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A BRIEF REVIEW OF THE EPIDEMIOLOGY OF
INFLUENZA AND RECENT ADVANCES IN THE
STUDY OF THE VIRUS

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**A Brief Review of the Epidemiology of Influenza
and Recent Advances in the Study of the Virus**

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Epidemics of influenza have occurred, so far as is definitely known, for the past 400 years, and possibly hundreds of years before that. From 1510 to 1930 some 30 of the outbreaks of influenza were considered clearly to be pandemic. The outbreak of 1743 was considered a virulent pandemic, and it has been stated that the actual number of deaths occurring in London during the height of the epidemic was as great in proportion to the population as it was in American cities in 1918. It was in this epidemic that the name "influenza" was derived from the Italian phrase attributing the origins of the disease to *un'influenza di freddo* (an influence of the cold).

Another epidemic occurred in 1782 and was considered a great pandemic in Asia and Europe with apparently a high frequency of complications. During the pandemic of 1889 to 1892 relatively accurate data were compiled and bacteriologic studies were undertaken to seek a specific etiologic agent. During this pandemic Pfeiffer in 1892¹ described *H. influenzae* and considered it the primary cause of influenza. The entire history of influenza was climaxed by the greatest of all pandemics, that of 1918-1919, which is estimated to have caused the deaths, directly or indirectly, of some 20,000,000 people. Although the causative agent was not identified at that time, it is generally presumed to have been a type A influenza virus. It was actually not until 1933 that successful isolation of influenza virus in ferrets from throat washings of patients was reported by Smith, Andrewes and Laidlaw.² This discovery prompted the institution of more effective studies in the hope of finding the means of preventing the widespread temporary disability and possible deaths resulting during future pandemics of influenza.

Three distinct types of influenza virus have been isolated and described to date. The first was labeled type A. A second type, designated as influenza virus B, was isolated independently by Francis³ and by Magill⁴ in 1940. A third type of influenza virus, type C, was found in 1947.^{5,6} More recently both influenza types A and B have been divided into subtypes such as A₁ and A₂, B₁, B₂ and B₃. Influenza type C virus exists as a single and stable antigenic type.

The most recent influenza pandemic occurred during the years of 1957 and 1958. It was caused by a new strain of type A influenza which originated in Asia and which has been designated A₂. About 80,000,000 cases occurred; the illnesses were generally mild, but the estimated number of

pneumonia-influenza deaths in the United States was about 60,000 above normal during the pandemic.

Smith's⁷ discovery in England that influenza virus could be cultivated in developing chick embryos increased the tempo of influenza research and permitted the production of large quantities of the virus for vaccines thus bringing the problem of the control of epidemics and pandemics one step closer to actuality. Many studies of the effect of vaccines have been made. During World War II the Commission on Influenza of the Army Epidemiological Board developed a vaccine containing strains of two serologic types (A and B) and presented experimental work, in man, showing that vaccinated individuals had a much lower incidence of influenza than control, unvaccinated groups.⁸ These vaccines were quite effective^{9,10} until new strains of virus emerged. These new strains proved capable of causing disease in individuals who had been previously vaccinated or had been previously infected with influenza strains of the older serological types.

It is believed that influenza vaccination is effective for only up to three months under the best of circumstances because influenza is essentially an external disease. Since the area affected is predominantly the upper respiratory tract, a much smaller quantity of antibody reaches these cells than is found in the blood. Following immunization the antibody level of the blood will peak within a month then decline so that after three months little antibody would be expected to be found on the cells of the upper respiratory tract.

Another point regarding influenza immunization relates to the epidemiology of influenza in temperate as opposed to tropical and semi-tropical climates. In the United States outbreaks of influenza usually occur during the winter months and continue through early spring. Thus, it is recommended that immunization be initiated in the fall during the months of October and November. However, in tropical and semi-tropical countries, such as the Philippines and Taiwan, outbreaks may occur at almost anytime of the year. For example, in 1961 two outbreaks of A₂ influenza occurred in Taipei, one in March and the other in June.¹¹ In addition, an epidemic of A₂ influenza also occurred in the Philippines in June of 1961. The following year a short epidemic, caused by a new influenza B virus strain, occurred in October. These observations suggest that in tropical and semi-tropical countries people who need to be protected against

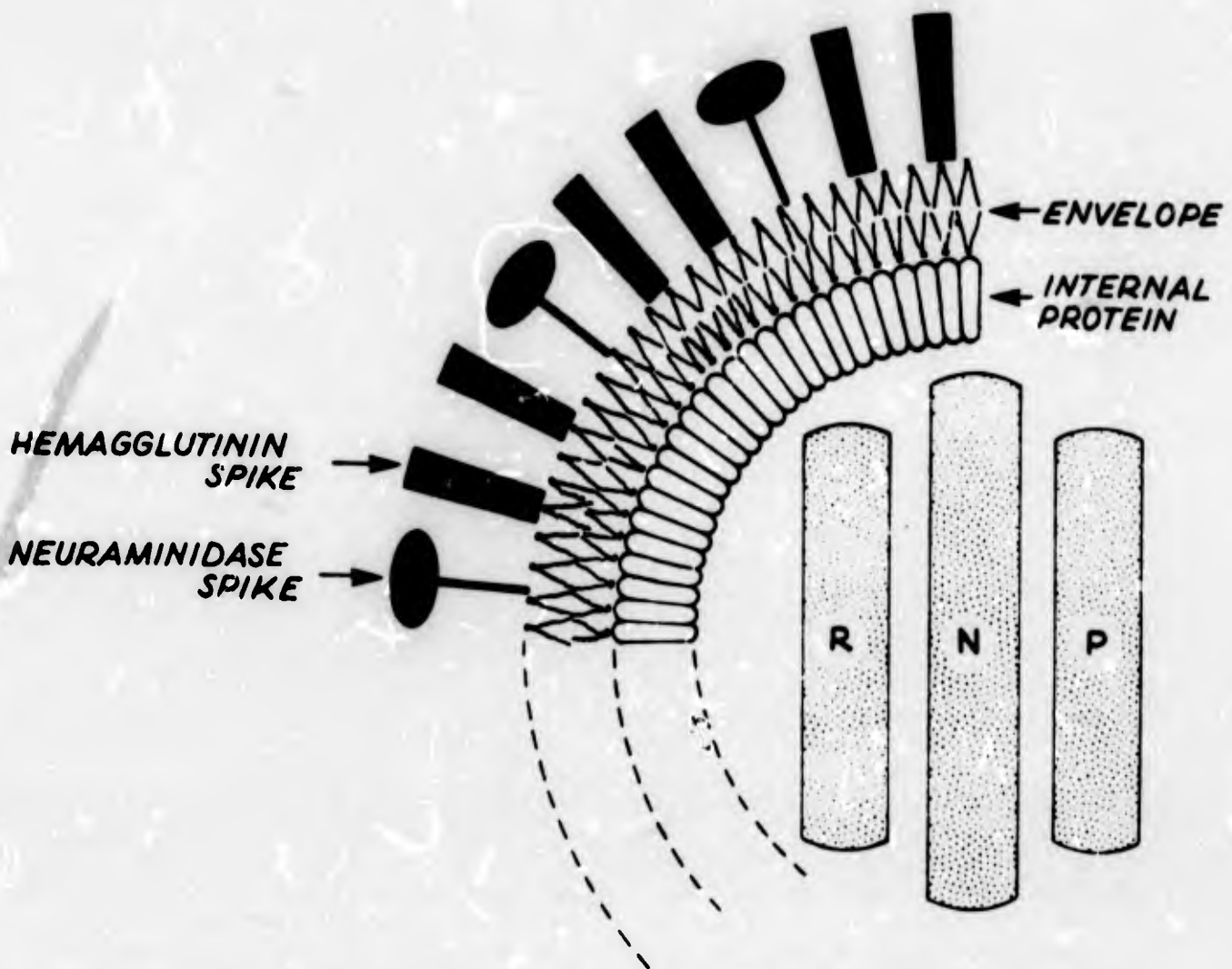


Fig. 1 - A model of the structure of the membrane of influenza virus. (After Compans & Choppin.)

influenza should be vaccinated twice a year, perhaps on the first of June and the first of December.

Some of the more recent knowledge relating to the physical, biochemical and immunological characteristics of the influenza viruses, and how these characteristics are used in the most recent classification of the myxoviruses will be discussed.

The components of an influenza virus are shown in Figure 1. The virus consists of spherical to pleomorphic particles having an external diameter of about 110 nm and an inner electron-dense core of 70 nm. The surface of the virus particle is covered with projections or spikes approximately 10 nm long which possess either the hemagglutinin or the neuraminidase activity of the virus. The heads of the neuraminidase spikes are considered the active enzymatic sites. The hemagglutinin and

neuraminidase antigens are now known to be immunologically distinct from one another and that they undergo independent antigenic variation. Therefore, an adequate description of influenza viruses requires that both these antigens be taken into account. The previous system used for the nomenclature of the influenza viruses implied that their antigens were unique for strains of virus isolated from a single animal species. Recently it has been found that hemagglutinin and neuraminidase antigens related to those of certain human influenza A viruses have been identified among strains isolated from non-human hosts. Viruses antigenically identical to the pandemic virus of 1968 were later isolated also from swine and other mammals. Therefore, a new system of nomenclature was proposed to more clearly define these immunological relationships. It was put into

use at the beginning of 1972.

Next to the layer of hemagglutinin and neuraminidase spikes is a lipid bilayer which comprises the viral envelope and is responsible for the virus' ether-sensitivity. Adjacent to the lipid envelope is an internal protein layer about 6 nm thick. The innermost component is the helical ribonucleoprotein, which is about 9 nm in diameter and 500 nm long. The helix appears to consist of hollow rings, each containing 5 or 6 spherical protein subunits 3 nm in diameter. The complement-fixing antigen, also known as the soluble antigen, used in typing the influenza viruses, is associated with the ribonucleoprotein. The nucleic acid of the influenza virus is not a single molecule, but consists of at least 6 distinct and separable components.

In addition, infectious virus particles of influenza have recently been reported to contain an RNA-dependent RNA polymerase that will catalyze in vitro RNA synthesis. Because of a divided genome, these viruses exhibit several biological phenomena such as high recombination frequency, multiplicity reactivation, and the ability to synthesize hemagglutinin and neuraminidase after chemical inactivation of viral infectivity. Since the viral RNAs are not known to be infectious, the RNA-dependent RNA polymerase may be located in the virion.

Since the influenza viruses have an affinity for mucins the term myxovirus was applied to the group. However, other viruses, including mumps, measles, respiratory syncytial and parainfluenza viruses, have been found to possess certain characteristics in common with the influenza viruses but to differ in other important respects. The myxoviruses, therefore, have been divided into two subgroups on the basis of the size of their inner ribonucleoprotein helix: 9 nm for the orthomyxoviruses which include all the influenza viruses and 18 nm for the paramyxoviruses which include the mumps, measles, parainfluenza, and Newcastle disease viruses.

The pneumonia virus of mice and the human respiratory syncytial virus possess ribonucleoprotein helices of 12-15 nm, a diameter between that of the orthomyxoviruses and paramyxoviruses. Therefore, as more new virus isolates are examined, it may be necessary to expand the present groupings of the myxoviruses into the ortho-meta-, and paramyxovirus subgroups.

In 1953 the WHO Expert Committee on Influenza recommended that influenza virus strains be

classified into types A, B, and C on the basis of their ribonucleoprotein antigens. It was also recommended that they be designated according to a uniform code. In 1959 this coding system was modified by making provisions for indicating influenza virus subtypes, e.g., A₂/Singapore/1/57, the use of which was considered to be optional (Table 1). The nomenclature of influenza A viruses of animals followed a similar pattern with the typespecific designation and species of origin being shown. A subtype designation was often included, e.g., A/equine-2/Miami/1/63, but this was inconsistently applied for viruses from other host species.

Table 1

WHO Uniform Coding System for Influenza Virus Isolates

<u>Old System (1959)</u>	<u>New System (1972)</u>
A ₂ /Singapore/1/57	A/Singapore/1/57 (H2N2)
A/equine-2/Miami/1/63	A/equine/Miami/1/63 (Hq2Nq2)
A ₂ /Hong Kong/1/68	A/Hong Kong/1/68 (H3N2)
A/swine/Taiwan/1/70	A/swine/Taiwan/1/70 (H3N2)

Since the neuraminidase antigen has been found to be morphologically and immunologically distinct from the hemagglutinating antigen, and both antigens are now known to undergo independent antigenic variation, an adequate description of influenza viruses requires that both these antigens be taken into account. Therefore, a new system of nomenclature was introduced at the beginning of 1972.¹² This system consists of two parts—namely, a strain designation, and a description of the hemagglutinin and neuraminidase antigens. The strain designation for types A, B, and C contains the following information:

- (1) a description of the antigenic type of ribonucleoprotein (A, B, or C);
- (2) the host of origin; this is not indicated for strains isolated from man but is indicated for all strains isolated from non-human hosts, e.g., swine, horse (equine), duck, chicken, turkey, quail, tern, etc.;
- (3) geographical origin;
- (4) strain number;
- (5) year of isolation.

For influenza A viruses the antigenic description follows the strain designation and includes, in parentheses, (1) an index describing the antigenic character of the hemagglutinin subtype, and (2)

an index describing the antigenic character of the neuraminidase subtype.

The host designation for the H and N antigens (e.g., Hq 1, Nav 1) refer to the source of the virus where the antigen was first characterized. It does not imply the existence of phylogenetic or evolutionary relationships between viruses containing a common H or N designation. Such designations are intended to indicate the possession of a common subtype of hemagglutinin or neuraminidase antigen, but it is implicit that a given H or N subtype will encompass strains showing a considerable degree of antigenic variation within the subtype.

Thus the main differences between the 1959 and 1972 methods of nomenclature are the omission of the subtype designations and the addition of the hemagglutinin and neuraminidase antigen designations.

On the 26th of August 1972 an outbreak of upper respiratory disease occurred among the personnel of a United States destroyer which was visiting the Philippine Islands. The ship arrived in the Subic Bay area about the 23rd of August and remained there until the 31st of August. In addition the ship traveled to Hong Kong arriving there on the 2nd of September.

Our original isolate was obtained from a man who had been restricted to the ship. He became

ill about the 3rd of September and reported to sick call on the 4th of September complaining of chills, fever, cough, headache, body ache, and chest pains. A throat swab specimen, as well as an acute serum sample, was obtained at this time. The throat swab specimen was suspended in brain-heart infusion broth containing antibiotics and frozen at -65°C until isolation could be attempted in tissue culture and embryonated eggs.

A hemagglutinating virus was isolated from the throat swab specimen following amniotic inoculation of 9-day old embryonated chicken eggs. The inoculated eggs were incubated at 34°C and harvested three days after infection. The hemagglutination titer of the pooled fluids was found to be 1:640, using 0.5% chicken erythrocytes.

To date, approximately 17 isolates have been obtained from 143 men who were sampled on the ship over a two-day period, the 4th and 5th of September. An additional 9 isolates were obtained from men on other ships during the same period. Of the 17 destroyer men from whom influenza virus was isolated, only *one* was *not* sick, and 4 of the 17 who were sick did not report to sick call while the remaining 12 did. Almost all of the men of the destroyer from whom virus was isolated received their last influenza immunization between the months of September 1971 and November 1971; however, one received his immunization as

Table 2

Antigenic relationships of selected Asian influenza virus strains as determined by HI test with hyperimmune rabbit serum against the 1972 NAMRU-2 prototype isolate.

Virus	Hyperimmune Rabbit Anti A/Taiwan/1/72 Serum			
	Hemagglutination-Inhibition Titer			
	Un Rx	Δ Rx	RDE Rx	KIO ₄ Rx
A ₂ /Japan/305/57	1:80	1:80	1:80	1:40
A ₂ /Legge/63	1:640	1:320	1:160	1:80
A ₂ /Ann Arbor/7/67	1:320	1:320	1:80	1:20
A ₂ /Hong Kong/1/68	1:1280	1:640	1:640	1:1280

Rx = treated.

Δ = heated for 1/2 hour at 56°C.

late as February 1972.

Four of the isolates were shown to be type A influenza virus by complement-fixation test using soluble antigens obtained from the chorioallantoic membranes of infected eggs and known positive influenza type A and B guinea pig sera obtained from the Communicable Disease Center, Atlanta, Georgia.

Antiserum prepared against our first isolate was used to establish the relationship of this strain with earlier influenza A strains. As can be seen in Table 2 antiserum prepared against our recent isolate showed only slight relationships to in-

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Table 3

Antigenic relationships between the 1972 NAMRU-2 prototype isolate and other recent influenza A isolates as determined in the HI test by the Center for Disease Control, Atlanta, Georgia.

Chicken Antisera*	Influenza Virus Strains					
	NAMRU-2 12727	A/HK/8/72	A/HK/5/72	A/Eng/42/72	A/Qld/5/72	B/Vic/ 98926/70
A/Aichi/2/68(H3)**	640	<u>1280</u>				
A/HK/8/68(H3N2)	320	<u>640</u>				
A/HK/5/72(H3N2)	80		<u>160</u>			
A/England/42/72	1280			<u>1280</u>		
A/Queensland/5/72	5120				<u>2560</u>	
B/Victoria/98926/70	0					<u>1280</u>

* Treated with RDE. Initial dilution 1:10.

** WHO reference chicken antiserum prepared against electrophoretically isolated H3 hemagglutinins of the recombinant strain A/Aichi/2/68(H3)-Bel/42(NI).

fluenza A virus strains isolated between 1957 and 1967 and a close relationship to the Hong Kong strain isolated in 1968.

The Center for Disease Control in Atlanta also characterized our original isolate by comparing it with some of the more recent influenza A isolates (Table 3). As can be seen from their results our isolate appears closely related to the A2/Hong Kong/8/68 strain and to more recent 1972 strains from England and Australia. Under the new 1972 system of nomenclature our isolate has been designated A/Taiwan/1/72 (H3N2).

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