylin-eosin staining. (× 600)

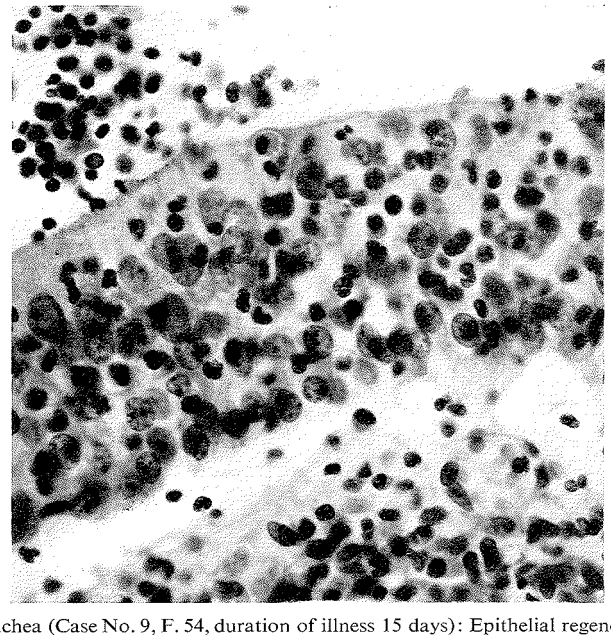


Fig. 38. Trachea (Case No. 9, F. 54, duration of illness 15 days): Epithelial regeneration almost completed although nuclear positions are still variable; no mucus production, but locally small cilia; dense lymphocytic infiltration of epithelium and tunica propria; some leucocytes. Haematoxylin-eosin staining. (× 600)

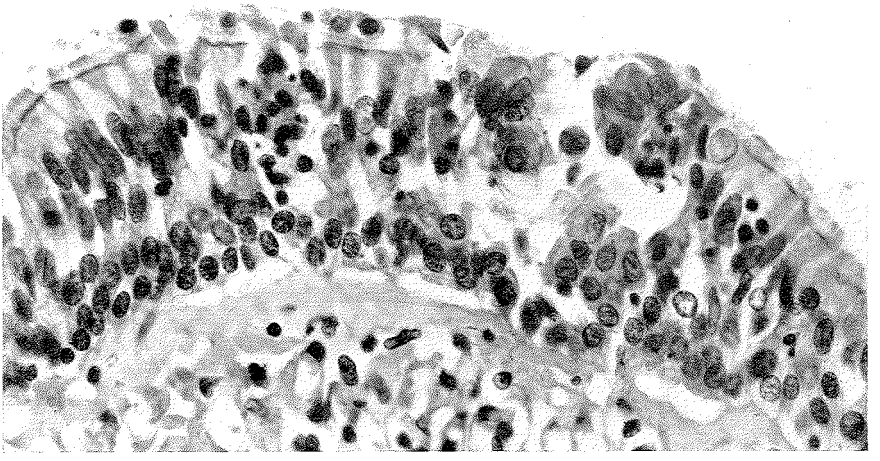


Fig. 39. Trachea (Case No. 11, F. 19, duration of illness 2 days): Beginning degeneration of the ciliated epithelium; swelling of cells and nuclei and loss of mucus production; desquamation of the necrobiotic cells to the depth of the basal cell layer, which, in itself remains intact; occasional leucocytes in epithelium and tunica propria; irregular swelling of the basement membrane. Haematoxylin-eosin staining. (× 600)

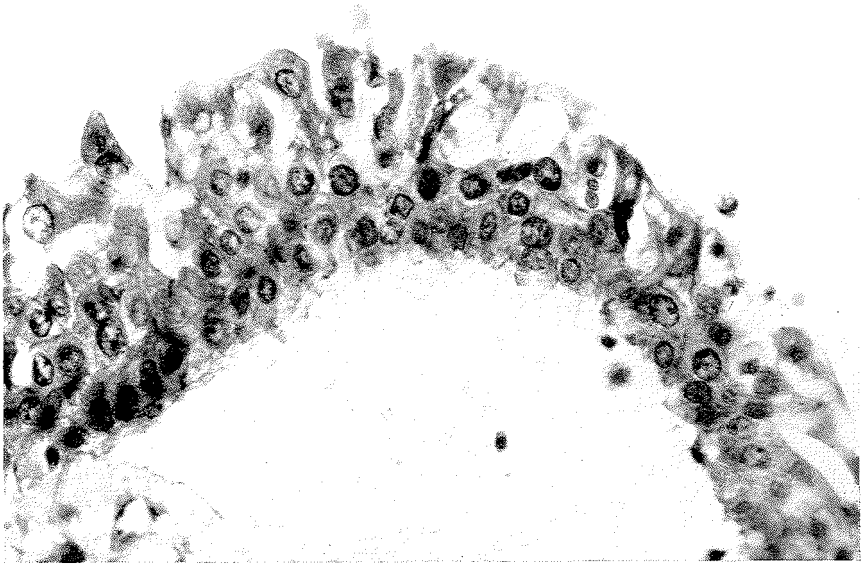


Fig. 40. Trachea (Case No. 11, F. 19, duration of illness 2 days): Beginning degeneration and desquamation to the depth of the intermediary cell layer with vacuolisation, loss of cilia and mucus production of the cells; the epithelium is upraised as a nodule above the basement membrane by an accumulation of oedema; no leucocytic infiltration. Haematoxylin-eosin staining. (× 600)

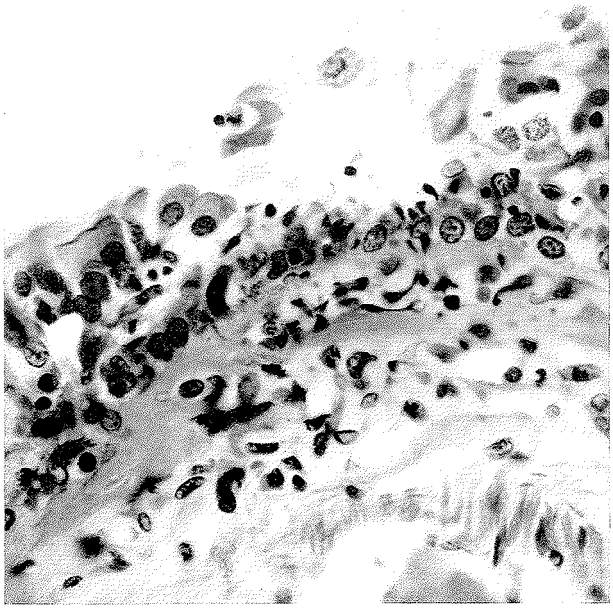


Fig. 41. Left main bronchus (Case No. 11, F. 19, duration of illness 2 days): Necrobiosis of the basal cells and desintegration of the basement membrane; formation of leucocytic exudate in the tunica propria. Haematoxylin-eosin staining. (× 600)

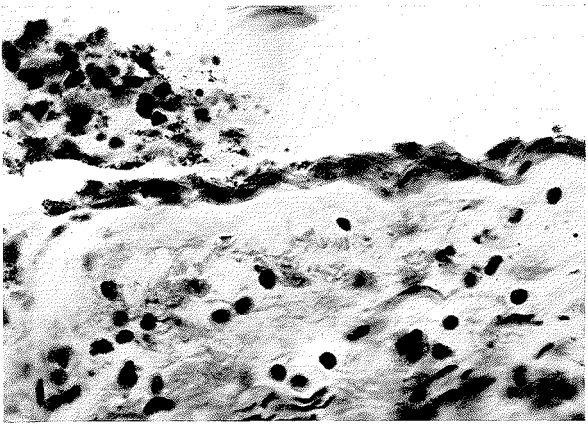


Fig. 42. Bronchus of left lower lobe (Case No. 10, M. 15, duration of illness 2 days): The surviving basal cells are filled with Staphylococci. Swelling of the basement membrane; no leucocytic infiltration in the tunica propria. Haematoxylin-eosin staining. (× 600)

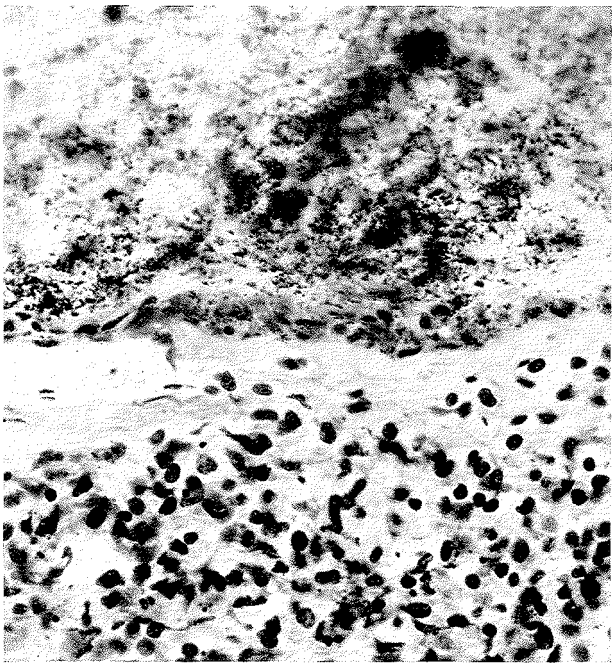


Fig. 43. Bronchus of right middle lobe (Case No. 11, F. 19, duration of illness 2 days): The basal cell layer and intermediary cell layer are strewn with Staphylococci; swelling of the basement membrane; leucocytic infiltration in the tunica propria; lumen of the bronchus filled with Staphylococci. Haematoxylin-eosin staining. (× 600)

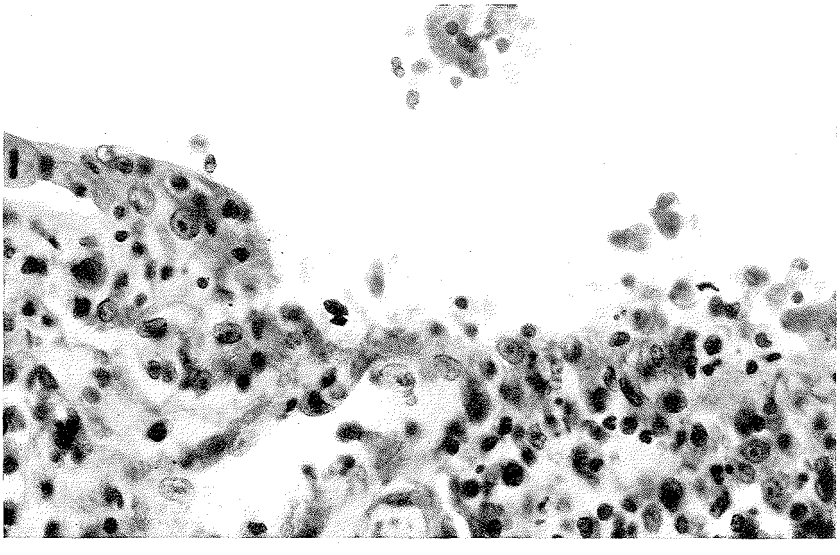


Fig. 44. Bronchus of right lower lobe (Case No. 11, F. 19, duration of illness 2 days): Absence of epithelium and basement membrane over a large portion; superficial necrosis of the tunica propria with necrotic capillary walls; dense leucocytic infiltration; just at the border of the surviving basal cells a mitosis is present. Haematoxylin-eosin staining. (× 600)

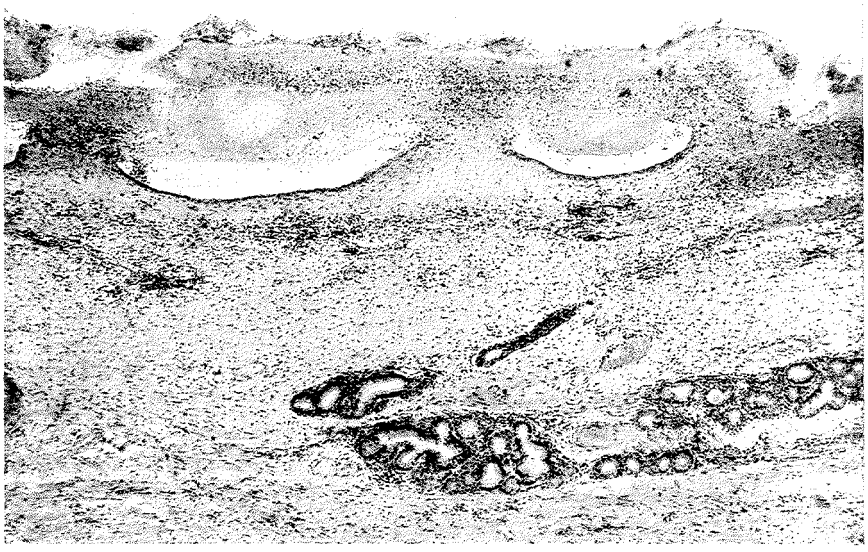


Fig. 45. Trachea (Case No. 11, F. 19, duration of illness 2 days): At three places explosive penetration of the exudate from the severely swollen tunica propria through the basal cell layer and the basement membrane. Haematoxylin-eosin staining. (× 150)

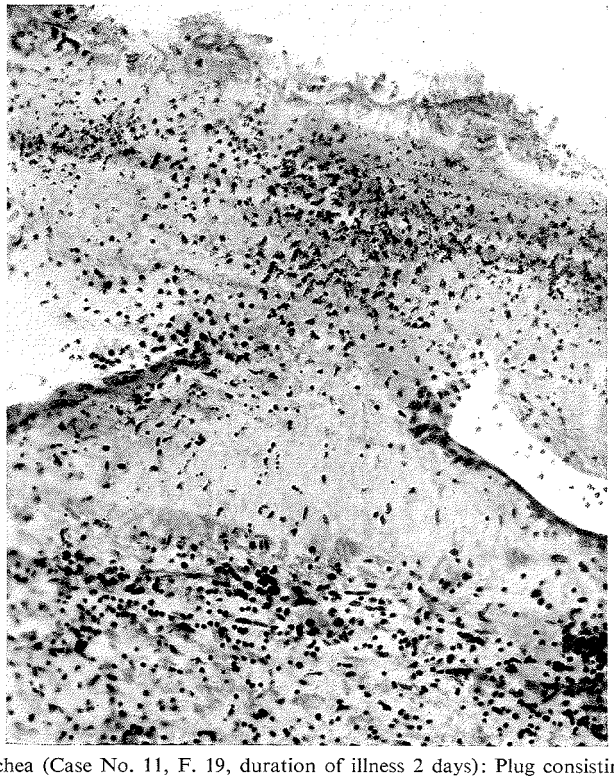


Fig. 46. Trachea (Case No. 11, F. 19, duration of illness 2 days): Plug consisting of protein-rich exudate and leucocytes due to which the basal cell layer is retracted to both sides. Haematoxylin-eosin staining. (× 350)

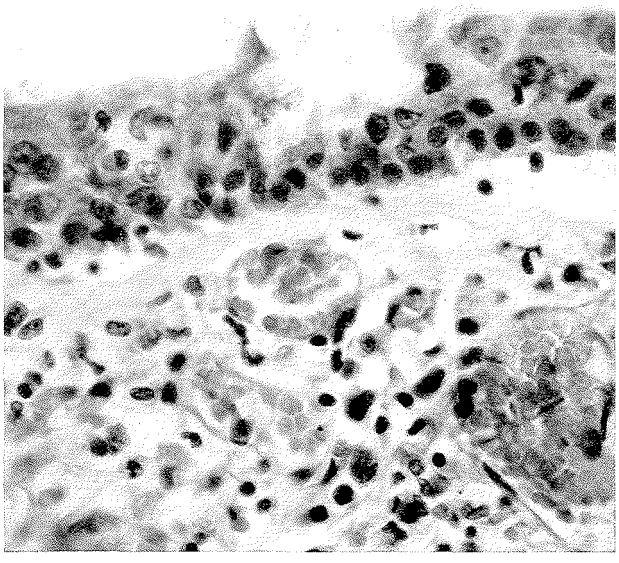


Fig. 47. Small bronchus of apical segment of right upper lobe (Case No. 11, F. 19 duration of illness 2 days): Beginning degeneration of the ciliated epithelium; loss of mucus production and cilia; local desquamation to the depth of the intermediary cell layer; hyperaemia of the tunica propria; occasional leucocytes and lymphocytes in tunica propria and epithelium. Haematoxylin-eosin staining. ($\times$ 600)

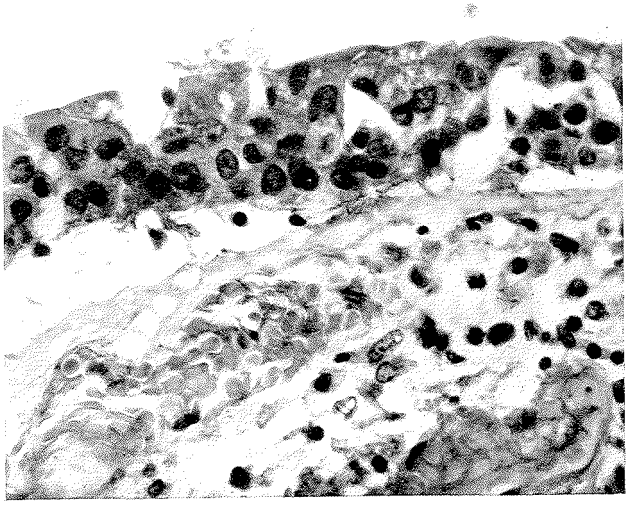


Fig. 48. Small bronchus of pectoral segment of right upper lobe (Case No. 11, F. 19, duration of illness 2 days): Swelling of cells and nuclei; vacuolisation and beginning desquamation; local oedema between epithelium and basement membrane; hyperaemia of the tunica propria without leucocytic infiltration of epithelium or tunica propria. Haematoxylin-eosin staining. ($\times$ 600)

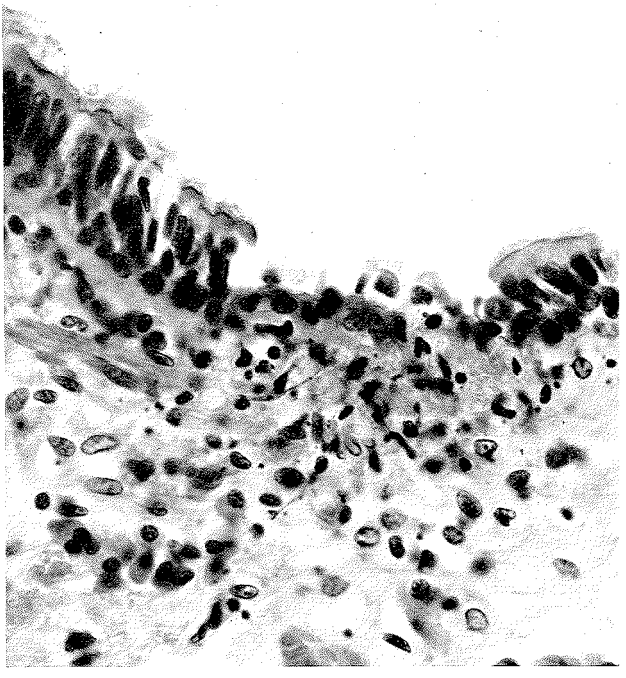


Fig. 49. Lobular bronchiole of apical segment of right upper lobe (Case No. 11, F. 19, duration of illness 2 days): Focal epithelial loss in normal epithelium to the depth of the basal cell layer which reveals necrobiosis at one place; swelling and fibrinoid degeneration of the basement membrane; beginning leucocytic infiltration of the tunica propria at the site of the epithelial lesion; no leucocytes in the normal epithelium or leucocytic exudate in the bronchiolar lumen. Haematoxylin-eosin staining. ($\times$ 600)

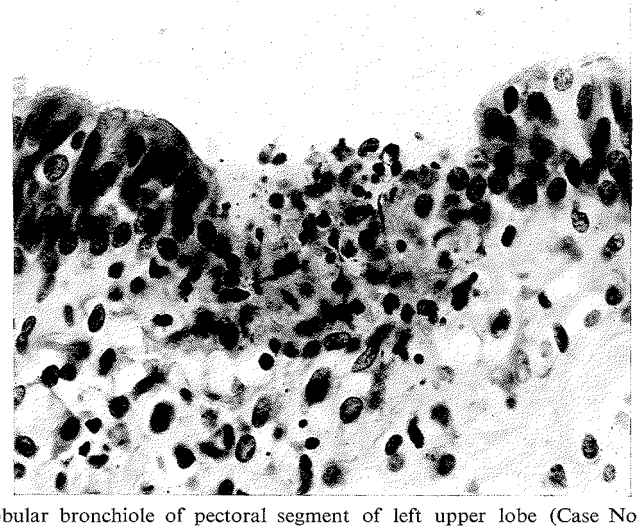


Fig. 50. Lobular bronchiole of pectoral segment of left upper lobe (Case No. 11, F. 19, duration of illness 2 days): Initial formation of an exudate plug issuing from the tunica propria and surrounded by normal epithelium. Haematoxylin-eosin staining. ($\times$ 600)

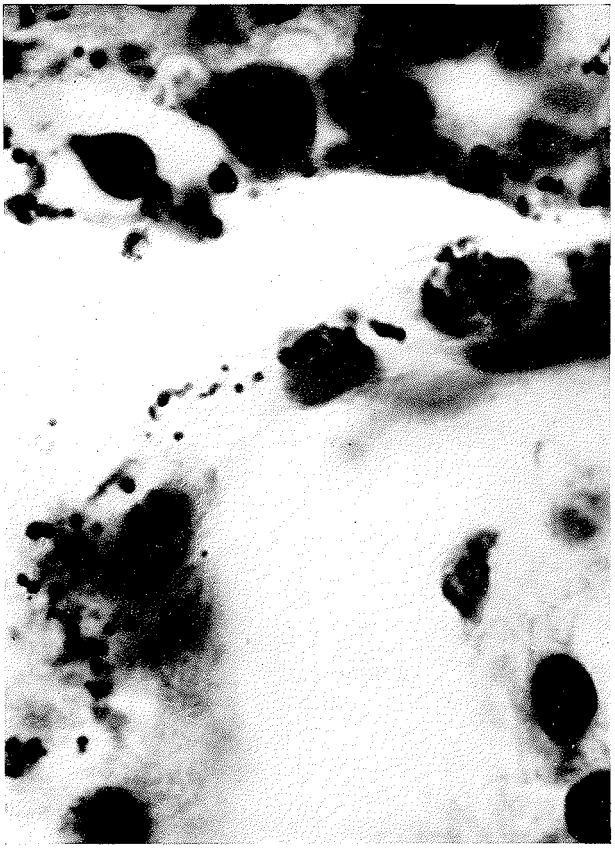


Fig. 51. (Case No. 11, F. 19, duration of illness 2 days): Staphylococci in the surviving basal cell layer of the epithelium. Gram-stain according to MacCallum. ($\times$ 1800)

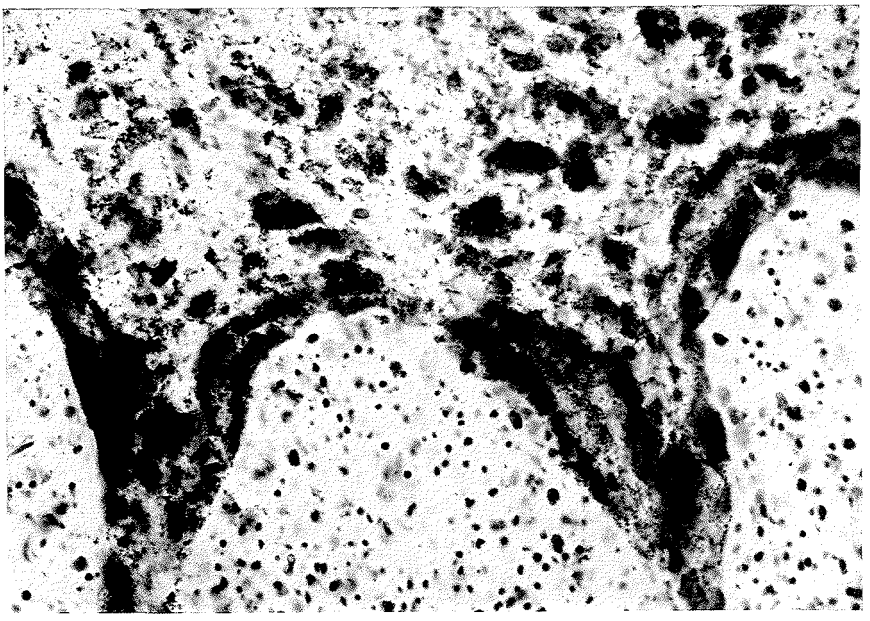


Fig. 52. (Case No. 11, F. 19, duration of illness 2 days): Staphylococcus colonies in the lumen of a bronchus and on the tunica propria denuded of epithelium. Gram-stain according to MacCallum. (× 400)

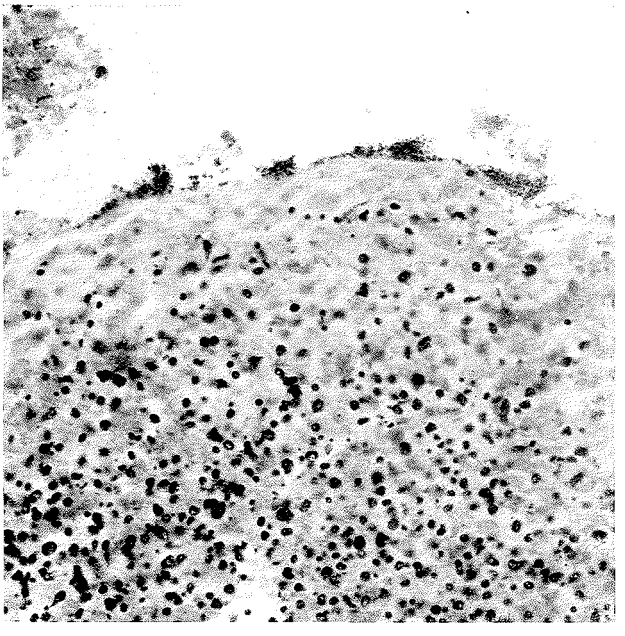


Fig. 53. (Case No. 11, F. 19, duration of illness 2 days): Staphylococcus colonies on the surface of an exudate plug. Gram-stain according to MacCallum. (× 400)

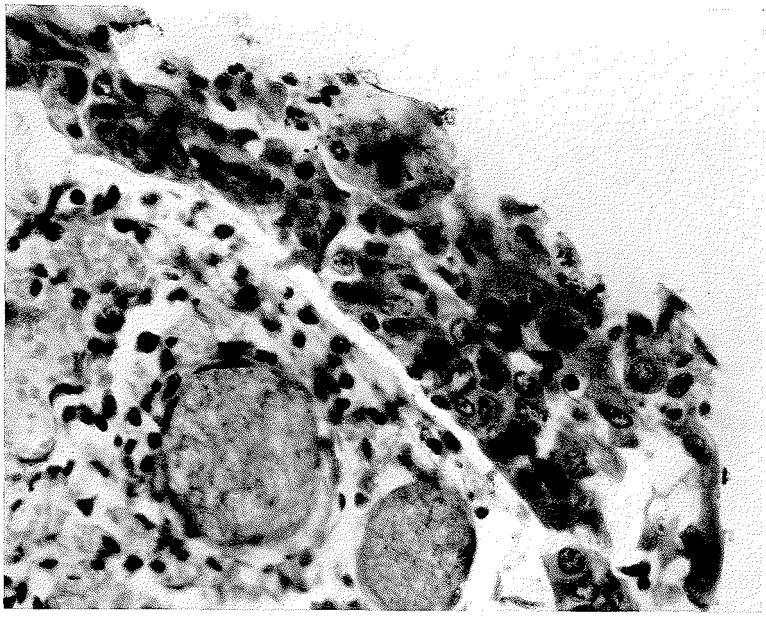


Fig. 54. Trachea (Case No. 13, F. 40, duration of illness 3 days): Coagulative necrosis of the superficial cell layer locally to the depth of and inclusive of the basal cell layer; hyperaemia and leucocytes in the tunica propria and in the epithelium. Haematoxylin-eosin staining. ($\times$ 600)



Fig. 55. Trachea (Case No. 13, F. 40, duration of illness 3 days): Exudate plug consisting of fibrin and leucocytes; regeneration to two or three layered undifferentiated epithelium on the right side; dense leucocytic infiltration of the tunica propria; focal necrosis in the tunica propria under the epithelial defect. Haematoxylin-eosin staining. (× 200)



Fig. 56. Trachea (Case No. 13, F. 40, duration of illness 3 days): Confluent exudate plugs consisting of fibrin and leucocytes; epithelial rests consisting of basal cells and a regenerating layer between the epithelial defects; fibrinoid necrosis in the tunica propria and dense leucocytic infiltration. Haematoxylin-eosin staining. (× 200)

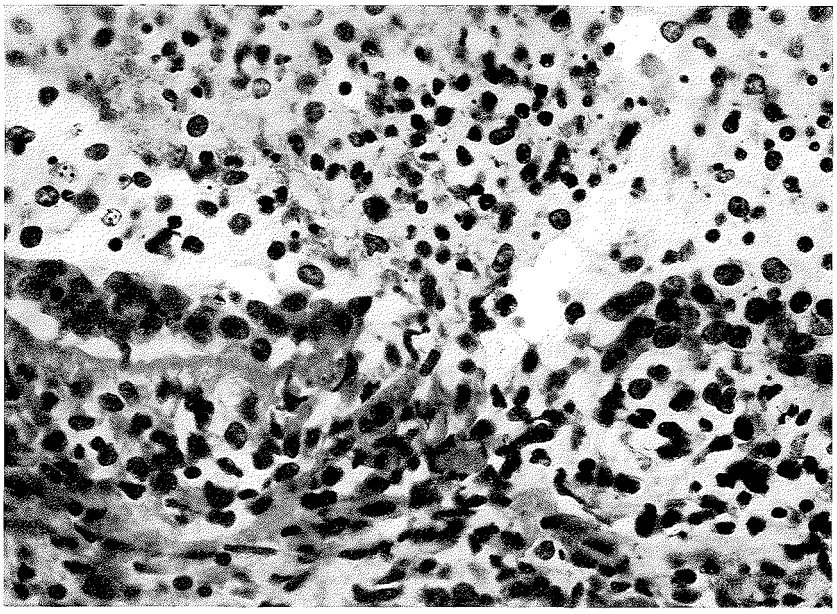


Fig. 57. Bronchus of antero-basal segment of left lower lobe (Case No. 12, M. 27, duration of illness 3 days): An exudate plug consisting of fibrin and leucocytes protrudes into the lumen; bilateral regenerative epithelium of two to three layers. Haematoxylin-eosin staining. (× 600)

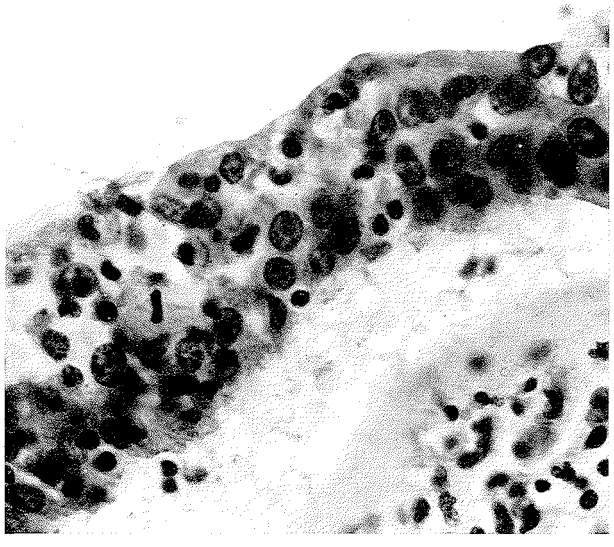


Fig. 58. Trachea (Case No. 14, F. 60, duration of illness 5 days): Regenerative epithelium of four layers; two mitoses; irregular nuclear position; swelling of cells and nuclei; oedema between epithelium and basement membrane; basement membrane frayed; leucocytes in tunica propria. Haematoxylin-eosin staining. (× 600)

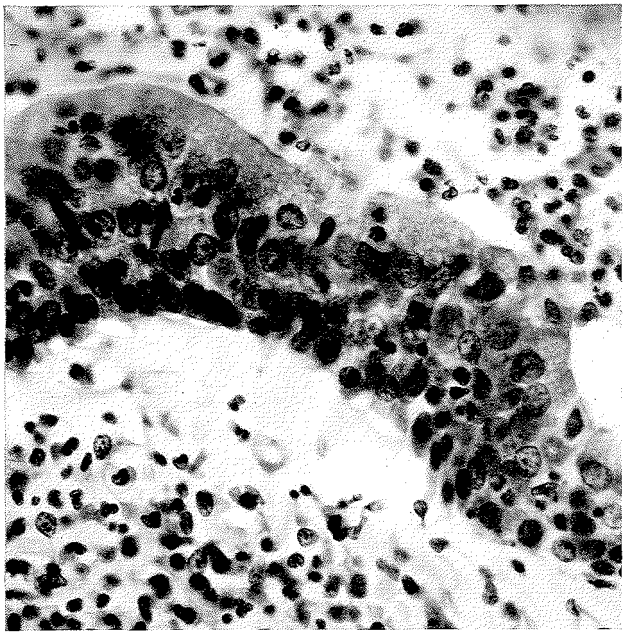


Fig. 59. Trachea (Case No. 16, F. 49, duration of illness 14 days): Almost normal ciliated epithelium; severely swollen nuclei and irregular nuclear position especially in the superficial cell layers; locally, meagre mucus production and dubious cilia; leucocytic infiltration of epithelium and tunica propria; leucocytic exudate in the lumen. Haematoxylin-eosin staining. (× 600)

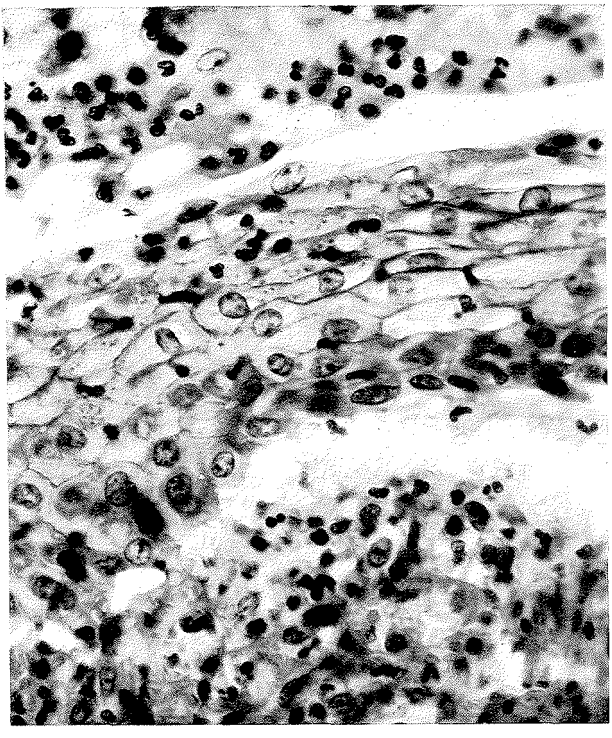


Fig. 60. Left main bronchus (Case No. 16, F. 49, duration of illness 14 days): "Metaplastic stratification" of the superficial layers of the undifferentiated epithelium; leucocytic infiltration in the epithelium and in the oedematous tunica propria; absence of a stratum granulosum and papilla formation; granules resembling prae-keratin in the superficial cells. Haematoxylin-eosin staining. (× 600)

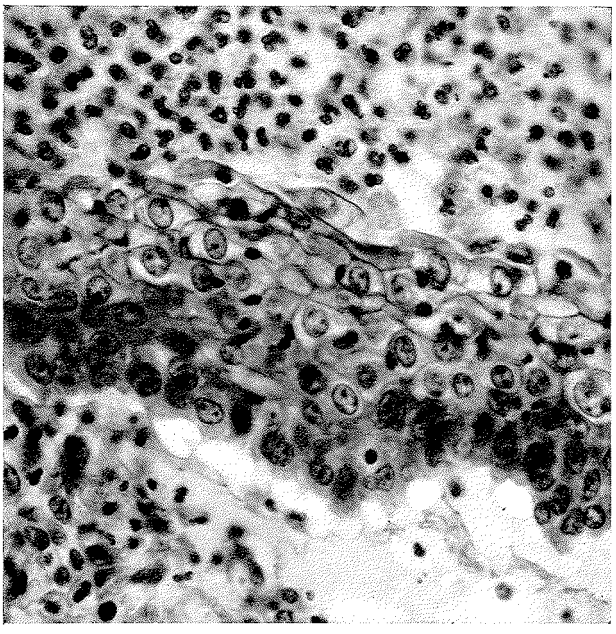


Fig. 61. Right main bronchus (Case No. 16, F. 49, duration of illness 14 days): "Metaplastic stratification" of the undifferentiated epithelium; leucocytic infiltration of the epithelium and in the oedematous tunica propria; leucocytic exudate in the lumen. Haematoxylin-eosin staining. (× 600)

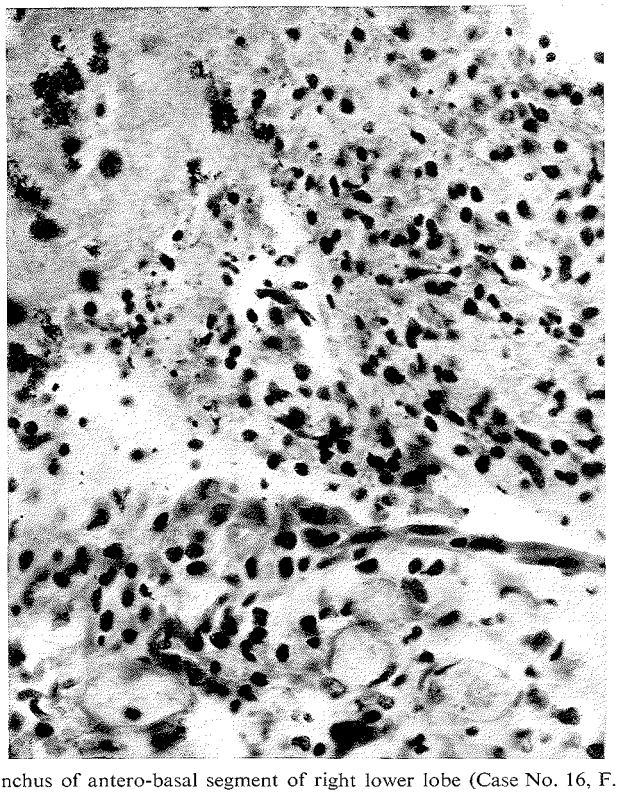


Fig. 62. Bronchus of antero-basal segment of right lower lobe (Case No. 16, F. 49, duration of illness 14 days): Locally lined only by the basal cell layer; leucocytic, purulent exudate with Staphylococcus colonies in the lumen. Haematoxylin-eosin staining. (× 600)

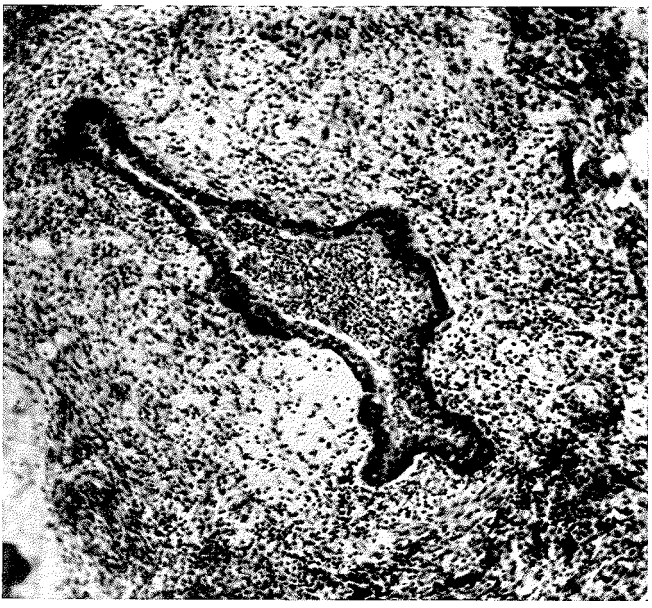


Fig. 63. Lobular bronchiole of left lower lobe (Case No. 16, F. 49, duration of illness 14 days): Lined by a two or three layered undifferentiated epithelium; lumen filled with a leucocytic exudate; proliferative cellular inflammation of the tunica propria. Haematoxylin-eosin staining. (× 200)

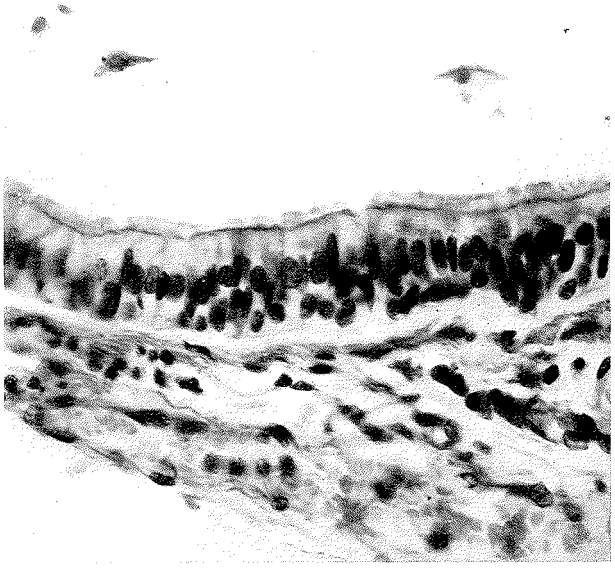


Fig. 64. Lobular bronchiole of pectoral segment of left upper lobe (Case No. 16, F. 49, duration of illness 14 days): Completely normal ciliated epithelium; slight hyperaemia of the tunica propria; no leucocytic infiltration of epithelium or tunica propria. Haematoxylin-eosin staining. (× 600)



Fig. 65a. Cytological preparation of tracheal epithelium (Case No. 1, F. 71, duration of illness 7 days; acute *H. influenzae* tracheo-bronchitis and bronchiolitis without influenza virus infection): Normal ciliated cells. Wet methylene blue preparation. (× 1200)

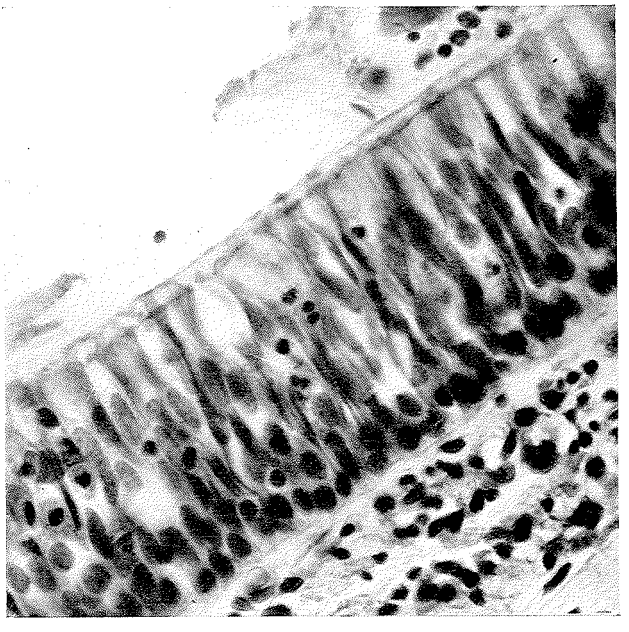


Fig. 65b. Corresponding section of trachea from Fig. 65*a* (Case No. 1): Normal ciliated epithelium; sporadic leucocytes in the epithelium and in the tunica propria. Haematoxylin-eosin staining. (× 600)

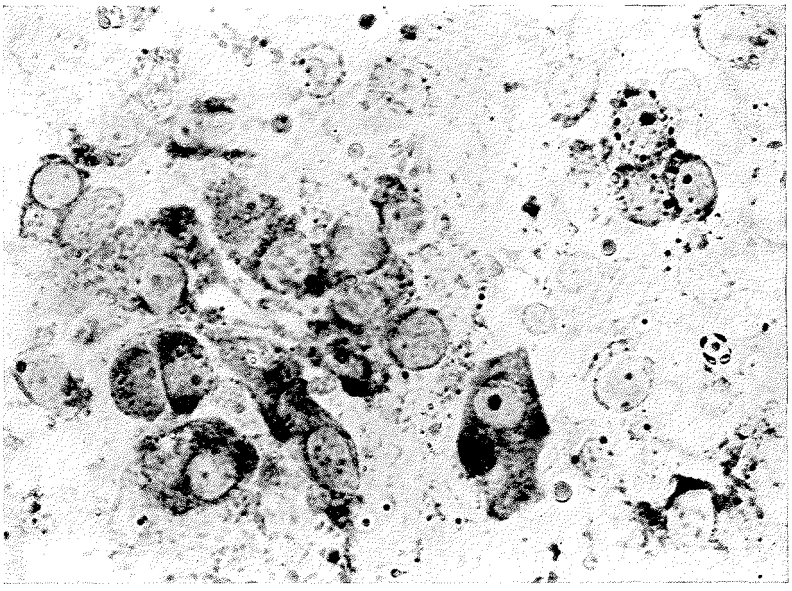


Fig. 66a. Cytological preparation of tracheal epithelium (Case No. 5, F. 69, duration of illness 3 days; influenza virus A infection, secondary invaders *H. influenzae* and Pneumococcus type 3): Strongly degenerative ciliated cells with swelling of cells and nuclei; hyperchromatosis of nuclear membranes. Wet methylene blue preparation. (× 1200)

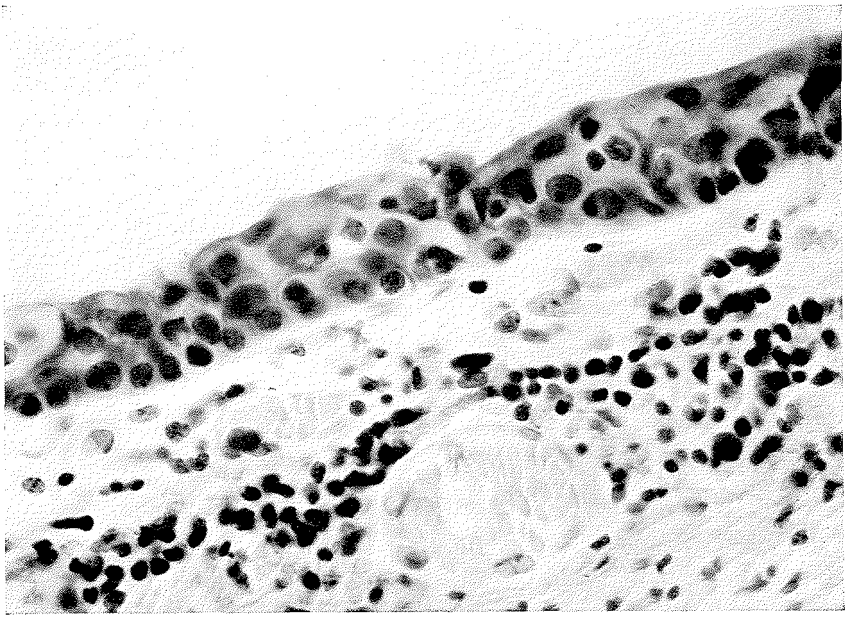


Fig. 66b. Corresponding section to trachea from Fig. 66*a* (Case No. 5): Surface displays necrobiotic epithelium without cilia or mucus production; no leucocytic infiltration of the epithelium or tunica propria. Haematoxylin-eosin staining. (× 600)

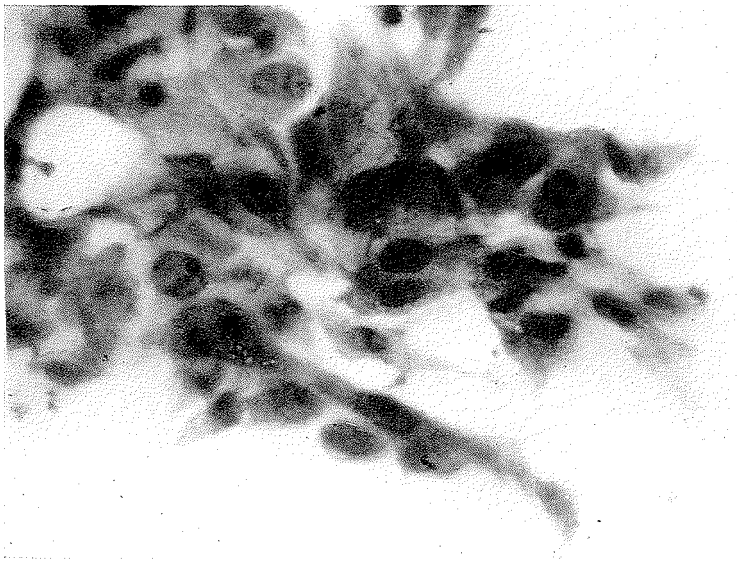


Fig. 67a. Cytological preparation of tracheal epithelium (Case No. 14, F. 60, duration of illness 5 days; influenza virus A infection, secondary invader *Staphylococcus aureus*): Undifferentiated epithelial cells without cilia or mucus production; two mitoses. Wet methylene blue preparation. (× 1200)

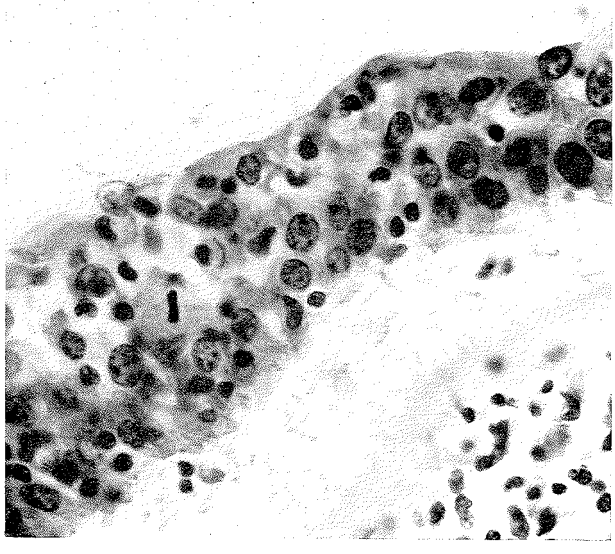


Fig. 67b. Corresponding section of trachea from Fig. 67*a* (Case No. 14): Regenerative epithelium with one mitosis; no leucocytic infiltration; oedema between epithelium and basement membrane. Haematoxylin-eosin staining. (× 600)

CHAPTER VII

SPUTUM CYTOLOGY IN INFLUENZA

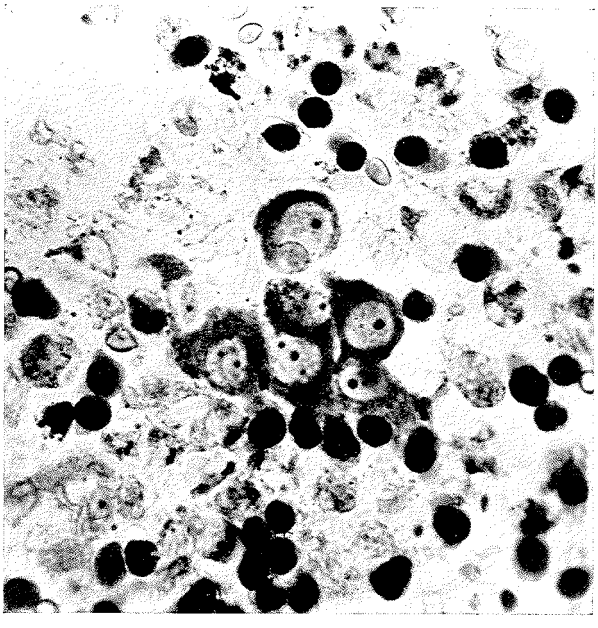


Fig. 68. Cytological sputum preparation from a patient with an uncomplicated influenza virus A-infection (1953-strain). Duration of illness 2 days. Groups of severely degenerated ciliated cells; swelling of cells and nuclei; no cilia; one cell shows a vacuole in the protoplasm. Wet methylene blue preparation. (× 1200)

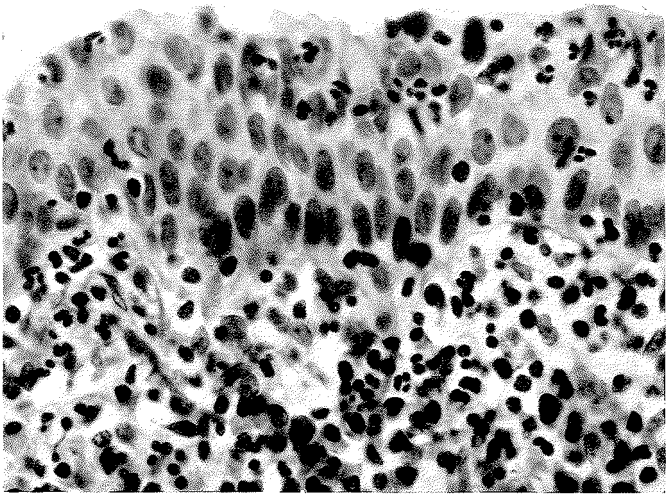


Fig. 69. Bronchus of antero-basal segment of right lower lobe (Case No. 18, F. 36, 57 days after influenza A (1949-strain); chronic *H. influenzae* infection): Ciliated epithelium which exhibited an irregular structure; decreased mucus production; no cilia; dense leucocytic and lymphocytic infiltration of epithelium and tunica propria. Haematoxylin-eosin staining. (× 600)

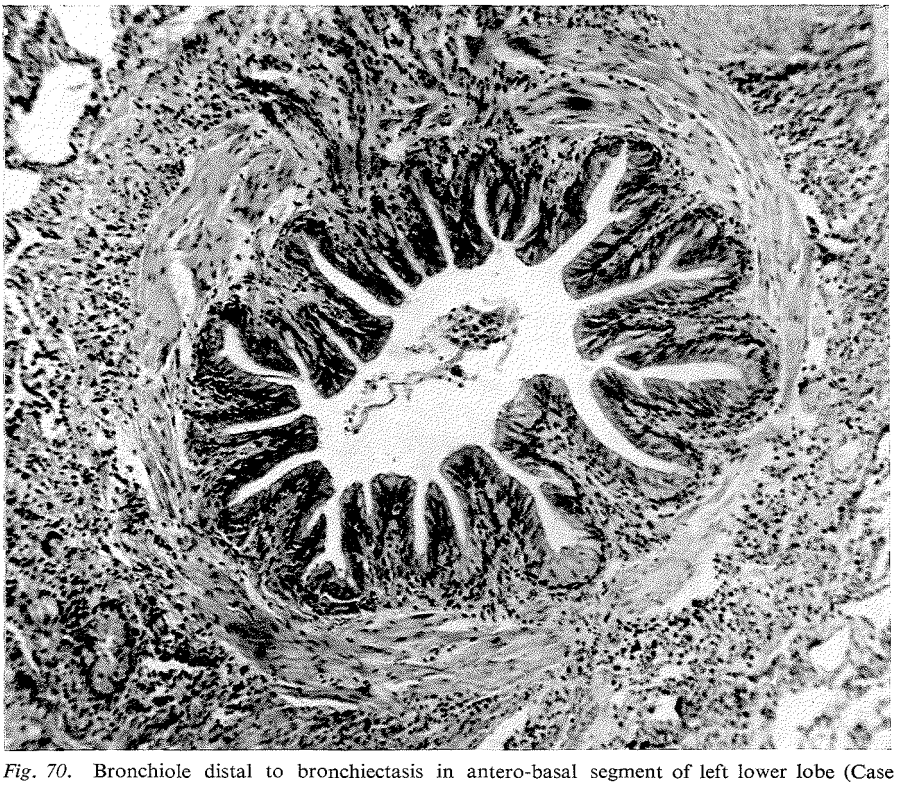


Fig. 70. Bronchiole distal to bronchiectasis in antero-basal segment of left lower lobe (Case No. 19, F. 29, 2 years and 3 months after influenza A (1949-strain) infection complicated by *Staphylococcus aureus* tracheo-bronchitis and bronchopneumonia): Normal epithelial lining; normal structure of the bronchiolar wall; moderate lymphocytic infiltration. Haematoxylin-eosin staining. (× 200)

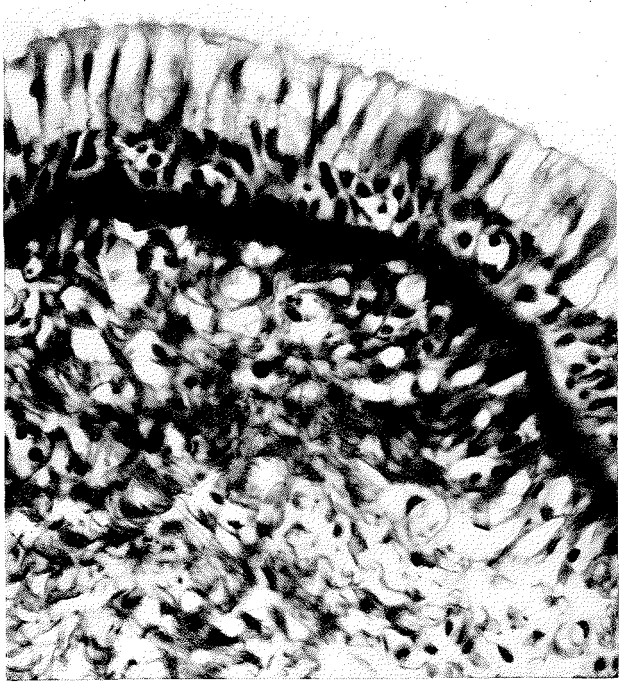


Fig. 71. Bronchus of antero-basal segment of left lower lobe (Case No. 19, F. 29, 2 years and 3 months after influenza A (1949 strain) infection complicated by *Staphylococcus aureus* tracheo-bronchitis and bronchopneumonia): Normal epithelial lining; hyalinisation of the thickened basement membrane and fibrosis of the tunica propria; no elastic fibres; small blood capillaries; slight lymphocytic infiltration of the tunica propria. Van Gieson-Weigert staining. (× 600)

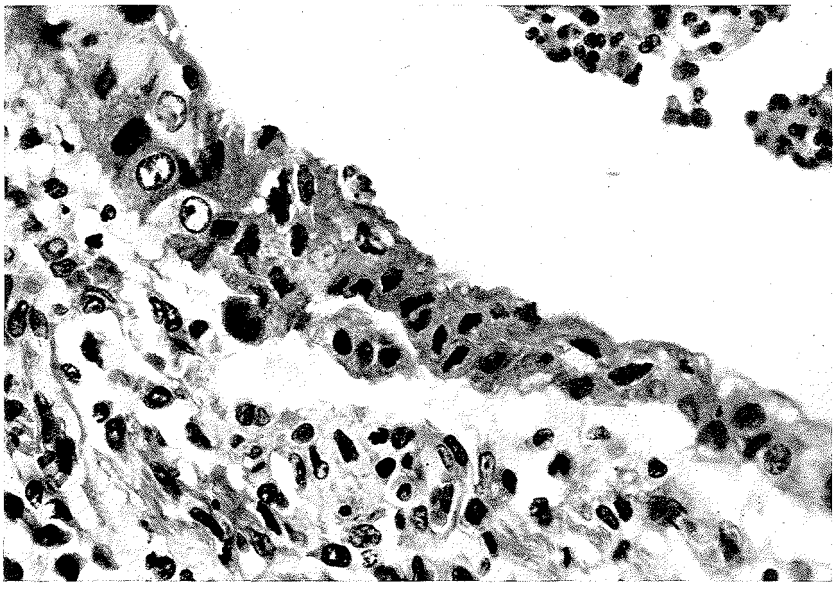


Fig. 72. Bronchus of postero-basal segment of right lower lobe (Case No. 6/1918, M. 12, duration of illness 7 days): Degeneration of the ciliated epithelium to the depth of the basal cell layer without leucocytic infiltration; occasional leucocytes and lymphocytes in the tunica propria. Haematoxylin-eosin staining. (× 600)

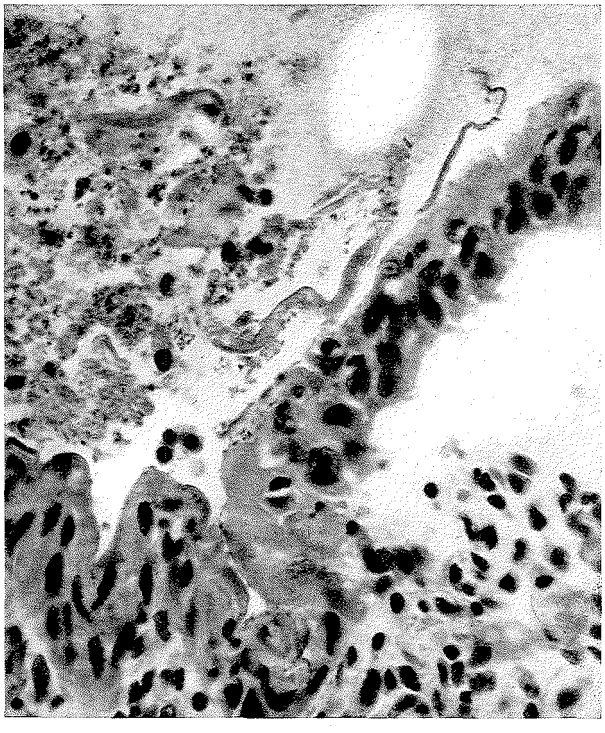


Fig. 73. Bronchus of antero-basal segment of left lower lobe (Case No. 4/1918, M. 40, duration of illness 12 days): Degeneration of the ciliated epithelium and basal cell layer. Oedema in the bronchial lumen and under the epithelium; occassional leucocytes; Staphylococci in the bronchial lumen and on the epithelial surface. Haematoxylin-eosin staining. (× 600)

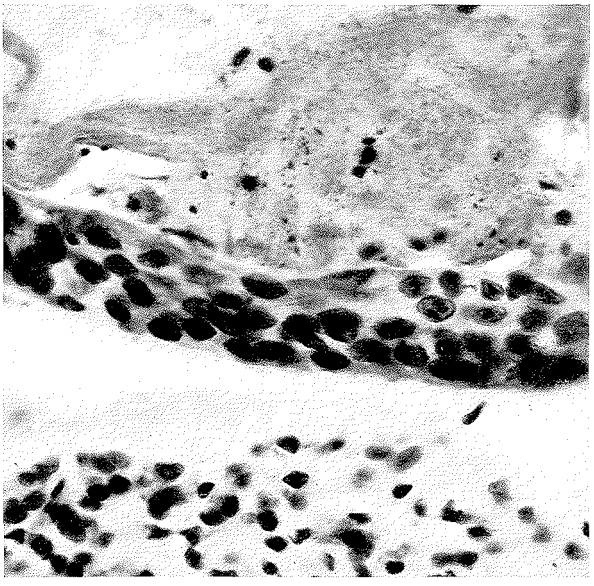


Fig. 74. Bronchus of antero-basal segment of left lower lobe (Case No. 4/1918, M. 40, duration of illness 12 days): Regeneration to a three layered undifferentiated epithelium; Staphylococci in the bronchial lumen. Oedema under the epithelium; occasional leucocytes in the tunica propria. Haematoxylin-eosin staining. (× 600)

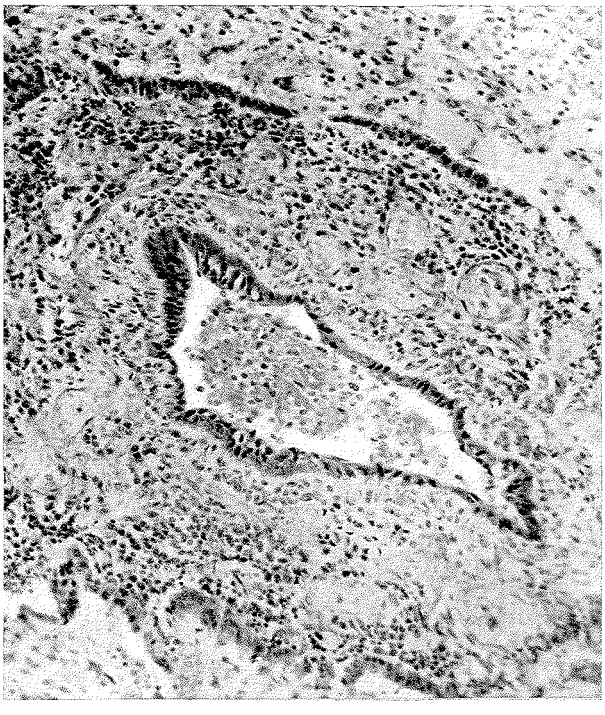


Fig. 75. Bronchiole of antero-basal segment of left lower lobe (Case No. 4/1918, M. 40, duration of illness 12 days): Regeneration to a two layered undifferentiated epithelium; chronic inflammation of the tunica propria, in which appear leucocytes and lymphocytes. Haematoxylin-eosin staining. (× 200)

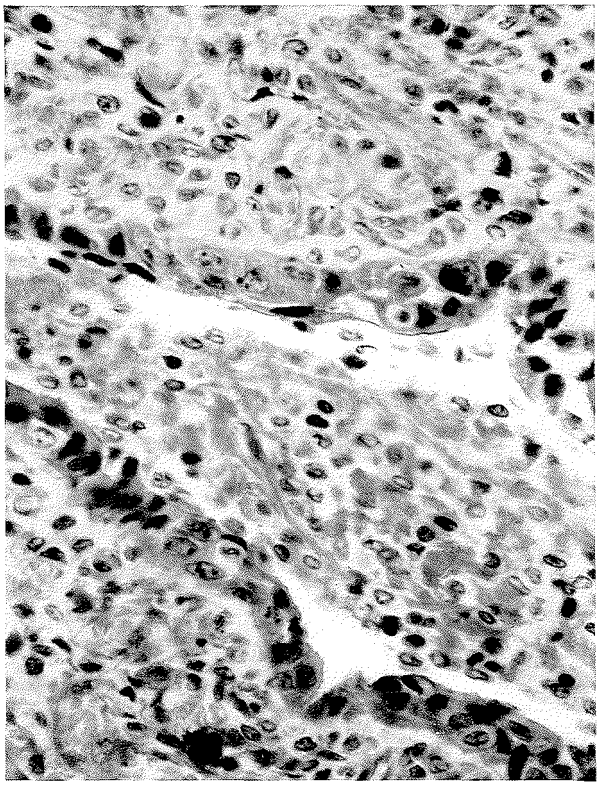


Fig. 76. The same bronchiole from Fig. 75: The basal cell layer shows foci of „young" epithelial cells, partly covered by a layer of flat cells. Haematoxylin-eosin staining. (× 600)

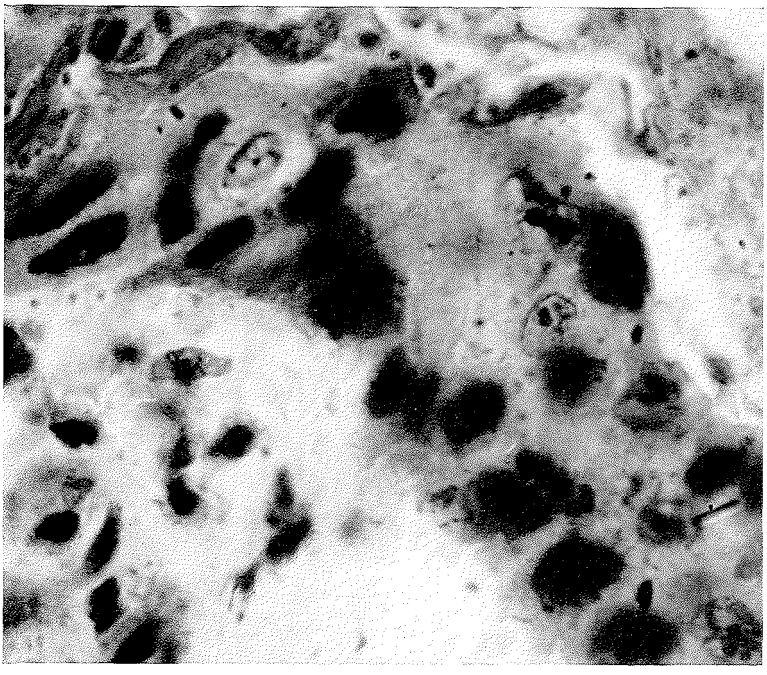


Fig. 77. Similar to bronchus of Fig. 72, but at a more caudal niveau (Case No. 6/1918, M. 12, duration of illness 7 days): Probably *H. influenzae* and occasional pneumococci situated in and under the epithelium. Haematoxylin-eosin staining. (× 1600)

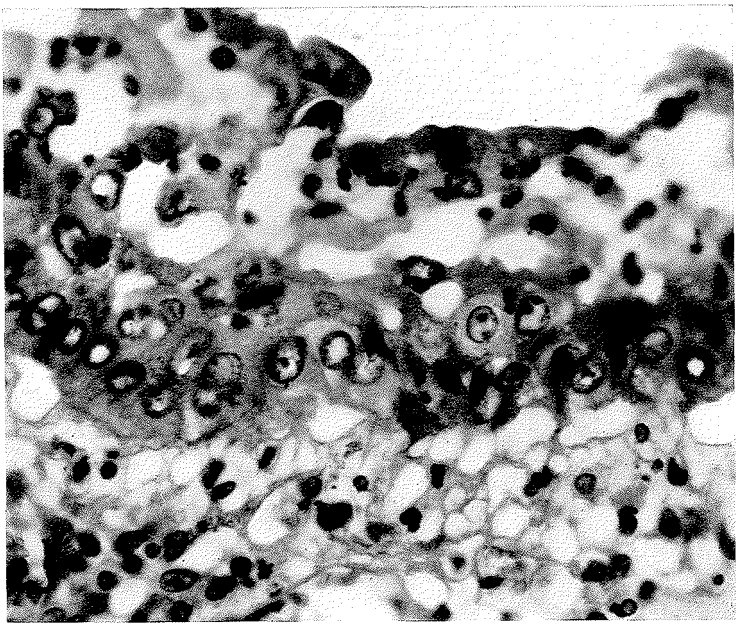


Fig. 78. Bronchus of right middle lobe (Case No. 1, F. 5, duration of illness 4 days. Infection with measles and *H. influenzae*): Degeneration of the epithelium; necrobiosis of the cells to the depth of the basal cell layer; no cilia or mucus production. Occasional leucocytes and lymphocytes in the tunica propria and in the epithelium. Haematoxylin-eosin staining. ($\times$ 600)



Fig. 79. Trachea (Case No. 7, M. 1, duration of illness 5 days. Infection with measles and *Staphylococcus aureus;* no influenza virus): Exudate plug consisting of fibrin and leucocytes; regeneration to two and three layered undifferentiated epithelium on both sides; dense leucocytic infiltration of the necrotic tunica propria. Haematoxylin-eosin staining. ($\times$ 400)

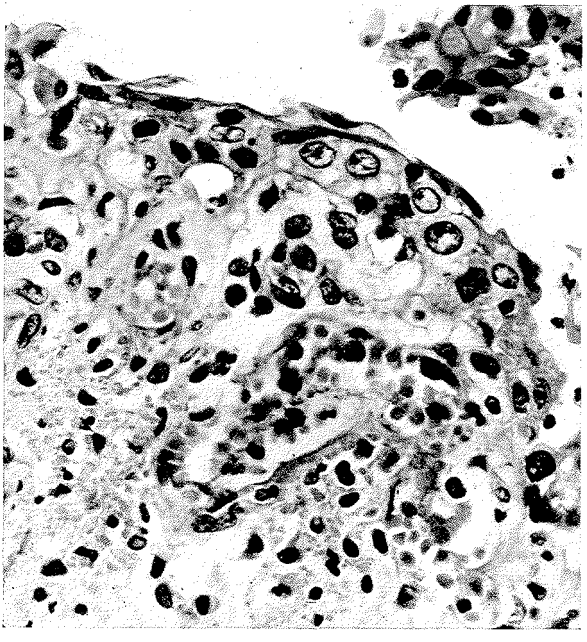


Fig. 80. Small bronchus of the left lower lobe (Case No. 8, F. 1½, duration of illness 5 days. Infection with varicella and *Staphylococcus aureus;* no influenza virus): Basal cell layer present but necrosis also apparent in these cells. Cells and nuclei locally swollen; vacuolisation of the protoplasm. Superficial to the basal cell layer a second layer of flat cells; Strong hyperaemia and oedema in the tunica propria. Capillaries exhibit endothelial necrosis. Infiltration with occasional lymphocytes and leucocytes. Haematoxylin-eosin staining. (× 600)