INFLUENZA

BY

J. Mulder M.D.
1901–1965

LATE PROFESSOR OF MEDICINE
THE UNIVERSITY OF LEIDEN, DEPARTMENT OF MEDICINE, THE NETHERLANDS

AND

J. F. Ph. Hers M.D.

WITH A FOREWORD

BY

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THOMAS FRANCIS, JR., M.D.
1900–1969
Photograph kindly provided by the University of Michigan, Ann Arbor, Department of Epidemiology and School of Public Health.

DEDICATED

to

Thomas Francis Jr., M.D.

1900–1969

LATE HENRY SEWALL UNIVERSITY PROFESSOR OF EPIDEMIOLOGY

AND

PROFESSOR OF MEDICINE

THE UNIVERSITY OF MICHIGAN, ANN ARBOR

DEPARTMENT OF EPIDEMIOLOGY AND SCHOOL OF PUBLIC HEALTH U.S.A.

J. MULDER M.D. 1901–1965 AND
SIR CHRISTOPHER H. ANDREWES M.D., F.R.C.P., F.R.S.

Photograph kindly provided by the CIBA Foundation, London, and taken during a coffee break of the Study Group on "Virus Virulence and Pathogenicity" held in honour of J. Mulder, June 15th, 1959. Chairman of the Study Group was Sir Macfarlane Burnet.

Foreword

When Professor J. Mulder died in 1965, he left behind an uncompleted book prepared in close collaboration with Dr. J. F. Ph. Hers which is the subject-matter of the present monograph. It has been Dr. Hers' main concern to preserve the thoughts, opinions and philosophy of Professor Mulder unmodified by his own interpretations. This has not been easy. The difficult task of arrangement of the sections of each of the Chapters and the avoidance of redundant material has been completed yet it seems likely that some sections will inevitably be regarded by readers as incomplete.

Professor Mulder was a clinician of the modern scientific school. For him, the clinical portrayal of disease and its interpretation demanded the most complete knowledge of the pathological lesions and of the causative agents of the disease. Pathogenesis meant for him the unfolding of the mechanism whereby the architecture of the normal body becomes translated into pathology. He was constantly aware of the limitations of a purely human approach and yet he was critical of the alternative approach through lesions in experimental animals. This was because he felt that each host species possesses peculiarities in terms of susceptibility and resistance even of apparently identical cells.

The great opportunity presented to him and the staff of the Influenza Research Team of the University Department of Medicine in Leiden by the advent of the Asian influenza pandemic in 1957 was fully seized. The epidemic brought many autopsies to be studied as fully as possible from the virological as well as the histopathological point of view. Pathologists from a wide area in the Netherlands responded to his appeal and sent a wealth of material to Leiden. This book is the result of the study thus set in motion. Journals have already published an account of the lesions of human influenza virus pneumonia uncovered so skilfully in Leiden in 1957. The scope of a book was required to give the full details including histological appearances together with a much needed historical perspective. For the latter, Mulder was equiped as no other influenza worker of his time, with a knowledge of the European as well as the American literature of the human disease encountered during the great pandemics of 1889 and 1918. For this reason, if for no other, the present monograph is unique and no excuse is offered for the inclusion of references to past writings, including original extracts and illustrations. "To look forward one must first look back" is a statement as applicable to modern medicine as to any form of human activity and Mulder's distillation of past happenings in influenza provides an essential perspective.

The 1957 pandemic of influenza brought Mulder much more than a collection of pathological specimens. He was a keen virologist whose foresight enabled him to collect serum specimens for examination before the A2 virus reached the Netherlands. Thus resulted the discovery of A2 antibodies in sera from persons of 70 years of age and over and the hypothesis (Mulder's *only* hypothesis as he used to say) that the 1889 pandemic was an attack by an influenza virus bearing the A2 antigen. During the Boerhaave Course on "Respiratory Virus Diseases", held at Leiden in April 1962, Mulder reintroduced

into his paper Werner Schäfer's term (1959) "archeology" in influenza research. This unpublished paper on "Facts and Speculations" reflects very well his philosophy and thoughts and is therefore added to this monograph as *Chapter IV* in part III.

Had Mulder lived, it seems likely that he would have expanded the hypothesis and included a chapter in this book of a speculative nature not only upon past epidemiological experiences but upon future happenings in man's struggle with the influenza viruses. No other could have written this chapter without contributing thoughts and opinions of his own and this has not, therefore, been attempted. Mulder conceived this book originally as an Atlas – a clinical and pathological portrayal of the last great plague of human illnesses. As he wrote, it became much more than this and others may judge whether or not its completion by another has concealed the peculiar genius of the author to see through a diseased body to the heart of the cellular problem induced by the attack of a virus. It is a privilege to have been consulted by Mulder in the undertaking of this, his last work.

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Dr. N. Masurel, who is now in charge as Lecturer of Virology and head of the Department of Clinical Virology, Medical School, University Hospital Rotterdam, was responsible for all virus isolations, the analysis of the antigenic structure of the virus strains and the serological findings in man and experimental animals as related to the influenza epidemics in general.

We acknowledge with gratitude the help of the many who collaborated in this investigation, the morbid anatomists who provided us with specimens and case records of fatal cases of influenza and the hospital specialists who kept us informed about the clinical and radiological features observed prior to death. This work could not have been published without their generous contribution, and in fact represents a massive joint effort in the Netherlands to obtain an insight into the causes of death in the influenza pandemic of 1957/1958.

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Miss E. P. Oostergo typed the first raw draft of the monograph; the final preparation was performed by Miss M. M. Hekert.

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THE AUTHORS

† deceased

Contents

General introduction

The pathogenesis of the lesions of the air passages and lungs in human influenza and its relation to the clinical symptomatology and epidemiology of the disease still requires further study. This is so even though more than thirty years have elapsed since the discovery of the human influenza virus A by Smith, Andrewes and Laidlaw in 1933.

It is for this reason that we have decided to publish a monograph describing the observations made over a period of some 25 years and including a critical review of the selected papers published by other authors. The future will doubtless reveal more adequate knowledge of the problem of the bronchotropism and the pneumotropism of influenza viruses in relation to host resistance than exists at present.

Viral cellular changes and inflammatory phenomena will be studied by immunofluorescent techniques, electronmicroscopy, and new cytochemical methods and will carry the subject much further. Yet, in 1957, when a new pandemic of influenza raged across the world, we then owed much to earlier writers on the subject, and perhaps in turn our observations may be of some help to later generations.

In the meantime, it is to be sincerely hoped that such studies as these may soon appear to have only historical significance and interest. For as virological knowledge in this field in human disease develops, it may lead to ultimate eradication of perhaps the last great pestilence of mankind and all the suffering that it brings with it.

THE AUTHORS

The study

THE COLLECTION OF MATERIAL

Before 1957 virologically proven studies on the histopathology of influenza in the Netherlands were limited in number. The investigations were restricted to those patients who were hospitalised and died at the Department of Internal Medicine of the University Hospital of Leiden. During the years 1947–1949 and subsequently when winter outbreaks of influenza were present, refined methods were developed to obtain optimal virological, bacteriological and histological information about the disease. (Straub and Mulder, 1948; Hers, 1954).

Early in September 1957 when influenza A2 became widespread in the Netherlands morbid anatomists throughout the country were asked to participate in an investigation of the causes of death from this disease. Their collaboration made it possible to collect a large volume of material in our laboratory for virological and bacteriological examination as well as for histopathological documentation. The greatest problem in a study such as this was the vast amount

TABLE I

Confirmed influenza virus A2, Hong Kong and B infection in suspected influenza deaths during 1957–1970

Year	No tested	No confirmed	Virological methods: No isolated strains C.E.	Virological methods: No isolated strains M.****	Virological methods: No I.R.F.****	Virus
1957	275	136*	99/275	5/32	28/157	A2
1958	55	20	18/55	0/1	2/10	A2
1959	26	4	4/26	...	...	A2(1), B(3)
1960	38	14	10/38	1/1	4/10	A2
1961	9	0	0/9	0/2	0/1	—
1962	40	5	2/40	1/1	3/7	A2(2), B(3)
1963	12	2	0/12	...	2/4	A2
1964	1	0	0/1	...	0/1	—
1965	0	0	...	...	...	—
1966	4	4	4/4	...	...	A2(1), B(3)
1967	1	0	0/1	...	0/1	—
1968	6	3	2/6	...	3/5	A2
1969	68	31	12/68**	...	30/65	Hong Kong
1970	42	26	17/42***	...	22/42	Hong Kong

C.E. = chick embryo
M. = mouse
I.R.F. = immune response in ferrets as measured by H.I. test
* = 11/11 cases confirmed by complement fixation and haemagglutination inhibition of human sera obtained by heart puncture after death.
** = 12/50 and *** = 12/30 cases isolated in tissue cultures as well
**** = positive results obtained of some of the negative isolations in chick embryo

TABLE II

Influenza virus infection in suspected influenza deaths as confirmed by histology and virology during 1957–1963

Influenza A2 virus infection in suspected influenza deaths	virologically confirmed (all tests)	virologically unconfirmed (all tests)	virologically not tested	total
Number of cases with histologic characteristics of influenza	150	84	7	241
Number of cases without histologic characteristics of influenza	11*	82	22	115
Total	161	166	29	356

* 6 lungpunctures in which no tissue was available, 5 cases in which a marked dyscrepancy was present between the histologic sections available and the virological data.

of material which had to be examined in a short space of time. Moreover, the number of strains of influenza virus to be isolated was so large that the possibility of false positive and false negative isolations cannot be completely ruled out. We have therefore omitted from this account a few cases in which the virological and histopathological findings were contradictory. (Hers *et al.*, 1957 and 1958).

PROOF OF INFLUENZA VIRUS INFECTION

The account which follows therefore is based upon the pathological findings in a series of patients from whom strains of influenza virus were isolated or in whom a reasonable assurance existed that influenza virus infection had occurred. Naturally, the isolation of virus from post-mortem material was generally considered proof that influenza virus infection was present in life. Simple aspiration of virus from the nasopharynx just before or even after death may have occurred and this may have accounted for the occasional isolation of virus from the lung even though influenza virus pneumonia was not present. Virus isolation had often to be attempted with the tracheal mucosa and/or the lungs sent by mail or transported by a special service. This situation created special problems both for the collaborating morbid anatomists elsewhere in the country and for our laboratory. Care was taken, whenever possible, to use methods of refrigeration (solid CO_2) during transport or storage in the laboratory which would provide the optimal conditions for virus isolation in the chick embryo. Passage of negative amniotic fluids from chick embryos inoculated with autopsy material was not, however, in all cases possible. Inevitably, therefore, negative results may have been encountered due to a variety of circumstances including the interval between death and actual storage at $-70°$ C and bacterial contamination of the autopsy material with organisms resistant to the antibiotics used in chick embryo inoculation.

Two expedients were used to strengthen the evidence of influenza virus infection. Lungs which were heavily infected or contaminated with bacteria were inoculated intranasally into ferrets or mice using an emulsion of the mucosa of the trachea, main bronchi or lung tissue. Sera from ferrets collected eighteen days after inoculation were examined for antibodies to influenza virus A2 in the haemagglutination-inhibition (H.I.) test using crude cholera filtrate to eliminate non-specific inhibitors. Secondly, heart blood collected at autopsy was requested but seldom obtained. The existence of antibodies demonstrated by the H.I. or complement-fixation (C.F.) tests in such blood could also establish the likelihood of influenza virus infection before death. Cases which showed the typical histopathological appearances of influenza but in whom virus infection was not definitely demonstrated have therefore been included in the investigation where appropriate. Table I and II show the results obtained.

BACTERIOLOGICAL INVESTIGATIONS

These were made on material collected by lung puncture immediately after death and at autopsy as described below. Aerobic and anaerobic cultures were made in the usual manner using selective media. Smears were also stained for bacteria. The bacteriological findings on autopsy material are subject to the same criticism as those referred to above in connection with virus isolation. Aspiration of saliva or pharyngeal mucus may result in the isolation of pathogens from the trachea or lung such as α-haemolytic streptococci, *Neisseria*, pneumococci, *H.influenzae* and *E.coli* which played no part in the actual lesions of these organs. Moreover, the original infecting bacteria may have been eradicated by the antibacterial therapy during life and have been replaced by a hospital organism. This applies particularly to staphylococci which have been isolated from the trachea even in cases of typical secondary pneumococcal infections. The

TABLE III

Bacterial pathogens cultivated from suspected influenza deaths during 1957–1963

Influenza A2 virus infection in suspected influenza deaths	*H. staph. aur.*	*H. strept. group A*	Pneum.	*H. infl.*	Indefinite growth*	Total	N.D.
Number of cases virologically confirmed	101	2	11	4	43	161	...
Number of cases virologically unconfirmed	79	0	15	2	70	166	...
Total	180	2	26	6	113	327	29

* no growth, presence of a single colony as a result of antibiotic treatment, or contamination during post-mortem.

TABLE IV

Type of bacterial pneumonia and bronchitis in suspected influenza deaths as confirmed by histology during 1957–1963

Influenza A2 virus infection in suspected influenza deaths	Histological characteristics of bronchitis or pneumonia due to						Total
	Staph.	Strept.	Pneum.	*H. infl.*	Indefinite**	Pure viral	
Number of cases virologically confirmed	80	2	16	3	24	25	150 (+ 11)* 161
Number of cases virologically unconfirmed	58	1	18	4	82	3	166
Number of cases virologically not tested	4	0	1	0	23	1	29
Total number of histologically characterised pneumonia or bronchitis	142	3	35	7	140	29	356

* see Table II.

** bacterial infection, the origin of which was unknown.

TABLE IVa

Type of bacterial pneumonia and bronchitis in suspected influenza deaths as confirmed by histology, virology and bacteriology during 1964–1970

Influenza A2, B and Hong Kong virus infection in suspected influenza death	Histological characteristics of bronchitis or pneumonia due to						
	Staph.	Strept.	Pneum.	*H. infl.*	Indefinite**	Pure viral	Total
Number of cases virologically confirmed	25	0	10	8	20	1*	64
Number of cases virologically unconfirmed	9	0	3	3	43	0	58
Total number of histologically characterised pneumonia or bronchitis	34	0	13	11	63	1	122

* Hong Kong virus

** bacterial infection, the origin of which was unknown.

interpretation of isolation from lung tissue is less difficult but even here aspiration and the effects of antibacterial therapy may cause difficulty. Table III, IV and IVa illustrate these findings.

THE AUTOPSY AND HISTOPATHOLOGICAL INVESTIGATIONS

The method of autopsy was of great importance because subsequent bacteriological, virological and histological investigations were all dependent upon it. Confirmed or suspected cases of fatal influenza were autopsied as soon as possible after death to facilitate these investigations and to preclude sloughing off through post-mortem autolysis of the respiratory tract epithelium. In some cases and immediately after death a lung puncture was performed with a thick needle to facilitate virological examination. In most cases 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 major vessels and the digestive tract before transection. Lungs, trachea and heart were then easily removed *en bloc*. The lungs were cut with a sterile knife into four slabs as nearly perpendicular to the bronchi as possible. A section of tracheal cylinder bordering on the bifurcation and about $1\frac{1}{2}$ cm in length was excised under sterile conditions for virological, bacteriological and cytological examination. Small pieces of lung tissue were cut from each pulmonary lobe for virological investigation.

A bacterial examination was done on exudate expressed from the cross-sections of the lungs and bronchi and bronchioli. A culture of heart blood was included in the autopsy of most patients and a blood sample taken by heart puncture was collected for serological methods in some of them. The trachea and bronchi were not sectioned but fixed *in toto*. Berblinger (1918), Askanazy (1919), Wätjen (1919 and 1921) and Straub (1948) pointed out that this method is necessary to avoid artefacts of the respiratory tract epithelium. After several days' fixation, each slice of lung tissue was split into two thinner