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THE HISTOPATHOLOGY OF THE RESPIRATORY TRACT IN HUMAN INFLUENZA

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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).

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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 1½ 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.

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