International Working Conference on HONG KONG INFLUENZA

ATLANTA, GEORGIA • OCTOBER 14-16, 1969

Auditorium B

NATIONAL COMMUNICABLE DISEASE CENTER

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Harvard Hospital
Salisbury, England

SESSION CHAIRMEN

I. Epidemiology of Hong Kong Influenza
Dr. Charles H. Stuart-Harris

II. Properties of the Hong Kong Influenza Virus
Dr. Preben von Magnus

III. Inactivated Influenza Virus Vaccines
Dr. Edwin H. Lennette

IV. Live Influenza Virus Vaccines
Sir Christopher Andrewes

V. Future Influenza Virus Vaccines
Dr. Thomas Francis, Jr.

VI. Chemotherapy and Chemoprophylaxsis of Influenza
Dr. David A. J. Tyrrell

International Working Conference on
HONG KONG INFLUENZA

TUESDAY, OCTOBER 14, 1969

ATLANTA, GEORGIA — OCTOBER 14-16, 1969

8:00 A.M. REGISTRATION

8:45 A.M. INTRODUCTION
DR. ROSLYN Q. ROBINSON
CONFERENCE CHAIRMAN

WELCOME TO PARTICIPANTS
DR. DAVID J. SENCER
DIRECTOR, NATIONAL COMMUNICABLE DISEASE CENTER

DR. ARTHUR P. RICHARDSON
DEAN, EMORY UNIVERSITY SCHOOL OF MEDICINE

DR. W. CHARLES COCKBURN
CHIEF MEDICAL OFFICER
VIRUS DISEASES
WORLD HEALTH ORGANIZATION

EPIDEMIOLOGY OF HONG KONG INFLUENZA

Dr. Charles H. Stuart-Harris, Chairman
Dr. A. T. Roden and Col. Edward L. Buescher, Rapporteurs

9:00 A.M. Origin and World Progress of the Epidemic
Dr. W. Charles Cockburn

9:15 A.M. National Experience – Hong Kong
Dr. W. K. Chang

9:30 A.M. National Experience – Japan
Dr. Hideo Fukumi

9:45 A.M. National Experience – United States
Dr. Robert G. Sharrar

10:00 A.M. National Experience – Czechoslovakia
Dr. Lubos Syrucek

10:15 A.M. National Experience – United Kingdom
Dr. A. T. Roden

10:30 A.M. Break

10:50 A.M. National Experience – U.S.S.R.
Dr. Victor M. Zhdanov

11:05 A.M. Experience with Hong Kong Influenza in Tropical Areas
Col. Edward L. Buescher

11:20 A.M. Discussants
Dr. Alan A. Ferris
Dr. N. Veeraraghavan
Dr. Jan Koztrzewski
Dr. Chaninthorn Suvongse
Dr. R. Sohier

11:45 A.M. Critical Evaluation of the Surveillance of Influenza
Dr. Alexander D. Langmuir

12:00 N. General Discussion

12:25 P.M. Concluding Comments
Dr. W. Charles Cockburn

Lunch

TUESDAY, OCTOBER 14 — SESSION II

PROPERTIES OF THE HONG KONG INFLUENZA VIRUS

Dr. Preben von Magnus, Chairman
Dr. Robert G. Webster and Dr. Fred M. Davenport, Rapporteurs

1:30 P.M. General Characteristics of the Hong Kong Virus
Dr. Marion T. Coleman

1:45 P.M. Antigenic Relationships of the Hong Kong Virus Hemagglutinin to That of Other Human Influenza A Viruses
Dr. Walter R. Dowdle

1:55 P.M. Antigenic Relationships of the Hong Kong Virus Neuraminidase to That of Other Human Influenza A Viruses
Dr. Edwin D. Kilbourne

2:05 P.M. Antigenic Relationships of the Hong Kong Virus Hemagglutinin to That of Animal Influenza Viruses
Dr. Bela Tumova

2:15 P.M. Antigenic Relationships of the Hong Kong Virus Neuraminidase to That of Animal Influenza Viruses
Dr. Geoffrey C. Schild

2:25 P.M. Experimental Influenza Virus Infections in Man and Horses
Dr. Julius A. Kasel

2:35 P.M. Discussants
Dr. Robert G. Webster
Dr. Martin M. Kaplan
Dr. B. C. Easterday

2:50 P.M. General Discussion

3:15 P.M. Break

3:35 P.M. Interpretation of Antibody Patterns of Man: I. In the United States
Dr. Fred M. Davenport

3:50 P.M. Interpretation of Antibody Patterns of Man: II. In Europe
Dr. N. Masurel

4:00 P.M. Interpretation of Antibody Patterns of Man: III. In Japan
Dr. Hideo Fukumi

4:10 P.M. Interpretation of Antibody Patterns of Man: Absorption of Human Serum with Hong Kong Variant, A/Equine-2, and Asian Influenza Viruses
Dr. William M. Marine

4:20 P.M. General Discussion
Opening Remarks
Dr. Dorland J. Davis

4:55 P.M. Concluding Comments
Dr. Charles H. Stuart-Harris

WEDNESDAY, OCTOBER 15 — SESSION III

INACTIVATED INFLUENZA VIRUS VACCINES

Dr. Edwin H. Lennette, Chairman
Dr. Frank T. Perkins and Dr. Harry M. Rose, Rapporteurs

8:30 A.M. Production of Influenza Vaccines
Dr. Roderick Murray

8:40 A.M. Problems of Influenza Virus Vaccine Standardization
Dr. Nicola M. Tauraso

8:50 A.M. Discussants
Dr. Albert V. Hennessy
Dr. Frank T. Perkins
Dr. Hideo Fukumi
Dr. Preben von Magnus

9:10 A.M. General Discussion
Opening Remarks
Dr. Charles B. Reimer

9:30 A.M. Effect of Dosage and Route of Inoculation upon Antibody Responses to Inactivated Hong Kong Influenza Virus Vaccine in Man
Dr. Nicola M. Tauraso

9:40 A.M. Antibody Responses to and Efficacy of Inactivated Vaccine
Major General Tadao Sonoguchi

9:55 A.M. Break

10:15 A.M. Studies with Inactivated Influenza Vaccines Purified by Zonal Centrifugation: I. Adverse Reactions and Serologic Responses
Dr. Steven R. Mostow

10:30 A.M. Studies with Inactivated Influenza Vaccines Purified by Zonal Centrifugation: II. Efficacy
Dr. Stephen C. Schoenbaum

10:45 A.M. Effect of Vaccination of a School-Age Population Upon the Course of an A2/Hong Kong Influenza Epidemic
Dr. Arnold S. Monto

11:00 A.M. An Evaluation of Influenza Immunization: Influence of Route of Administration and Vaccine Strain
Dr. Parker A. Small

11:10 A.M. Antibody Responses to and Efficacy of Inactivated Spray Vaccines
Dr. A. S. Beare

11:20 A.M. Discussants

Dr. William J. Mogabgab
Dr. Theodore C. Eickhoff
Dr. J. Thomas Grayston
Dr. Floyd W. Denny
Dr. Howard C. Goodman
Col. Edward L. Buescher

11:50 A.M. General Discussion

Opening Remarks

Dr. Harry M. Rose

12:25 P.M. Concluding Comments

Dr. Edwin H. Lennette

Lunch

WEDNESDAY, OCTOBER 15 — SESSION IV

LIVE INFLUENZA VIRUS VACCINES

Sir Christopher Andrewes, Chairman
Dr. Gordon Meiklejohn and Dr. A. S. Beare, Rapporteurs

2:00 P.M. Antibody Response to and Efficacy of Live Virus Vaccines
Dr. David A. J. Tyrrell

2:20 P.M. The Efficacy of Live Virus Vaccines
Dr. Anatoli A. Smorodintsev

2:40 P.M. Laboratory Characteristics of Attenuated Strains of Influenza Virus
Dr. Huenin F. Maassab

2:55 P.M. Laboratory Characteristics of Attenuated Influenza Virus Strains
Dr. A. S. Beare

3:10 P.M. Evaluation of Influenza Virus Mutants for Use in Live Virus Vaccine
Dr. John Mills V

3:25 P.M. Discussant
Dr. Gordon Meiklejohn

3:30 P.M. General Discussion

3:55 P.M. Concluding Comments
Sir Christopher Andrewes

CONFERENCE RECEPTION AND DINNER

Wednesday, October 15, 6:30 PM

Top of the Mart

Atlanta, Georgia

Honoring

Sir Christopher Andrewes

and

Dr. Thomas Francis, Jr.

6:30 Reception given by Emory University School of Medicine

7:30 Banquet

Master of Ceremonies
Dr. David J. Sencer
Director, National Communicable Disease Center

Principal Speaker
Professor Charles H. Stuart-Harris
Sir George Franklin, Professor of Medicine
The University of Sheffield
Sheffield, England

"37 Years Onward"

FUTURE INFLUENZA VIRUS VACCINES

Dr. Thomas Francis, Jr., Chairman
Dr. Maurice R. Hilleman and Dr. Edwin D. Kilbourne, Rapporteurs

8:30 A.M. Adjuvant Vaccines
Dr. Charles H. Stuart-Harris

8:40 A.M. The Role of Early Alert and Adjuvant in the Control of Hong Kong Influenza by Vaccines
Dr. Maurice R. Hilleman

8:50 A.M. Influenza Immunization: Clinical Studies with Ether-Split Subunit Vaccines
Dr. Frank B. Brandon

9:00 A.M. Desoxycholate Split Vaccines
Dr. M. F. Warburton

9:10 A.M. Future Influenza Vaccines and the Use of Genetic Recombinants
Dr. Edwin D. Kilbourne

9:20 A.M. The Role of Antineuraminidase Antibody in Immunity to Influenza Virus Infection
Dr. Jerome L. Schulman

9:30 A.M. New Criteria for the Selection of Vaccine Strains
Dr. S. Fazekas de St. Groth

9:45 A.M. General Discussion

10:15 A.M. Concluding Comments
Dr. Thomas Francis, Jr.

10:25 A.M. Break

CHEMOTHERAPY AND CHEMOPROPHYLAXSIS OF INFLUENZA

Dr. David A. J. Tyrrell, Chairman
Dr. Robert B. Couch and Dr. Gordon M. Williamson, Rapporteurs

10:45 A.M. The Antiviral Activity of the Isoquinolines Famotine (UK 2054) and Memotine (UK 2371) in Respiratory Infections in Man
Dr. Gordon M. Williamson

11:00 A.M. The Evaluation of Amantadine Hydrochloride in the Treatment of Influenza
Dr. Richard B. Hornick

11:10 A.M. The Prophylactic Effectiveness of Amantadine in Volunteers Challenged with A2 Viruses and in Populations Experiencing an A2/Hong Kong Influenza Epidemic
Dr. Anatoli A. Smorodintsev

11:20 A.M. Study of 1-Adamantanamine Hydrochloride Used Prophylactically during the Hong Kong Influenza Epidemic in the Family Environment
Dr. Alan W. Galbraith

11:30 A.M. Observations on the Use of Interferon in the Prophylaxsis of Influenza
Dr. V. D. Soloviev

11:45 A.M. The Effect of an Interferon Inducer on Influenza Virus
Dr. David A. Hill

12:00 N. Discussants
Dr. Robert B. Couch
Dr. Maurice R. Hilleman

12:10 P.M. General Discussion

12:25 P.M. Concluding Comments
Dr. David A. J. Tyrrell

12:30 P.M. Final Remarks
Dr. Roslyn Q. Robinson

ABSTRACTS

Session I

EPIDEMIOLOGY OF HONG KONG INFLUENZA

TUESDAY, OCTOBER 14, 1969

8:45 A.M.—12:30 P.M.

Session I

Origin and World Progress of the Epidemic

by

W. Charles Cockburn, P. J. Delon, and W. Ferreira; Geneva, Switzerland

The World Health Organization's information on influenza is obtained through its Influenza Programme which was established in 1947. Its objectives are the identification, rapid characterization, and distribution of emerging strains, and the collection and dissemination of virological and epidemiological information in the *WHO Weekly Epidemiological Record*. Collaborating in the programme are 85 national influenza laboratories in 55 countries and two international influenza centres.

The Hong Kong strain of virus A2 may have originated somewhere in mainland China, but this is not certain. It first came to general notice when an epidemic of 500,000 cases occurred in Hong Kong in mid-July 1968. Strains isolated in Hong Kong were characterized in the international centres and distributed to interested laboratories by mid-August.

Infection spread rapidly to Singapore, the Philippines, Taiwan, Vietnam, Malaysia, Thailand, India (Madras and Bombay), and the Northen Territories of Australia—as happened in the 1957 pandemic. Later, however, progress slowed down, and except in the United States of America little spread to new areas occurred until the beginning of 1969. In the U.S.A. an extensive epidemic commenced in November and was associated with a large number of "excess deaths." This experience was different from that of all other countries. In Europe several large epidemics were reported, but in general the incidence as measured by school-absence and sickness-absence in industry was relatively low and no general rise in numbers of deaths was reported.

In the southern hemisphere, outbreaks have been reported recently in Australia, New Zealand, South Africa, and several countries in South America. The disease has been clinically mild and the reported incidence not particularly high.

The most important epidemiological observations so far are, first, the contrast between the rapid spread in south and southeast Asia and the smouldering spread in most other areas and, second, the contrast between the high mortality in the U.S.A. and the low mortality in other countries. A satisfactory explanation of these differences would lead to increased understanding of the natural history of influenza and might also lead to the development of better measures for prevention, control, or treatment.

The WHO Influenza Programme attained its main objective—the early isolation, characterization, and distribution of the strain—but there is scope for improvement of the epidemiological part of the programme.

Session I

National Experience—Hong Kong

by

W. K. Chang; Hong Kong

Hong Kong has experienced two large epidemics of influenza in the past 12 years. On both occasions, the epidemic was caused by a new variant of influenza A virus and was followed by a pandemic spread. The Asian flu of 1957 apparently originated in the central mainland of the People's Republic of China, and the epidemic of 1968, possibly, could have come from the same source. Prior to the outbreak in Hong Kong, travellers reported an increase in the incidence of influenza-like infection in the neighboring Chinese province. However, virus isolation from the incoming travellers was not attempted to confirm the reports.

The frequent daily communications by cargo boats and passenger trains from China predisposes Hong Kong to influenza spread. Being a tourist centre and a free port, Hong Kong is also a likely place for virus exchange with other parts of the world by air and sea. The major factor facilitating the local dissemination of virus is over-population. In urban areas, the average population density is 500 per acre. Under this crowded living condition, an epidemic due to a new variant is explosive and readily recognizable. Overcrowding can provoke an outbreak in a sub-tropical country even in the hot summer season.

The 1968 epidemic was first recognized on July 13 when the number of patients with influenza-like symptoms turning up at the government clinics suddenly increased. The increase was greatest in the week of July 27 and subsided gradually in the following 3 weeks. The outbreak lasted for about 6 weeks. An estimated one-fifth of the population was involved, and the mortality rate was very low. All age groups were equally affected. The clinical symptoms were considered mild, lasting from 3 to 5 days.

The first virus strain was isolated in primary monkey kidney tissue culture on July 17. It was preliminarily identified as influenza A2 virus. The virus was inhibited to a titre of 1 in 80 by the polyvalent A2 antiserum which had an HI titre of 1 in 640 against A2/67 strains. In view of its antigenic deviation, the virus was immediately dispatched in the form of infected tissue culture to the World Influenza Centre in London. Five more lyophilized strains were sent to the World Influenza Centre and the International Influenza Centre for the Americas, Atlanta, during the following week. The strain was later shown to be a distinct antigenic variant of the A2 virus, and on August 16, the World Health Organization issued a warning of the potential spread of the disease.

Session I

National Experience—Japan

by

Hideo Fukumi; Tokyo, Japan

During and since the unusual influenza epidemic in Hong Kong in July 1968, many cases of influenza were imported into Japan, not only from Hong Kong but also from areas where the epidemic had spread. A few more than ten virologically confirmed cases were scattered from their entrance ports to various parts of the country. Except for those which were imported, no cases of influenza due to the Hong Kong virus were reported anywhere in Japan until the end of September.

At the beginning of October 1968, the first outbreak of influenza, confirmed virologically as due to the Hong Kong virus, was reported in a primary school in Tokyo; thereafter, the number of such outbreaks, mostly in primary, middle, and high schools in the Tokyo-Yokohama area, gradually increased. By the end of October, Osaka and some other cities were involved. The epidemic thus consisted of multicentric outbreaks.

Generally speaking, influenza outbreaks due to the Hong Kong virus started to occur in Japan at the beginning of October 1968, but the speed at which the virus spread did not appear to be as rapid as is usually the case when a new antigenic variant has appeared. Moreover, the areas to which the virus was reported to have spread were mainly cities with large populations; namely, the Tokyo-Yokohama, Osaka-Kyoto-Kobe, and Nagoya areas. Though scattered outbreaks, confirmed as due to the Hong Kong virus, were reported in other areas, the epidemic seems to have been reluctant to spread to neighboring country places. This contrasts sharply with the pattern of the type B influenza epidemic which occurred in Japan at approximately the same time and spread to both urban and rural areas.

The extent of the spread of the Hong Kong influenza epidemic in Japan was estimated by means of serological surveys, i.e., by calculating the morbidity rate of Hong Kong influenza in various populations on the basis of antibody rises against the new variant after the epidemic subsided at about the end of March 1969. Such experiments were carried out in several populations whose living and environmental conditions varied. Our experience suggests that in populations having a certain level of antibody against the A2 influenza virus due to earlier experience, the disease ceased to spread and the epidemic subsided. For example, in the Self-Defense Forces camps, 70 to 80% of the total population had experienced infection by the end of the second wave of A2 influenza, whereas among people working in health centers the rate was about 40 to 50%. Compared to these rates, the attack rate during the Hong Kong influenza epidemic was much lower: for instance, serological tests performed in April 1969 showed the rate in Self-Defense Forces camps to be 30 to 40%.

In conclusion, after Hong Kong influenza appeared, there were many chances for the virus to be imported into Japan from epidemic areas abroad. Nevertheless, in Japan the epidemic had a fairly delayed start, spread rather slowly, and was limited in extent.

Session I

National Experience—United States

by

Robert G. Sharrar; Atlanta, Georgia, U.S.A.

Documented A2/Hong Kong/68 influenza activity began in early September as isolated outbreaks in military installations in the Pacific Division and isolated cases in the civilian population predominantly in eastern metropolitan areas. Seeding of the virus continued during the following 6 weeks, and by October 17, 1968, 15 States reported isolated documented cases. Most of these patients were, or had contact with, persons returning from the Far East.

The first community-wide outbreak, as determined by school and industrial absenteeism in the continental United States, began in Needles, California, during the week ending October 19. Activity developed in five additional States in the Mountain and Pacific Divisions during the following 3 weeks and in two States in the Middle Atlantic Division and one State in the West North Central Division during the following fourth week. Outbreaks were reported in eight of the nine geographic divisions by December 7. By December 28, all 50 States experienced influenza outbreaks. Twenty-nine States reported that peak activity occurred between December 15 and January 4, and activity in an additional 11 States peaked during the week ending January 11. During January influenza activity declined except for sporadic outbreaks in populations not previously involved.

The National Health Survey reports that 53.5 million persons, or 27% of the noninstitutionalized population, experienced an influenza-like illness between October 1 and December 31, 1968. Data for 1969 are not available. Schools were reporting absenteeism rates as high as 50%, and some communities were experiencing attack rates of approximately 20%. Preliminary data from Kansas City indicate that the age-specific attack rates were relatively uniform for all age categories.

Pneumonia-influenza mortality statistics from 122 selected U.S. cities can be used as an indicator of influenza activity. Excess mortality was first evident during the week ending December 7, rose sharply over the following 6 weeks, and peaked during the week ending January 11 when a total of 1,166 excess deaths were reported. This peak occurred 1-3 weeks after most States reported greatest activity. Pneumonia-influenza mortality returned to expected levels during the week of March 29. Total mortality statistics also reflect influenza activity. During this influenza season, total excess mortality for 122 U.S. cities was estimated to be 19,500 compared to a total excess mortality of 26,800 from 114 U.S. cities during the 1957-58 pandemic.

Isolated cases of influenza B were documented in November and December; however, outbreaks did not become evident until late January and February. The reported illness predominantly involved elementary school children, and caused absenteeism rates of 25-45% in some areas. Twenty States reported one or more outbreaks, and some influenza B activity was documented in 37 States. The most widespread B activity occurred in eight contiguous States in the Midwest.

Session I

National Experience—Czechoslovakia

by

Lubos Syrucek; Prague, Czechoslovakia

Session I

National Experience—United Kingdom

by

A. T. Roden; London, England

The Hong Kong variant of influenza virus A2 was isolated in Britain for the first time from a child resident in London who developed a respiratory illness early in August 1968. The first outbreak was reported in September from a residential school in the South of England. From September to December outbreaks occurred in several residential institutions in various parts of the country, but there was no evidence of general spread in the community until after Christmas.

In the last week of December 1968 there was a steep rise in new claims to sickness benefit, particularly in the West Midland region, where a maximum for the winter was reached in the week ending January 7, 1969. During mid-January new claims to sickness benefit fell in all regions, but rose again, in the Northern region during the last week of January and in all other regions of England and in Wales and Scotland during the first half of February. A peak for the country as a whole was reached in the week ending March 4, 1969, and new claims to sickness benefit continued to remain substantially above their usual levels until the first half of April.

The incidence of influenza in practices reporting to the Royal College of General Practitioners began to increase in the first week of January 1969 and rose slowly to a peak of just over 150 cases per 100,000 population in the week ending March 11. In England and Wales (population approximately 48.7 million) deaths assigned to influenza and influenzal pneumonia rose very slowly from the beginning of the year to reach a maximum of 125 in the week ending March 28.

The numbers of influenza virus A infections, confirmed by virus isolation or serological investigation, which were reported by the Public Health Laboratory Service, began to increase in the last week of December 1968 and rose rapidly to a peak of 161 cases reported in the week ending January 31, 1969. After a fall in the first half of February, the number of reported cases again rose and was maintained for six successive weeks, from late February to early April, within a range of 125 to 161 per week. The numbers then fell steeply, though isolations of influenza virus A continued to be reported from specimens received as late as May. All strains of influenza virus A investigated were similar to A2/Hong Kong/1968. Throughout the winter there was little evidence of influenza virus B infection.

Compared with previous epidemic winters, the prevalence of influenza during that of 1968/69 rose to only a moderate height in any one week, but extended over an unusually long period—from late December to early April. The total morbidity, as estimated by excess new claims to sickness benefit, was similar to that of the previous winter of 1967/68 in which a sharp outbreak of influenza virus A2 infection occurred. The relatively leisurely progress of the 1968/69 epidemic was accompanied by no sudden or excessive demands either on medical practitioners or on the hospital services. The mortality was substantially lower than in previous influenza winters.

National Experience—U.S.S.R.

by

V. M. Zhdanov and I. V. Antonova; Moscow, U.S.S.R.

A new influenza epidemic broke out in Hong Kong in 1968 against a background of the waning influenza A2 epidemic which had spread over 40 countries and territories of the world. This epidemic was caused by a new variant of influenza A2 virus designated as A2/Hong Kong/68 virus. Epidemic outbreaks associated with this virus were registered in almost all countries of southeast Asia and in Oceania during July-September. Later on the new variant was transported to the United States by air and sea. In September of 1968 separate foci, more often of a family character, appeared in European countries. During the first quarter of 1969, the United States, Canada, and all countries of Europe were involved in an epidemic wave of influenza due to the A2/Hong Kong/68 virus.

The appearance in 1957 of the pandemic strain of influenza A2 virus resulted in four epidemics in the U.S.S.R. The fifth pandemic wave was observed in the winter of 1967, but it was of a mixed etiology, since influenza B virus circulated at the same time as influenza A2 virus in many towns. Cases of illness associated with influenza virus A2/Hong Kong/68 in the U.S.S.R. were registered in the middle of December, 1968, in one of the schools in Moscow and among adult contingents in the Moscow region. However, the infection did not spread rapidly. Almost simultaneously, i.e., during the second 10-day period of December, an increase in influenza morbidity was observed in the central Asiatic towns of Frunze and Dushanbe where strains of influenza virus A2/Hong Kong/68 were recovered. A peak in the incidence of the disease was registered in the third 10-day period of December. In January the epidemic involved the Republics of Central Asia and the Caucasus and began movement through the center of the European part of the U.S.S.R. to Moscow, Leningrad, and Tallin. In February, the epidemic spread over the Baltic area, Byelorussia, Ukraine, and Moldavia and subsequently spread along travel routes.

In Moscow and Leningrad influenza morbidity started to increase from approximately January 6, 1969. A peak in incidence was registered in Moscow on January 27 and in Leningrad on January 28, 1969. The duration of the epidemic in towns was approximately 50-80 days. On the whole, in the U.S.S.R. the epidemic lasted about 4 months. Therefore, the intensity of the rise and fall of the epidemic wave was relatively gradual. The age distribution of the disease was not in the least usual, i.e., in most towns children under seven made up 14 to 25 percent of the total number of the registered cases.

Dynamics of the curve of influenza incidence, a fluctuation of the percentage of the population affected, as well as an unusual age distribution of the disease apparently might be explained by the appearance of a new variant of influenza virus A2.

Session I

Experience with Hong Kong Influenza in Tropical Areas

by

Edward L. Buescher; Washington, D. C., U.S.A.

Although influenza has been repeatedly recognized in resident populations of tropical areas, descriptions of major epidemics have been confined to incidences of reported disease, its sequellae and complications, and its chronological progression in and between various countries. Reports of clinical disease prevalence during the 1957 (Asian) epidemic suggested that attack rates in tropical countries were higher, complications more frequent, and dissemination of virus more rapid than in countries of the north and south temperate zones.

During the late summer and early fall of 1968, introduction of the significantly variant Hong Kong virus into tropical Asia and Central America made possible two as yet unpublished studies of influenza infection and disease. One of these from the South East Asia Treaty Organization Medical Research Laboratory (Smith, T. J., Olson, L. C., Kandel, G. E., and Snitbhan, R.) describes the introduction and progression of infection through a population of approximately 6,000 U. S. servicemen resident in a single community in Thailand. Hong Kong virus was apparently introduced into the community in early August by flight crews returning from other countries in Asia where influenza was occurring. During the next 3 months, 8% of the population contracted disease severe enough to require relief from duty, and by November 1, at least 12% of the population (as determined by follow-up serological survey) had been infected. Disease was relatively mild, and there were no untoward sequellae or complications. Earlier routine immunization with vaccines which did not contain the epidemic virus strain had no apparent effect upon occurrence or severity of disease. The epidemic tended to peak in late August and early September, but significant numbers of new cases continued to occur daily through October; thus the epidemic propagated slowly and was different from the more precipitous epidemics seen during the winter of 1968 in temperate zones.

In contrast, another study from the Middle America Research Unit (Zachary, I. H., and Johnson, K. M.) showed that Hong Kong virus was rapidly disseminated through both indigenous and U.S. populations of the Panama Canal Zone during September-November 1968. Apparently introduced by steamship crews returning from Asia, the virus rapidly established itself in both populations. Clinical attack rates were higher (approximately 50%) in both adult indigenous and select U.S. personnel of the Zone than were those observed in Thailand, and these higher disease rates were supported by the results of a postepidemic serological survey.

Infection was less frequent in children (approximately 30%), but a greater proportion of infected children became ill than adults, and the reported incidence of complications in both children and adults was less than that observed in Panama during the 1957 epidemic.

Thus, the rates at which the same virus was disseminated among populations residing in different tropical areas varied and, in this respect, patterns of infection and disease are similar to those seen in temperate zone populations.

notes

DISCUSSANTS

Dr. Alan A. Ferris; Fairfield, Victoria, Australia

Dr. N. Veeraraghavan; Coonoor, Nilgiris, India

Dr. Jan Kostrzewski; Warsaw, Poland

Dr. Chaninthorn Suvongse; Bangkok, Thailand

Dr. R. Sohier; Lyon, France

Session 1

Critical Evaluation of the Surveillance of Influenza

by

Alexander D. Langmuir and Jere Housworth; Atlanta, Georgia, U.S.A.

Surveillance, as recently defined by the World Health Organization, consists of the current collection, the careful evaluation, and the prompt dissemination of all pertinent data regarding the occurrence of a disease. The information developed through surveillance should be distributed not only to health officers and others responsible for or concerned with the control of disease, but also in appropriate form to the general public.

A global event of the magnitude of the 1968-69 pandemic of Hong Kong influenza presents a massive challenge to the adequacy of the existing surveillance system. It has varied in the extreme. The collection of essential laboratory information about the virus and its immunological characteristics has been coordinated through the WHO and its international centers and collaborating laboratories. The prompt, accurate, and extensive knowledge resulting therefrom has made possible the manufacture in large quantity of vaccines containing the specific new strain.

The collection of pertinent and clinical epidemiological data has been neither as adequate nor as consistent. Little information regarding the relative severity of the clinical disease or the character or seriousness of bacterial complications has been made available, even in the United States where an extensive surveillance network for epidemiological and laboratory data existed. From information available from many parts of the world it has not been easy to discriminate sporadic single cases or small localized outbreaks from community-wide epidemics or widespread national involvement. Present methods of influenza surveillance need much improvement.

The most practical, widely applicable, and internationally comparable index of the extent and severity of epidemic influenza is excess mortality. William Farr developed this method to a high degree of perfection during an epidemic in London in 1847. Essentially the same method was used to describe the pandemics of 1889-92 and 1918-19. Since 1918 excess mortality has been a standard surveillance procedure in the United States, but it is not in common use elsewhere.

A comparison of excess mortality in the United States with England and Wales since 1957 reveals that influenza epidemics have been generally associated with markedly higher mortality in England and Wales, with one notable exception. During the past year in the U.S.A. the Hong Kong strain has caused an extensive epidemic with high excess mortality, whereas in England and Wales the increase in mortality has been minimal in spite of widespread identification of the virus. The reasons for these differences remain obscure. Experience during the winter of 1969-70 will be watched with interest.

More extensive use of current mortality data throughout the world would greatly enhance the effectiveness of global surveillance of influenza.

ABSTRACTS

Session II

PROPERTIES OF THE HONG KONG INFLUENZA VIRUS

TUESDAY, OCTOBER 14, 1969

1:30 P.M.—5:00 P.M.

General Characteristics of the Hong Kong Virus

by

Marion T. Coleman and Walter R. Dowdle; Atlanta, Georgia, U.S.A.

Experience with the Hong Kong influenza variants in 1968-69 permitted observation of the biological characteristics of these strains under laboratory conditions. Some of their properties which have practical usefulness in the laboratory are reported.

Unlike A2 strains of the past few years, Hong Kong virus strains were readily isolated in both eggs and primary rhesus monkey kidney tissue cultures. Hemagglutinins were detectable with both chicken and guinea pig erythrocytes. The hemagglutinin titers of allantoic fluids on initial harvest were usually adequate for identification of most isolates by hemagglutination inhibition (HI) tests.

Early electron micrographs of the Hong Kong variants revealed both spherical and filamentous forms with morphological structure typical of influenza virus particles. Antigenic structure of the variants has been defined by complement fixation (CF) as sharing the soluble antigen common to viruses in the influenza A group. The neuraminidase of the Hong Kong strains resembled other A2 strains, and varying relationships were found with human A2 and animal influenza viruses by HI tests.

The spectrum of nonspecific serum inhibitors of Hong Kong hemagglutinins found in normal sera of humans and several animal species was different from that of earlier A2 strains. Human and rabbit serum inhibitors were removed by treatment with receptor destroying enzyme (RDE). Periodate or trypsin-periodate reduced nonspecific inhibitors from ferret and horse sera. Kaolin absorption was required to remove inhibitors from guinea pig sera. Nonspecific inhibitors were not encountered in monkey and goat sera and rarely in chicken sera. Some low passage level Hong Kong strains were more sensitive to nonspecific serum inhibitors than other strains.

The efficiency of HI and CF tests for diagnosing infection was evaluated on paired sera of 163 adults from populations experiencing Hong Kong influenza outbreaks. Serodiagnosis by HI was the more efficient method in that 22% of conversions (fourfold or greater increases in antibody titer) detected by HI were not recognized by CF. Since 8% of the conversions were detected by CF only, the use of both tests was required for maximum efficiency in serodiagnosis.

Session II

Antigenic Relationships of the Hong Kong Virus Hemagglutinin to That of Other Human Influenza A Viruses

by

Walter R. Dowdle, Marion T. Coleman and Elmer C. Hall; Atlanta, Georgia, U.S.A.,

and

Violeta Knez; Cordoba, Argentina

The magnitude of antigenic change of the Hong Kong strains from the earlier influenza A2 strains was compared with previous antigenic changes among the type A viruses. Representative type A strains recovered from humans during the years 1933 through 1969 were examined by reciprocal hemagglutination-inhibition (HI) tests with antisera produced in chickens. Phenograms or family trees were constructed by using numerical taxonomic methods based on cluster analysis of similarity coefficients.

A phenon line corresponding to negative or zero correlation in the HI test phenogram divided the 27 influenza A strains into two major groups—one consisting of all strains isolated prior to the 1957 Asian pandemic and the second consisting of all strains isolated since that time. Bases for additional subdivision were less clear, but strains could be further divided by arbitrarily selecting certain levels of correlation. A phenon line drawn at the next major level of correlation divided the strains into three groups corresponding generally to subtypes A0, A1, and A2. A phenon line at the correlation level separating the Hong Kong strains from earlier A2 strains resulted in a further subdivision of the 27 type A strains into a minimum of six or a maximum of 10 groups.

A phenogram constructed from reciprocal neutralization (N) tests with a smaller number of influenza A strains was similar to that obtained by HI. The neuraminidase-inhibition (NI) phenogram was also similar in that only two major groups were observed.

The use of numerical taxonomic methods to establish the antigenic position of Hong Kong-like strains further illustrates the inadequacy of the present system of nomenclature. The antigenic dissimilarity between A0 and A1 seen in phenograms constructed from reciprocal HI, N, and NI tests is of far less magnitude than that between A1 and A2 and should not be given equal emphasis. The degree of antigenic dissimilarity between the Hong Kong-like strains and the early A2 strains is even less than that between A0 and A1, and the A3 appellation proposed by some investigators cannot be supported on antigenic grounds.

Yet there is an undeniable need to distinguish PR/8-like strains from FM/1-like strains and Hong Kong-like strains from earlier A2 strains, as well as others, past and future. To do this, some new system of identification is required. Application of numerical taxonomic methods provides a basis for such a system.

Session II

Antigenic Relationships of the Hong Kong Virus Neuraminidase to That of Other Human Influenza A Viruses

by

Jerome L. Schulman and Edwin D. Kilbourne; New York, New York, U.S.A.

Genetic recombination of influenza viruses may result in the formation of antigenic hybrids combining in 'the envelope of the recombinant virus the hemagglutinin of one parental virus and the neuraminidase of the other. Such reciprocal recombinants have greatly facilitated the study of antigenic relationships among influenza viruses by segregating and dissociating the two surface proteins of the virus that are primarily responsible for the serologic reactions of intact viruses. This result is important, for although the neuraminidase is quantitatively less represented on the virion surface than the hemagglutinin and does not participate in conventional pre-inoculation virus neutralization reactions, specific antiserum to the enzyme may cause hemagglutination inhibition (HI) of intact virus; conversely, antiserum specific for the hemagglutinin may inhibit the hydrolytic activity of viral neuraminidase (EI) through steric hindrance when intact virus is the enzyme source.

Hybridization of six influenza A2 strains (A2/Japan/305/57, A2/Taiwan/1/64, A2/Itsukaichi/1/65, A2/Montevideo/2208/67, A2/England/10/67, A2/Texas/2/68) and Hong Kong/16/68 virus with A0/NWS led to the isolation of seven recombinants bearing the NWS hemagglutinin (A0) and the neuraminidase (E) of each of the A2 or Hong Kong viruses (A0E1, A0E2, etc.) and seven reciprocal recombinants containing A2 or Hong Kong hemagglutinin in combination with the NWS enzyme (e): (A21e, A22e, etc.). Specific antisera were then produced to all parental and recombinant viruses, and their antigenic relationships were defined by HI, EI, plaque inhibition (PI), and plaque size reduction (PSR) tests. (PI and PSR permit the comparison of HI and EI activity, respectively, in the same test system). Antisera to all six A2 viruses inhibited the neuraminidase activity of all A0E recombinant viruses including A0E(HK). However, as reported earlier, (Schulman and Kilbourne, Proc. Nat. Acad. Sci. 1969) great variation in enzyme cross-reactivity was demonstrated between earlier (1957-64) strains and later (1965-68) viruses, including Hong Kong. Drift of the hemagglutinin antigen was also demonstrated in HI tests, but this variation was independent of and not concordant with the change in enzyme antigenicty.

Similarity coefficients of HK virus with the six A2 strains determined by HI and EI tests revealed identity of the HK neuraminidase with the enzyme of the 1967-68 A2 viruses but little cross-reactivity of the hemagglutinins. These findings were confirmed in the plaque system in which antisera to all A2 parental viruses induced PSR of A0E(HK) virus (to greater titer with 1967-68 antisera), but no PI of either HKe or the parental HK strain was induced at the lowest dilutions tested. Furthermore, high titer antiserum to the parental HK virus had no plaque inhibiting (PI) activity with any of the A2e recombinants (which contained hemagglutinin of the 1957-68 period but not the A2 enzyme).

In summary, cross-reactivity of HK virus and virus of the A2 sub-types is entirely dependent upon and mediated through the viral neuraminidase and not through the hemagglutinin. The neuraminidase of the HK virus is slightly cross-reactive with the neuraminidase of older A2 strains and is identical to the enzyme of the recent 1967-68 A2 variants.

Session II

Antigenic Relationships of the Hong Kong Virus Hemagglutinin to That of Animal Influenza Viruses

by

Bela Tumova; Prague, Czechoslovakia, and B. C. Easterday; Madison, Wisconsin, U.S.A.

The origin and evolution of antigenic changes in influenza virus type A are still poorly understood, and several proposed hypotheses do not satisfactorily explain the appearance of either the variant or the novel subtypes. The hypothesis of a common origin of animal and human type A influenza viruses has received considerable support in recent years with the recovery of a number of strains from different animal species related to human influenza viruses of the A2 subtype by one or both envelope antigens—hemagglutinin and neuraminidase.

The present study demonstrates relationship in envelope antigens of six human A2 strains isolated in the years 1957-1968 (including A2/Hong Kong/1/68), two strains of A/Equine-2 virus isolated in 1963, and 7 avian influenza viruses isolated in Europe, North America, and the Ukraine in the years 1960-1967.

Antigenic relationship among the strains was determined on the basis of hemagglutination-inhibition, virus-neutralization, strain specific complement-fixation, and neuraminidase-inhibition tests.

North American avian influenza strains Turkey/Massachussets/65, Turkey/Wisconsin/66, Turkey/California/64, and the Italian strain Duck/Italy/574/66 are antigenically related to human A2 influenza viruses by hemagglutinin and neuraminidase. None of these viruses is antigenically related to the A/Equine-2/63 strains, Duck/Ukraine/2/60 and 1/63, and A2/Hong Kong/1/68. However, A2/Hong Kong/1/68 has neuraminidase similar to that of other A2 strains isolated in previous years.

Definite relationship was shown between the hemagglutinin of A/Equine-2/63 strains, A2/Hong Kong/1/68, Duck/Ukraine/2/60 and 1/63 in hyperimmune and postinfectious sera by means of three different serological tests. Common neuraminidase was demonstrated only between A/Equine-2/63 strains and the duck strains from the Ukraine.

The significance of these findings and its interpretation with respect to the ecology of influenza viruses is discussed.

Session II

Antigenic Relationships of the Hong Kong Virus Neuraminidase to That of Animal Influenza Viruses

by

Geoffrey C. Schild; London, England

There is now much evidence that the haemagglutinin and neuraminidase form immunologically and morphologically distinct components of the influenza virus envelope. To be comprehensive, studies on immunological relationships between subtype specific antigens of different influenza viruses should therefore involve studies on virus enzyme in addition to haemagglutinin. In the present study the neuraminidase of the A2/Hong Kong/1/68 variant was compared with that of former A2 viruses and of influenza viruses of avian, equine, and porcine origin.

The neuraminidases of representative A2 virus strains isolated in 1957, 1964, 1966, and 1967 variants were compared with that of the Hong Kong variant in enzyme-inhibition tests with antisera prepared by hyperimmunizing rabbits with purified neuraminidase or with concentrated and purified virus particles. The results were expressed as the serum dilution inhibiting enzyme activity by 50% after incubation for 16 hours at 4°C. The tests demonstrated considerable immunological differences in the enzymes of A2/Singapore/1/57 and A2/Hong Kong/1/68. High-titred antiserum against A2/Singapore/1/57 reacted to only low titres against A2/Hong Kong/1/68 enzyme, whereas antiserum to Hong Kong virus failed to neutralize the enzyme of A2/Singapore/1/57. Virus strains A2/England/12/64, A2/England/66, and A2/Tokyo/3/67 contained enzymes which cross-reacted to some extent with both 1957 and 1968 variants. Immunological relationships between the enzymes of the various A2 viruses were also studied in immuno-double-diffusion tests with antiserum prepared against purified neuraminidase preparations using methods described by Schild and Pereira (1969). In these tests all A2 viruses reacted similarly. The precipitin lines showed reactions of complete identity when the different A2 virus strains were compared in the same test. Human A0 or A1 virus strains failed to react with anti A2 neuraminidase antiserum. The differences in specificity of the immunoprecipitin and enzyme-inhibition tests are difficult to understand. However, the results of the immunoprecipitin tests suggested that all the A2 viruses tested shared enzymes of similar basic structure, whereas enzyme-inhibition tests appeared to indicate possibly minor immunological differences in the enzymes.

In previous studies enzyme-inhibition tests have indicated immunological relationships between human and animal influenza A virus which were not apparent from the results of haemagglutination-inhibition tests. Pereira, Tumova, and Webster (1967) found that an avian virus A/Turkey/Massachusetts/65 contained an enzyme immunologically related to that of A2/Singapore/1/57 virus and Schild, Pereira, and Schettler (1969) described an influenza virus of duck origin which contained an enzyme immunologically related to that of human influenza A0 and A1 viruses. Reciprocal enzyme-inhibition tests were carried out in which Hong Kong virus neuraminidase was compared to that of representative strains of avian, equine, and porcine influenza A viruses. Unlike A2/Singapore neuraminidase, the enzyme of A2/Hong Kong showed no cross-reaction with that of A/Turkey/Massachusetts/65. However, two different duck influenza virus strains were found to contain enzymes related to that of the Hong Kong virus, but these failed to cross-react with Hong Kong in haemagglutination-inhibition tests. Although in haemagglutination-inhibition tests A2/Hong Kong cross-reacts with Equine-2 virus strains, enzyme-inhibition tests failed to indicate serological relationships between the enzymes of these viruses or that the enzyme of Hong Kong was related to that of a swine influenza virus (Swine/Cambridge/39).

Schild, G. C. and Pereira, H. G., (1969). J. Gen. Virol., 4, p. 355.

Pereira, H. G., Tumova, B. and Webster, R. G., (1967). Nature, Lond., 215, p. 982.

Schild, G. C., Pereira, H. G. and Schettler, C. H., (1969). Nature, Lond., 222, p. 1299.

Session II

Experimental Influenza Virus Infections in Man and Horses

by

J. A. Kasel; Bethesda, Maryland, U.S.A., and R. B. Couch; Houston, Texas, U.S.A.

The recognition of an antigenic relationship between the hemagglutinins of A/Equine-2 virus isolated from horses in 1963 and a type A2 virus which caused epidemic illness in human populations in 1968 and 1969 suggested that interspecies spread of influenza virus may occur in nature and result in the emergence of new strains of virus in man and horses.

Because of the possible biologic importance of this relationship for human influenza, an investigation was conducted to assess the effect of inoculation of volunteers with equine influenza virus and of subsequent challenge with human type A2 virus. A similar study was conducted in horses except that the virus challenge sequence was reversed.

Fifteen serum neutralizing antibody-negative adult male volunteers were inoculated with A/Equine-2/Miami/63 influenza virus, and virus shedding ensued in all subjects. Illness developed in 13 persons, the most common clinical response being a febrile illness indistinguishable from naturally occurring human influenza. Fourteen of the 15 individuals exhibited a rise in serum antibody titer to the infecting strain and, of these, nine developed a heterologous antibody response to A2/Hong Kong/68 influenza virus. After administration of A2/Hong Kong/68 virus to 10 serum antibody-negative Chincoteague ponies *(Equus caballus)*, there was virus shedding from nine and a febrile response in six. Four of the animals developed a rise in antibody titer to the human strain, but none exhibited an antibody response to A/Equine-2 virus.

When the 15 human subjects previously inoculated with equine virus were challenged with A2/Hong Kong/68 virus, the frequency of illness and the extent of virus shedding was lower than that which occurred among 20 control individuals. Immunity against challenge was found to be related to the level of serum antibody titer to A2/Hong Kong/68 virus which developed after equine virus infection. Only those volunteers who lacked or had low levels of serum antibody experienced infection. Challenge with A/Equine-2/Miami/63 virus of 10 ponies previously inoculated with A2/Hong Kong/68 virus in the absence of any measurable levels of serum antibody to the human strain and five control animals resulted in significantly less shedding of virus among the former group of animals. Two ponies with a prior exposure to A2/Hong Kong/68 virus and one control developed a febrile response.

DISCUSSANTS

Dr. Robert G. Webster; Memphis, Tennessee

Dr. Martin M. Kaplan; Geneva, Switzerland

Dr. B. C. Easterday; Madison, Wisconsin

Session II

Interpretation of Antibody Patterns of Man: I. In the United States

by

F. M. Davenport, E. Minuse, A. V. Hennessy, and T. Francis, Jr.;

Ann Arbor, Michigan, U.S.A.

It has been shown that the major antigens of the strains of the initial infections of childhood permanently orient the antibody forming mechanisms so that on subsequent exposure by vaccination or infection with different strains of influenza virus of the same type, antibody against the original infecting strain is vigorously reinforced. Owing to antigenic shifting, the indelible imprint of different cohorts of the population is oriented to different families of strains. Consequently, a serologic recapitulation of a population's past experiences with influenza viruses may be constructed by delineating the age distribution of specific antibodies now reacting with prototype strains.

Criteria for the delineation of an antibody pattern have been progressively developed. At present four are in use. The age distribution of a particular antibody should correlate with what is known about the periods of epidemic prevalence of influenza. Moreover, with the passage of time, the antibody pattern should shift in concordance with the number of years that have elapsed between the two serological surveys. The specificity of the antibodies should be established by the use of more than one serologic method. Neutralization tests and Drescher's photometric test for defining strain specific antibody have proven to be highly useful for differentiating between antibody patterns found with homologous and heterologous strains. Finally, a direct test of the validity of the recapitulation can be carried out by ascertaining the antibody response of different cohorts of the population to monovalent influenza virus vaccines.

In the present study the age distribution of HI Hong Kong antibodies was ascertained with the Aichi strain in sera collected in 1958 and in 1966. In both collections a high frequency of antibodies was found in the sera of persons born before the early 1890's. The antibodies of this cohort were shown to neutralize virus in ovo and to react specifically in the Drescher photometric test. The antibody response to monovalent A2/Aichi/2/68 and A/Equine-2/Milford/2/63 vaccines was ascertained in subjects born in the intervals 1928-1937, 1888-1901, and 1879-1888. After administration of both vaccines the Aichi antibody titers were the highest in specimens obtained from persons born between 1879 and 1888.

Clearly, Hong Kong-like viruses have existed during a period of past prevalence, and Hong Kong antigens were recycled in the pandemic of 1969. The significance of these findings will be discussed.

Session II

Interpretation of Antibody Patterns of Man: II. In Europe

by

N. Masurel; Leiden, The Netherlands

Analyses of strains of influenza A in man and animals suggest that viruses derived from animal sources have played an important role in human epidemics. The presence of antibody against such an influenza A strain in sera of persons in a certain age group may indicate that this virus, or a related influenza strain, has caused previous epidemics in man.

Results of virologic and serologic investigations permit the following conclusions and suggestions:

1. There is strong evidence that the antigen of influenza A/Swine/1930 is identical or related to the influenza strain that caused the pandemic of 1918-1919. From 1933 until 1957 all human A strains showed a distinct antigenic relationship to the A/Swine/1930 virus.

2. It is highly probable that the antigen of the pandemic A2 influenza virus strain of 1957 is identical or closely related to the virus responsible for a pandemic in 1889-1890.

3. The A/Equine-2/63 virus demonstrates an antigenic relationship with the human A2 strains in the antibody response to vaccination and in infection and re-infection experiments in man and ferrets. However, no relationship was found in the cross haemagglutination-inhibition test. Antibody against the equine strain has been found in man in age groups which suggest that the A/Equine-2 virus or an A/Equine-2-like virus may have caused epidemics in man around 1900, that is, 10 years after the A2 pandemic of 1889-1890.

4.a). Four different experiments (the cross HI test, the antibody response to vaccination and infection, and the re-infection experiments in ferrets) demonstrate an antigenic relationship between the Hong Kong virus and the A2 viruses and between the Hong Kong strain and the A/Equine-2 viruses.

b). Exposure to A2 influenza viruses during the period 1957-1967 increased the level and frequency of antibody titres against the A/Equine-2 virus in sera from older age groups.

c). The pattern of antibody against the Hong Kong virus in sera collected before the 1957 pandemic of A2 influenza suggests that a virus resembling the Hong Kong/68 strain was prevalent in man around the beginning of this century, or about 10 years after the 1889-1890 pandemic.

Session II

Interpretation of Antibody Patterns of Man: III. In Japan

by

Hideo Fukumi; Tokyo, Japan

Sera from residents in old people's homes were examined for their antibody patterns by using five strains of type A influenza virus: A/Swine/30, AO/PR/8/34, A1/Ohmachi/1/53, A2/Kumamoto/1/67, and A2/Aichi/2/68 (Hong Kong variant), in the hemagglutination-inhibition test. The sera were collected prior to the occurrence of Hong Kong influenza in Japan. The frequency distribution of the antibody titers against each of these antigens is more or less characteristic for each, but the most characteristic of them is that for Hong Kong virus. As previously shown by many workers, the prevalence of Hong Kong antibody in the general population prior to the Hong Kong influenza epidemic was relatively low; only a small percentage possessed the Hong Kong antibody, mostly in a titer of 1:32 or, at most, 1:64. However, in people over 70, the frequency of antibody titers against Hong Kong type virus was fairly high, and it was even greater in people over 80. Not only was the frequency of Hong Kong antibody fairly high, but the average HI titer was considerably elevated, and in some individuals, it was as high as 1:256 to 1:512.

It was also found that though Hong Kong antibody titer was definitely correlated with the old A2 virus antibody titer in younger people, there was no correlation between these titers in people over 70. For example, in the old people, the frequency distribution of Hong Kong antibody titer was found to be independent of their A2 antibody titers, whereas in younger people, this was not the case.

These findings suggest that about 70 years ago there was an era during which the main antigen of type A influenza virus was the Hong Kong antigen independent from the main antigen of the old A2 virus. As for the worldwide epidemic eras of influenza, some authors have suggested that influenza eras have been occurring successively from swine to AO, A1, and then A2, and that now the era of Hong Kong influenza has just started. Previously, some Dutch workers proposed that there had been an era about 80 years ago of an influenza virus whose main antigen was like that of the old A2 virus. Here a further suggestion is made that immediately following the early A2 era, which lasted about 10 years, an early Hong Kong influenza era occurred.

Session II

Interpretations of Antibody Patterns of Man: Absorption of Human Sera with Hong Kong Variant, A/Equine-2, and Asian Influenza Viruses

by

William M. Marine and Wilton M. Workman; Atlanta, Georgia, U.S.A.

Antigenic interrelationships among the Hong Kong variant (HK), A/Equine-2 (Eq 2), and Asian influenza A viruses were explored in sera obtained from nursing home and young adult populations before and after the 1968-69 Hong Kong influenza epidemic. A2/Hong Kong/8/68, A/Equine-2/Milford/2/63, and A2/Japan/170/62 strains were used in microtiter hemagglutination-inhibition (HI) and antibody absorption techniques.

Persons in the nursing home population had birthdates from 1869-1901. HK and Eq 2 antibodies, 85% and 20% respectively, were almost exclusively found in sera obtained in 1964 from the 118 persons born in the years 1869-1891 (pre-1892). The prevalence of Asian antibody was high, regardless of age. HK vaccine in the pre-1892 group caused a fivefold rise in HK antibody, a rise in Eq 2 in two-fifths of the group, and no rise in Japan/170 titer. In 71 young adults the pre-epidemic prevalence of HK, Eq 2, and Japan/170 antibody was 3%, 0%, and 93%, respectively. HK vaccine produced a moderate response in HK, no response to Eq 2, and a sixfold rise in Japan/170 titer.

Antibody absorption experiments were performed on individual sera diluted to contain two HI antibody units. The sera came from (A) the nursing home group in 1964,(B) the nursing home pre-1892 group with $\geq$ fourfold rise in HK antibodies after HK vaccine, (C) seven of the nursing home group with Eq 2 antibody rises to 1:64 after HK vaccine, and (D) the young adult group before and after $\geq$ fourfold rise in HK antibodies after HK vaccine.

HK virus consistently removed Eq 2 antibody from Groups A and C when the sera contained HK antibody (12/12 sera). Eq 2 virus also removed HK antibody from Groups A and C when the sera contained Eq 2 antibody (15/16 sera); however, Eq 2 virus infrequently removed HK antibody from sera in Groups A and D which had no Eq 2 titer (4/16 sera).

When HK antibody was not present, HK virus removed Japan/170 antibody occasionally from sera in Group A as well as from pre-vaccine sera in Group D (8/29 sera). Further, there was little improvement when HK antibody was present in Groups A and B (13/30 sera). Japan/170 virus removed HK antibody from Groups A and B at a comparable low frequency (10/28 sera). However, after use of HK vaccine in Group D a striking improvement occurred in the absorption of Japan/170 antibody with HK virus (9/9 sera) and in the absorption of HK antibody with Japan/170 virus (8/8 sera).

Eq 2 virus removed Japan/170 antibody poorly in sera from Group A with or without Eq 2 antibody (0/10 and 2/9 sera, respectively) as well as in Group C (1/7 sera). Japan/170 virus partially removed Eq 2 antibody from Groups A and C (6/13 sera) but not to the same degree that HK removed Eq 2 antibody from these same sera.

These studies suggest a close relationship between HK and Eq 2 strains. A more distant relationship is seen between HK and Asian strains in the nursing home sera; however, the dual response of a young adult group to HK vaccine confirmed by reciprocal antibody absorption supports a definite association between these strains. A different sequence of exposure of the two groups to HK and Asian strains might explain the contrasting responses of the two groups to HK vaccine.

Both antibody prevalence and antibody absorption studies in these populations support the hypothesis that the HK virus is the best existing prototype of the human antigenic group of influenza A viruses that circulated before 1900.

ABSTRACTS

Session III

INACTIVATED INFLUENZA VIRUS VACCINES

WEDNESDAY, OCTOBER 15, 1969

8:30 A.M.—12:30 P.M.

Session III

Production of Influenza Vaccines

by

Roderick Murray; Bethesda, Maryland, U.S.A.

Session III

Problems of Influenza Virus Vaccine Standardization

by

Nicola M. Tauraso and Thomas C. O'Brien; Bethesda, Maryland, U.S.A.

The lack of reliable laboratory methods for determining the antigenicity of inactivated influenza virus vaccines prompted our laboratory to reinvestigate the reproducibility of the tests used for measuring the antigenic content of influenza vaccines, namely, the CCA (chick cell agglutinating unit) and mouse potency tests.

The data obtained in the second part of the mouse potency test, that is, the neutralization test performed either in the mouse or embryonated egg, statistically demonstrated protective differences between two vaccines differing in antigenic mass by as little as twofold. However, the dependence upon a single egg or mouse neutralization test to provide the correct vaccine/reference ratio was based upon an assumption rendered invalid by "biological" variation. Further, the test was long and tedious, and it would be impractical to perform the number of tests needed to obtain statistically significant results. Thus, the extreme variability observed between individual mouse potency tests and the impracticality of performing this test in statistically sufficient numbers precluded its usefulness for measuring the antigenic content of inactivated influenza vaccines.

The simpler CCA test, on the other hand, did provide the reproducibility required for the correct determination of the vaccine/reference ratio once a stable CCA reference vaccine was prepared. This test was easily reproducible, and results obtained were sufficient for drawing a meaningful and reliable conclusion about vaccine potency.

The problems of measuring the relative content of several components in multivalent vaccine preparations and of finding a test which positively correlates with vaccine potency in man, however, remain unsolved.

DISCUSSANTS

Dr. Albert V. Hennessy; Ann Arbor, Michigan

Dr. Frank T. Perkins; London, England

Dr. Hideo Fukumi; Tokyo, Japan

Dr. Preben von Magnus; Copenhagen, Denmark

Effect of Dosage and Route of Inoculation upon Antibody Responses to Inactivated Hong Kong Influenza Virus Vaccine in Man

by

Nicola M. Tauraso, Frank A. Pedreira, and Rachel Yahwak; Bethesda, Maryland, U.S.A., and Richard A. Gleckman, Morton A. Madoff, and Jacobo Sabbaj; Boston, Massachusetts, U.S.A.

In 1947 Van Gelder et al showed that a 0.1 ml intradermal (ID) injection of inactivated influenza vaccine was superior to 1.0 ml administered by the subcutaneous (SC) route. From studies conducted 10 years later (Boger et al and McCarroll et al), the opposite was concluded, that is, the smaller ID dose offered no advantage or was inferior to the larger SC dose. It is difficult to know whether the data and conclusions of these earlier studies would be valid with the more potent vaccines available today. With the advent of the 1968-69 Hong Kong influenza epidemic it seemed worthwhile to re-evaluate whether a smaller ID dose would elicit antibody responses comparable to those following the larger SC dose. Three doses—0.1 ml (65 CCA), 0.25 ml (160 CCA), and 0.5 ml (320 CCA) of zonal-purified vaccine—were evaluated. The 0.1 ml dose was administered by both the ID and SC routes, and the other doses were given by the SC route only. The effect of "booster" inoculation by the same route 2 and 4 weeks later was also studied. Sera were examined by the hemagglutination-inhibition test and the antibody response was determined by the percent showing fourfold or greater titer rises and by the increase in geometric mean titer (GMT).

The antibody response to the first inoculation was highest in the 0.1 ml/ID groups and the lowest in the 0.1 ml/SC groups. The responses in the 0.5 ml/SC and 0.25 ml/SC groups were intermediate, with the responses of the former averaging the better of the two. All groups receiving a second inoculation 2 weeks after the first experienced an increase in antibody response. Responses to the second inoculation given 4 weeks after the first were variable. Considering the overall effect of all combinations of doses and routes, the ID groups appeared to achieve the best antibody response, the 0.1 ml/SC groups were least effective, and the 0.5 ml/SC and 0.25 ml/SC groups were intermediate, with the former appearing slightly better.

There appeared to be an inverse relationship between antibody response and preimmunization antibody titer.

Our data show that using vaccine of similar CCA content, 0.1 ml intradermally, would be a reasonable, if not better, alternative to the higher 0.5 ml SC dose. The limitations of this approach are discussed.

Session III

Antibody Responses to and Efficacy of Inactivated Influenza Vaccine

by

Tadao Sonoguchi; Tokyo, Japan

In October 1968 about 3,000 Ground Self-Defense Force (GSDF) personnel in five camps were inoculated subcutaneously with various doses of monovalent Hong Kong influenza vaccine containing 300 chick cell agglutinating units (CCA)/ml of the A2/Aichi/2/68 virus.

The paired sera from half of the subjects obtained before and 1 month after the inoculation were examined for their hemagglutination-inhibition (HI) antibody titers. The frequency distribution of HI antibody titers in prevaccination sera was $<$1:16 in 92%; 1:16 in 3.8%; 1:32 in 3.6%, and 1:64 in 0.5% (HI titers are shown in final dilution).

Percentages of the subjects possessing an HI antibody titer of $<$1:16, and the geometric mean of those HI titers of $\geqq$ 1:16 one month following inoculation of various doses were: 40-50% and 1:30-1:50 for the groups given 0.2-0.25 ml in a single dose; 7-50% and 1:45-1:84 for those given 0.5 ml in a single dose; 7-26% and 1:56-1:119 for those given 1.0 ml in a single dose, and 1-19% and 1:49-1:111 for those given 0.5 ml in two separate doses. In one of the camps where the antibody response was extremely high, it was serologically proven that there were more or less sporadic Hong Kong influenza cases within 1 month after vaccination.

An epidemic due to Hong Kong virus occurred from January through March 1969 in a vaccinated camp of approximately 2,000 GSDF soldiers in Tokyo. In a unit consisting of 980 men of whom 90% were living on the post, 80% were vaccinated. The infectivity rate was 16% in the vaccinated group (7% for the ones given 1.0 ml, 13% for these given 0.5 ml, and 43% for those receiving 0.2 ml), and it was 26% in the unvaccinated group, living in the same barracks with the vaccinated. The rate in the control group living in another barrack was 63%. The number of paired sera with fourfold rises in HI antibody titer to Hong Kong virus was 21 of 134. The second serum specimen was taken 1 month after vaccination. Before the epidemic, 20 of the 21 had an HI titer of $\leqq$1:32 and one had a titer of 1:64.

During the same period an epidemic of Influenza B occurred in this camp, and the infectivity rate was 14% for the vaccinated group as well as for the control group. Therefore, exposure in the two groups was apparently equal, indicating that differences in infectivity rates for Hong Kong virus between vaccinated and unvaccinated groups were due to the Hong Kong vaccine.

In contrast to the aforementioned findings on infectivity, there was no significant difference in incidence rate between the two groups.

In another unit of about 1,200 men, half of them living in the camp, about one-third were inoculated with two doses of commercial vaccine containing 200CCA/ml of Hong Kong virus and 100CCA/ml of a B strain given in a single dose of 0.3 ml. There was no difference in the infectivity rate between the vaccinated and unvaccinated individuals. The inability to detect a vaccine effect in this group was ascribed to the relatively small number of subjects living in the camp. The infectivity rate of those not vaccinated who lived in the camp was 37%, whereas that of those, also not vaccinated, living outside the camp was 26%.

Studies with Inactivated Influenza Vaccines Purified by Zonal Centrifugation: I. Adverse Reactions and Serologic Responses

by

S. R. Mostow; Cleveland, Ohio, U.S.A., S. C. Schoenbaum; Boston, Massachusetts, U.S.A., W. R. Dowdle, M. T. Coleman, and H. S. Kaye; Atlanta, Georgia, U.S.A.

High (3000 CCA unit) and standard low (300 CCA unit) doses of formalin inactivated influenza vaccines purified by zonal ultracentrifugation were evaluated in a double-blind manner for adverse reactions and antibody responses in three populations. Monovalent A2/Japan/170/62 and A2/Aichi/2/68 vaccines at both dosage levels were administered to volunteers among residents of a retirement community, students in junior and senior high schools, and inmates at the Georgia State Prison. The latter two populations were also given high and low doses of highly purified B/Massachusetts/3/66 vaccines as controls. Commercially available Sharples centrifuged bivalent and polyvalent vaccines containing the 1967-68 formulation were included as additional controls in the two adult populations. All vaccines were administered subcutaneously in 1 ml volumes by jet injector gun. Sera were collected before and 3 weeks after immunization.

Local and systemic reactions were fewest with the low doses of all three purified vaccines. The high doses of A2/Japan and A2/Aichi vaccines produced a two- to threefold greater incidence of adverse reactions. Nevertheless, the percentage of adverse reactions produced by the high doses did not exceed and was generally less than that observed with the commercial vaccines in the two adult populations where both zonal centrifuged and Sharples centrifuged vaccines were used. Unlike the high doses of the A2 vaccines, 3000 CCA units of B/Massachusetts administered to prison volunteers produced more frequent and more severe systemic reactions than the polyvalent vaccine. The same 3000 CCA units of B/Massachusetts caused very severe systemic reactions in the school populations and resulted in a sharp increase of absenteeism. The reasons for the high toxicity of B/Massachusetts vaccines have not been fully evaluated. Adverse reactions to all vaccines were more frequent in the school-age population.

The A2/Japan vaccines and the commercial vaccines stimulated moderate to high geometric mean titers by hemagglutination-inhibition tests against the homologous A2/Japan antigen. However, these vaccines did not stimulate a significant antibody response to A2/Aichi/2/68 or to other Hong Kong variants. The homologous serum antibody response to the Aichi vaccines was excellent in all three populations; nearly 80% receiving the low dose showed a fourfold or greater increase in antibody titer and over 90% of those receiving the high dose had a fourfold or greater response. A2/Aichi vaccines in the two adult populations produced A2/Japan titers equal to or greater than that produced by the A2/Japan vaccines. The high doses of all three vaccine strains produced geometric mean titers two to three times greater than those produced by standard doses.

Thus it was demonstrated that high doses of purified influenza vaccines could be given safely, that purification neither enhanced nor decreased antigenicity, and that tenfold greater doses of vaccine produced two- to threefold greater geometric mean antibody titers.

Studies with Inactivated Influenza Vaccines Purified by Zonal Centrifugation: II. Efficacy

S. C. Schoenbaum; Boston, Massachusetts, U.S.A., S. R. Mostow; Cleveland, Ohio, U.S.A., W. R. Dowdle, M. T. Coleman, and H. S. Kaye; Atlanta, Georgia, U.S.A.

High (3000 CCA unit) and standard low (300 CCA unit) doses of monovalent purified vaccines described in Part I were evaluated in a double-blind manner in two adult populations for protective effectiveness against illness caused by the Hong Kong influenza virus. A sharp influenza outbreak of 3 weeks' duration, with an overall clinical attack rate of approximately 40%, occurred in late December 1968 among the inmates of the Georgia State Prison. A smaller outbreak beginning in late December, lasting 5 to 6 weeks, with an overall clinical attack rate of approximately 10% occurred among the residents of the retirement community. Epidemics in both populations occurred 4 to 6 weeks after single injections of vaccine were given. No epidemics occurred in the school-age population.

The attack rates among recipients of low or high doses of A2/Japan vaccines in the prison were virtually identical to those of the B/Massachusetts control groups. The attack rate among those receiving the 3000 CCA units Aichi vaccine was nearly 70% less than the rate for the A2/Japan or B/Massachusetts vaccine groups. Although no monovalent influenza B vaccine was given to the residents of the retirement community, a similar 60-70% reduction in attack rate was observed among the 3000 CCA unit Aichi recipients when compared to the A2/Japan vaccine recipients.

Furthermore, in the high Aichi groups in both populations, those who became ill tended to have less morbidity, fewer and lower fevers, and shorter stays in bed. The attack rates among the groups receiving the low doses of Aichi vaccine were somewhat less than the rates among those receiving the A2/Japan or B/Massachusetts vaccines, but the effect was not statistically significant in either population. The bivalent and polyvalent commercial vaccines were also without protective effect; and, in fact, in both populations the polyvalent vaccine recipients experienced the highest attack rate.

Effect of Vaccination of a School-Age Population Upon the Course of an A2/Hong Kong Influenza Epidemic

by

A. S. Monto, F. M. Davenport, J. A. Napier, and T. Francis, Jr.; Ann Arbor, Michigan, U.S.A.

In influenza outbreaks, the greatest morbidity is experienced by children of school age. These children are also responsible in great part for the dissemination of the virus through the community. Vaccination of school children should, therefore, decrease morbidity in the most susceptible segment of the population and also curtail transmission to the adults and younger children.

To control the anticipated outbreak of influenza in the winter of 1968-1969, Hong Kong variant influenza vaccine was offered to the school children of Tecumseh, Michigan, a community which has been under study since 1959. More than 85% of the school children were vaccinated subcutaneously with 0.5 ml of a zonal ultracentrifuge-purified preparation of inactivated A2/Aichi/2/68 (800 CCA per ml). Systemic reactions to the vaccine were rare. Pre- and post-vaccination blood specimens were obtained from approximately 5% of those inoculated, and 92.4% exhibited a fourfold or greater rise in HI titer.

Surveillance of respiratory disease had been underway in Tecumseh since 1965. A group of families was contacted weekly and the illnesses occurring in the past week were recorded. Specimens for microbial isolation were also collected when illnesses were reported. The city of Adrian, Michigan, 12 miles from Tecumseh, also had a surveillance program underway, using the same questions on respiratory disease in a somewhat different format. Adrian, therefore, appeared to be a suitable comparison community against which to measure the effectiveness of the vaccination program in Tecumseh.

The first isolation of Hong Kong variant influenza virus was made in Tecumseh 2 weeks after the period of vaccination. It continued to be isolated in both Tecumseh and Adrian for 9 additional weeks. Through this 10-week-period, mean rates of illness, adjusted for differences in pre-epidemic reporting, were 2.8 times higher than in Tecumseh. School absenteeism in Tecumseh did not exceed pre-established threshold levels, whereas it was markedly increased in Adrian. The protection from illness was not limited to the school children; illness rates in Tecumseh were also lower for adults and younger children. So many individuals in the school-age group were vaccinated that protection even extended to those few who were not vaccinated. The rates of illness in Tecumseh were shown to be markedly lower than those in two other Michigan cities where similar surveillance studies were underway.

Session III

An Evaluation of Influenza Immunization: Influence of Route of Administration and Vaccine Strain

by

Robert H. Waldman and Parker A. Small, Jr.; Gainesville, Florida, U.S.A., James O. Bond, Eldert C. Hartwig, E. Charlton Prather, and Robert L. Baratta; Jacksonville, Florida, U.S.A., Lawrence P. Levitt and John S. Neill; Tampa, Florida, U.S.A.

Approximately 2,000 school teachers from the Tampa, Florida, school system volunteered to participate in a study designed primarily to assess three variables in influenza immunization: 1) route of administration: aerosol *vs.* injection, 2) strain of virus used in vaccine: bivalent (A2 and B) *vs.* A2/Hong Kong, and 3) number of doses: one *vs.* two. The experimental groups were as follows:

Study Group	Volunteers Per Group	First (December 12-13, 1969)		Second (January 3, 1969)	
		Injection	Aerosol	Injection	Aerosol
1	235	H	S	H	S
2	230	H	S	S	S
3	231	B	S	B	S
4	240	B	S	S	S
5	240	S	H	S	H
6	239	S	H	S	S
7	237	S	B	S	B
8	234	S	B	S	S
9	237	S	S	S	S

S = saline; B = bivalent vaccine; H = A2/Hong Kong monovalent vaccine.

Each person, therefore, received in a double-blind manner both an injection and an aerosol administration on two occasions 3 weeks apart. Approximately half of the study population gave two or three blood and two nasal wash specimens. A brief history, including smoking habits, was obtained. Each teacher then kept a daily record of symptoms for the next 11 weeks. Shortly after the initial immunization, an influenza epidemic occurred, as shown by isolations of A2/Hong Kong virus, antibody rises, and increased absenteeism in the local industries and schools.

Over 2.5 million pieces of data were collected. Computer programs were developed to determine illness rates defined in a variety of ways, side effects associated with the different vaccines and their modes of administration, and correlations between smoking habits and illness rates. Infection rates were determined by serologic analysis.

Session III

Antibody Responses to and Efficacy of Inactivated Spray Vaccines

by

A. S. Beare; Salisbury, England

Volunteers were sprayed intranasally with killed influenza B vaccine and the extent of their protection assessed by means of a deliberate challenge with living, homologous virus.

Two types of trials were performed. In the first of these, 19 volunteers sprayed themselves intranasally on either one or two occasions with 1.0 ml of vaccine containing 5,500 haemagglutinating units by means of a coarse hand spray. A control group of 10 received phosphate-buffered saline by the same method. All the volunteers were subsequently admitted to the Common Cold Research Unit and challenged with 100,000 egg-infecting doses of the virus used in the preparation of the vaccine. Infections were assessed from the incidence of clinical reactions, isolations of virus from nasal washings taken on the second, third, and fourth days after the challenge and from serum antibody rises 2-3 weeks later. No reduction of infections occurred in the vaccinated volunteers as compared with those in the controls.

In the second trial series, the protective effects of intranasal spray of inactivated influenza B vaccine were compared with those of conventional vaccines. Groups of about 20 factory workers were allocated at random to each of the following vaccine groups, (1) aqueous parenteral influenza B vaccine, (2) split parenteral influenza B vaccine, (3) intranasal killed influenza B vaccine given twice in the same dose as before by means of a fine hand spray, and (4) a heterologous parenteral influenza A vaccine, which served as an unprotected control. All the volunteers were later challenged with 10,000 egg-infecting doses of live virus of the kind used in the preparation of the first three vaccines. Infections were diagnosed by rises of serum antibody 2 weeks later. While the control group showed the expected incidence of infections and the two parenteral vaccine groups showed appreciable degrees of protection, the protective effect seen in the group receiving intranasal spray was apparently negligible. It is possible, however, that the vaccine was not wholly inert.

No estimations were made of local antibody formation after the intranasal vaccinations.

Whether killed intranasal vaccines are potentially able to protect against influenza B is not known, but in the doses used in these trials they were relatively ineffective.

DISCUSSANTS

Dr. William J. Mogabgab; New Orleans, Louisiana

Dr. Theodore C. Eickhoff; Denver, Colorado

Dr. J. Thomas Grayston; Seattle, Washington

Dr. Floyd W. Denny; Chapel Hill, North Carolina

Dr. Howard C. Goodman; Geneva, Switzerland

Col. Edward L. Buescher; Washington, D. C.

ABSTRACTS

Session IV

LIVE INFLUENZA VIRUS VACCINES

WEDNESDAY, OCTOBER 15, 1969

2:00 P.M.—4:00 P.M.

Session IV

Antibody Response to and Efficacy of Live Virus Vaccines

by

D. A. J. Tyrrell and A. S. Beare; Salisbury, England

Live influenza virus vaccination has potential advantages and disadvantages compared with killed virus vaccine given by intramuscular injection. A smaller quantity of live virus would be needed to vaccinate each individual but, if the virus were very attenuated, a large dose might be needed to infect, and, in addition, some activity might be lost in freeze-drying the virus. Nevertheless, live virus vaccination is more likely than any other method to stimulate antibody in the respiratory mucosa. An important difficulty is that there is no agreed method for regularly inducing the attenuation, nor are there laboratory "markers" to indicate that the attenuation has occurred. These would be essential if live virus vaccination were to be used in the face of an epidemic. Some attenuation probably takes place in the first few passages, but it is not enough to make the viruses suitable as vaccine strains.

We have therefore tried to attenuate completely influenza A2 and B strains by up to 30 serial passages in the allantoic cavity of eggs at 33°. No attenuation occurred. We have recently tried the method of passing viruses in the presence of an increasing concentration of horse serum, thus selecting inhibitor-resistant variants. Serial passage was shown to induce an increasing degree of inhibitor resistance in the virus and an increasing degree of attenuation for volunteers. This was observed with both an Asian strain and a Hong Kong strain.

The Hong Kong virus was also passed serially at 25° in eggs, but it did not seem much further attenuated as a result; however, a virus strain supplied by Dr. Maassab with a much higher degree of adaptation to low temperature is now being tested, and the results will be reported at the conference.

Partly or completely attenuated strains have been used to induce or detect protection. Influenza B strains gave partial protection after one dose and almost complete protection after two doses, and protection persisted for 7 months. A comparative study showed that there was protection after vaccination with killed virus by the parenteral route and with live virus by the nasal route.

The relative susceptibility and efficiency of all these vaccines should be further assessed.

Session IV

The Efficacy of Live Virus Vaccines

by

Anatoli A. Smorodintsev; Moscow, U.S.S.R.

Laboratory Characteristics of Attenuated Strains of Influenza Virus

by

Huenin F. Maassab; Ann Arbor, Michigan, U.S.A.

Attenuation of influenza virus prior to 1967 was achieved by gradually lowering the incubation temperature until optimal growth, at 25°C, was obtained.

The process of attenuation of a Hong Kong strain was modified and shortened considerably. The temperature of incubation was changed abruptly from 35°C to 25°C, with selection of a cold variant by the plaque assay system.

A set of genetic markers for the cold variant was developed for assessing the potential virulence of cold-passaged lines. The cold variant of the Hong Kong strain was temperature sensitive and acid labile and produced a small-sized plaque in primary chick kidney cells incubated at 35°C. Additional differentiating biological properties relating the adaption of the virus to growth at 25° to loss of virulence in a susceptible host will be presented.

The cold-adapted line was found to be relatively avirulent and highly antigenic for mice and ferrets. Virus was recovered from the nasopharynx of infected ferrets during the first 3 days. The virus recovered still had the impaired capacity to grow at 41°C (rct/41-), was sensitive to acid pH, and produced small plaques at 35°C and larger ones at 25°C.

After a series of plaque purifications, the cold variant showed further loss of virulence to mice, more vigorous growth at 25°C, complete failure of reproduction at 41°C, and good antigenic potency.

Markers of the plaque-purified cold variant were stable after at least 10 consecutive passages in either tissue culture at 35°C or in mice.

Cold variants of type B influenza virus have a narrower range of temperature sensitivity compared to type A strains. Attenuated B strains showed an impaired plaquing efficiency and reproductive capacity at 35°C (rct/35-) when compared to the type A strains at 41°C.

Session IV

Laboratory Characteristics of Attenuated Influenza Virus Strains

by

A. S. Beare; Salisbury, England

Difficulties in preparing satisfactory live influenza vaccines are allegedly due to genetic instability of the viruses. It has long been said that simple passage in an alien host will rapidly abolish their pathogenicity for man, and rather less rapidly, their human infectivity and antigenicity. Host-derived material associated with the haemagglutinins can usually be detected in viruses passed in embryonated hens' eggs, but no other major changes have been reported. So far, however, the host components have not been shown to possess any biological activity. One influenza A virus and two influenza B viruses with different growth characteristics were passed repeatedly in eggs in an effort to produce attenuated mutants. Although minimal changes were at first induced, there were no obvious differences in the human pathogenicity and infectivity of early and late passes of the viruses when these were given to volunteers of comparable susceptibility to infection at the Common Cold Research Unit. Nor could attenuation be hastened by passing one of the viruses repeatedly at low dilutions. In trials previously reported, the effects of virus inoculations were assessed from virus excretions, incidence of clinical reactions, and rises of circulating antibody, and it was clear that pathogenicity and infectivity for man are relatively stable characteristics of influenza viruses when these are propagated in the laboratory at nearly optimal temperatures.

Experiments were then performed with a virus known as A2/England/501/68, which is antigenically similar to A2/Tokyo/3/67, and with the strain A2/Hong Kong/1/68. By passing the former in eggs in the presence of heated horse serum, it was possible to obtain a virus totally resistant to serum inhibitor. When this virus was compared with the parent virus in the same volunteer trial, it was clearly much attenuated although it remained infective and antigenic. Comparable success was not, however, achieved with A2/Kong Kong/1/68. Most of the sensitivity to inhibitor was abolished by prolonged passage in the presence of horse serum, and the virus was appreciably attenuated, but the little sensitivity which remained was apparently associated with human pathogenicity. As in the case of A2/England/501/68, the modified viruses were infective and antigenic.

The inhibitor-sensitive strain of A2/Hong Kong/1/68 was also attenuated by serial passages in embryonated eggs at 25°C. Virus titres were, however, low, and it was necessary to propagate the viruses at limit dilution at 33°C before they could be used in volunteer trials. It is not yet clear whether suitable degrees of attenuation and infectivity can be achieved simultaneously, and there is no information on the stability of the low temperature mutants.

When wild and attenuated viruses were examined in the laboratory, the inhibitor-resistant viruses eluted more rapidly from red blood cells than the inhibitor-sensitive viruses. This is a well known characteristic of inhibitor-resistant viruses and is probably not a marker of attenuation. Studies on the characters of the attenuated influenza viruses are as yet incomplete.

Session IV

Evaluation of Influenza Virus Mutants for Use in Live Virus Vaccine

by

John Mills V, John VanKirk, and Robert M. Chanock; Bethesda, Maryland, U.S.A.

A safe, immunogenic live attenuated influenza virus vaccine would possess a number of theoretical advantages over inactivated vaccines for the immunoprophylaxis of influenzal disease. Chief among these are more effective stimulation of protective local respiratory secretory antibody and greater ease of administration. At present, an acceptable live influenza virus vaccine is not available; however, several recent developments suggest that this goal may be within sight.

Adaptation to growth at abnormally low temperatures has resulted in the emergence of attenuated mutants of poliovirus, respiratory syncytial virus, measles virus, and other viruses. Similarly, Dr. Maassab has shown that growth of influenza A virus in eggs at low temperature (25°C) results in a loss of viral virulence for mice. Stimulated by these observations, we investigated the infectivity and virulence of Hong Kong influenza A2 which had been propagated and terminally diluted at 25°C in bovine kidney (BK) tissue culture. When $10^8 TCD_{50}$ of this 25°C grown virus (BK passage 13) was given to eight serum neutralizing antibody free volunteers, we were unable to recover virus from these men, nor did illness develop; however, three volunteers developed a low level rise in serum antibody. Another group of eight seronegative volunteers exhibited a similar response to $10^5 TCD_{50}$ of virus. In contrast, when $10^6 TCD_{50}$ was administered to eight antibody free men, each man shed virus, six men developed influenzal disease (five with fever), and antibody responses were more marked than those seen previously. In this circumstance, it appeared that propagation at low temperature had led to the emergence of a mutant with decreased infectivity for man. Unfortunately, when a moderate level of infection did occur, it was associated with influenzal symptoms. Because of this failure to dissociate virulence from infection, we have turned our attention to another approach to attenuation of influenza A virus.

The newest approach involves the selection of conditional lethal, temperature-sensitive (ts) mutants of influenza A virus. With other respiratory viruses and mycoplasma, workers in our laboratory have shown that certain ts mutations are associated with attenuation. In part, this results from the marked growth restriction exhibited by such mutants at 37°C (the temperature of the lower respiratory tract); at 32°-33°C (the temperature of the nasopharynx) these mutants grow in an unrestricted fashion. We succeeded in isolating two ts mutants from BK influenza A2 virus which had been grown in the presence of 5-fluorauracil, a potent chemical mutagen. One of the influenza A2 ts mutants exhibited marked restriction of plaque formation at 37°C, whereas the other mutant was restricted at 38°C and above. In contrast, the wild type (parent) virus exhibited a high efficiency of plaque formation at 39°C. Preliminary complimentation studies suggest that the two mutants were affected in different cistrons. The behavior of the two mutants is being studied in hamsters, and these findings will be reported in detail.

DISCUSSANT

Dr. Gordon Meiklejohn; Denver, Colorado

ABSTRACTS

Session V

FUTURE INFLUENZA VIRUS VACCINES

THURSDAY, OCTOBER 16, 1969

8:30 A.M.—10:25 A.M.

Session V

Adjuvant Vaccines

by

Charles H. Stuart-Harris; Sheffield, England

Enhancement of the antibody response to injected antigens by incorporation of the latter in an oily emulsion has a respectable background of experience. The use of emulsified inactivated influenza virus vaccines has now been pursued for 18 years. The goal of enhanced serological response persisting for 2 or more years has been attained. The safety of the method in relation to immediate pyrogenic reactions has been demonstrated and no carcinogenic effects are known to have occurred in man. The problem of delayed local reactions after the injection of mineral oil vaccines has not, however, been solved. British experience of adverse reactions to commercial adjuvant influenza vaccine will be quoted.

New methods for obtaining adjuvant action without the risk of local abscess formation are needed both for inactivated whole virus and for split haemagglutinin vaccines. The peanut oil A65 emulsion of Woodhour et al. (1964) is one solution, but experience in Britain with this material is limited. The suggestion of a reversal of the ordinary water-in-mineral oil emulsion to an oil-in-water emulsion made by Herbert (1965) has the advantage of reducing the viscosity and permitting diffusion of the depot injection. A serological trial in Britain in 1966 by Taylor and others (1969) has shown equally good adjuvant properties of the reversed emulsion incorporating influenza virus vaccine so far as serological response is concerned. The results will be described. Too few persons have yet received the material for adequate evaluation of the likelihood of delayed local reactions. Even with the commercial mineral oil emulsion vaccine, the frequency of these has been of the order of 1:5000 to 1:10,000 of inoculated persons. Lack of the ability to reproduce such reactions in experimental animals hampers developments.

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Session V

The Role of Early Alert and Adjuvant in the Control of Hong Kong Influenza by Vaccines

by

Maurice R. Hilleman; West Point, Pennsylvania, U.S.A.

Aqueous influenza vaccine may be quite effective in the control of the disease. The minor antigenic variations in the virus which occur during the interpandemic periods can usually be handled adequately by periodic revision of the strain formula for the vaccine. However, the major antigenic change in influenza A virus which occurs at approximately 10-year pandemic intervals precludes the usefulness of the previous formula vaccine and taxes the ingenuity of those who would hope to control pandemic influenza by vaccines.

There are two principal approaches to the solution of this predictable and continuing dilemma. One is to detect the emergence of a new variant in one part of the world as far as possible in advance of its general spread in order to produce as much vaccine as possible and to carry out a vaccination program. The other is to improve the utility of the old formula prepandemic vaccine to afford at least some degree of protection against the new strain before its major pandemic spread. Both of these approaches were put to test in the 1968 Hong Kong influenza pandemic.

Vaccine preparation. The first alert to our laboratories of the existence of the new Hong Kong influenza virus came in a letter, sent on August 16, 1968, from the World Health Organization in Geneva. By September 13, new facilities were being readied for vaccine production, and actual production began in our laboratories on September 23. The problem of low yield of virus in embryonated hens' eggs from the new strain was solved by Dr. H. Fukumi who furnished a well-adapted, high yielding Hong Kong influenza strain. The first lot of vaccine made in our laboratories was released on November 19 and 6,167,000 doses were released by the end of 1968. A total of 9,750,000 doses were released by January 20, 1969. A total of 21,900,000 doses were produced by all vaccine manufacturers in the United States combined. Meantime, the pandemic itself had progressed rapidly in the population and had largely spent itself by the end of 1968. Hence, for the vast majority of persons in the United States, the new Hong Kong influenza vaccine was too little and too late. This performance, in spite of heroics, was far less spectacular than in the 1957 Asian influenza pandemic in which there was a 5-month alert prior to the pandemic event in contrast with the short 3-month alert in the Hong Kong influenza pandemic. Vaccine prepared too late for use in the United States was, however, in good time for use in the southern hemisphere, where the seasons are opposite to those in the United States, and in certain parts of Europe in which the major event was delayed.

Adjuvant 65 vaccine. One means for improving the performance of influenza vaccine is by incorporating the aqueous material into an adjuvant. Studies were made by our group in man of 1967 formula influenza vaccine in metabolizable emulsified peanut oil adjuvant 65. It was previously shown that incorporation of aqueous vaccine into the adjuvant increased the antibody titer response in man from fourfold to sixteenfold. This same response was achieved with only ¼ the antigen dose, or even less, making it possible to stretch the vaccine supply fourfold or more in time of urgent need. The increased antibody effect afforded by the adjuvant may persist in man for long periods—at least 6 years following the last previous dose of vaccine.

Importantly, incorporating the influenza vaccine in adjuvant 65 effects a considerable broadening of antibody response in human beings against diverse influenza virus serotypes. For example, incorporating aqueous 1962 influenza A2 virus vaccine in adjuvant 65 provided high level antibody response against the 1957 and the 1964 influenza A2 variants, rendering these interpandemic

changes in antigenic structure of the virus relatively unimportant from the immunization standpoint. An extreme example of broadening of antigenic response was shown for the recent Hong Kong influenza pandemic virus in persons given old formula vaccine in the adjuvant. Only 1.4% of persons who received the 1967 formula pre-Hong Kong influenza vaccine developed antibody against the 1968 Hong Kong virus. In contrast, 55% of persons given the same vaccine in adjuvant 65 responded. The antibody titers ranged from 1:10 to 1:160, and it seems reasonable that general application in 1968 of the old 1967 vaccine in adjuvant 65 might have afforded substantial protection against Hong Kong influenza in a significant portion of the human population.

Another magnitude of antibody response enhancement to influenza vaccine in adjuvant 65 was recently demonstrated in studies in which polynucleotides were added to the adjuvant formulation. These polynucleotides, which also induce interferon, do enhance antibody responses for a short period when given alone with aqueous antigen. Incorporation of the polynucleotide into adjuvant 65 formula influenza vaccine may give a fourfold-to-eightfold further increase in antibody, which is beyond that achieved by the adjuvant alone. The implications of this synergistic hyperpotentiation of antibody response is obvious. The activity of the polynucleotides in increasing antibody was separate and distinct from its capacity to induce interferon.

Influenza vaccine in adjuvant 65 has been given to more than 16,000 persons without untoward effect. In a recent follow-up of 504 persons given adjuvant 65 influenza vaccine in one to three doses up to 5 years previously, only three, or 0.6%, showed very small, barely palpable persistent nodules.

notes

Session V

Influenza Immunization: Clinical Studies with Ether-Split Subunit Vaccines

by

F. B. Brandon and I. W. McLean, Jr.; Detroit, Michigan, U.S.A.

The clinical evaluation of ether-split Type A2 influenza virus subunit (antigen) vaccines began shortly after 1957 when this virus first appeared. Monovalent, bivalent, and polyvalent antigen vaccines have been studied in infants, working age adults, and senior citizen groups. Because the specific strain of A2 virus varied from year to year, in accord with recommendations of the Division of Biologics Standards, our experience includes the complete spectrum of Type A2 isolates from the earliest to the current Hong Kong virus. Generally, antigen vaccines were tested in parallel with conventional vaccines containing equal quantities of the same components as their subunit counterparts. The report summarizes our total serologic experience with the A2 virus, including recent studies with the Hong Kong strain.

Pediatric studies with the Type A2 subunit vaccines, showing their immunological effectiveness and pyrogen-free character in small children, have been reviewed in earlier publications. Simultaneously with these studies, serologic evaluation of these vaccines was started in an adult population. The pre-Hong Kong studies included 17 pairs of conventional and antigen vaccines tested in over 1,600 adult volunteers. Antigen vaccines containing the Hong Kong virus were also studied in a hospital employee group and in a senior citizen retirement community.

Subjects received one dose of either antigen or conventional vaccine and hemagglutination inhibiting (HI) antibody titer was measured in serum samples taken at the time of and from 2 to 4 weeks following immunization.

In the studies conducted before 1968, 1,671 subjects received one or another of the 17 paired vaccines. The overall geometric mean pre-vaccination HI antibody titer against the A2 virus in this group was 20.5. Eight hundred and seventy-four volunteers were given conventional vaccine. These had a 3.5-fold increase in geometric mean HI antibody titer with a final titer of 72.6. The 747 antigen vaccinees displayed an eightfold increase in HI antibody with a final titer of 163.5. Overall conversion rates in the two groups were 67 and 83%, respectively.

Two additional antigen vaccines containing the Hong Kong virus have been studied. The first trial compared monovalent antigen and conventional vaccines in the residents and employees of an East Coast retirement community. This study was started just before the Hong Kong epidemic. The conventional and antigen vaccines were of equal immunogenicity in both populations; however, resident serologic response greatly exceeded employee response, presumably because of the former group's experience with a Hong Kong-like virus around the turn of the century. No influenza-like disease was seen in the community during the epidemic period.

The second Hong Kong study was conducted in a small group of hospital employees approximately 2 months after the epidemic peak. A fourfold increase in HI antibody titer against the Hong Kong virus was observed.

Desoxycholate Split Vaccines

by

M. F. Warburton; Victoria, Australia

Webster and Laver first showed that influenza viruses disrupted by treatment with sodium desoxycholate still retained most of their antigenicity for rabbits as well as their neuraminidase activity. With the hope of developing a relatively non-toxic influenza virus vaccine preliminary experiments were conducted in both children and adults using a desoxycholate split A2/Asia/57 vaccine. The promising results from these experiments led to the development of large-scale methods for the production of desoxycholate split influenza virus vaccines.

A number of outbreaks of influenza associated with A2 virus strains have occurred in Australia in recent years, and it was found that many infants had experienced infections with these viruses. Accordingly, to evaluate the effectiveness of desoxycholate disrupted vaccines, a vaccine was prepared from the A/Swine (Shope) strain and used for further studies in humans. The anti-haemagglutinin antibody response to this split vaccine in mice was somewhat lower than that of the untreated vaccine when measured after single doses were injected. The difference was more pronounced with the higher dilutions (lower doses), but after second injections at the same dose levels the antibody responses were comparable with both the split and the untreated vaccines.

No adverse reactions were observed when this desoxycholate split vaccine was injected into infants, even when a dose equivalent to 500 CCA (chick cell agglutinating units) was used. The anti-haemagglutinin antibody response following a single injection was low but was detectable in all cases. Excellent antibody titres were obtained, and no adverse reactions occurred when second doses of the split vaccine were given. Similar results were obtained when the split vaccine was studied in young adults.

The equivalent of six million adult doses of desoxycholate disrupted A2/Hong Kong vaccine have recently been prepared and used in Australia. A small number of possible reactions (including mild soreness at the site of injection) have been reported. The absence of toxicity of the desoxycholate split vaccine has again been demonstrated in infants. Although clinical influenza associated with A2/Hong Kong viruses has occurred sporadically ever since these viruses reached Australia in August 1968 and although there has been some increase in the number of cases with the advent of winter in 1969, no outbreak of epidemic proportions has occurred in this country. A number of field trials have been set up to study the effectiveness of the desoxycholate split A2/Hong Kong vaccine, but whether the incidence of influenza will be large enough to provide a meaningful answer is not yet clear.

Session V

Future Influenza Vaccines and the Use of Genetic Recombinants

by

Edwin D. Kilbourne; New York, New York, U.S.A.

It is important to recognize that influenza vaccines of the past and present are, in fact, empirically selected genetic viral variants with desirable properties; i.e., optimal growth characteristics, "attenuation" of virulence, thermal stability, and requisite antigenicity. These variants have usually been selected unsystematically, on a hit or miss basis, from uncloned viral stocks of variable history by such empiric maneuvers as mouse lung passage to increase viral yield in chick embryos.

Obviously, it is more sensible to attempt a correlation of identifiable genetic attributes (markers) of the virus with requisite vaccine properties. Then one can proceed to genetically manipulate the virus for selection of optimal clones: 1) by screening of multiple viral clones, 2) by chemically induced mutation and/or selection through environmental pressures (e.g., temperature sensitive mutants) or 3) by genetic recombination of viruses.

Recombination is analogous to sexual reproduction in its potential for the immediate, one-step reassortment and combination of genes and gene products. Thus, deliberate mating of two or more viruses—each bearing a desired trait—can be effected and appropriate progeny virus selected (without need for tedious "adaptation" until appropriate mutants—if any—are manifest). It is predictable that recombination, with its redistribution of genes, will lessen the probability of expression of any polygenic characteristic such as virulence. Therefore, a kind of "instant attenuation" can be provided by recombining wild type with established laboratory strains of lesser virulence and then selecting out virus of the required antigenicity, e.g., Ax Vir1 Vir2 Vir3 (virulent) X A2 Avir1 Avir2 Avir3 → Ax Avir1 Vir2 Avir3 (avirulent). However, new phenotypes can arise with recombination, so the possibility of increased virulence cannot be completely excluded.

In practice, recombination has already produced vaccine virus of requisite antigenicity (Hong Kong-like) and chick embryo growth capacity (A0/PR8-like) by dual infection with these viruses and selection against the A0/PR8 parent. This recombinant, X-31, was isolated within 2 weeks of the initiation of the experiment. Its yields in chick embryos exceed that of any empirically adapted commercial strain that we have examined, and the recombinant grows equally well at 33° and 35°C.

Other potential and "anticipatory" applications of recombination to vaccine production include: 1) establishment of a "library" of prefabricated recombinants in an attempt to anticipate recombination in nature of antigens presently known which may lead to the evolution of new variants; 2) storage in the library of pedigreed high yield parental viruses ready for recombination with new strains (human or animal) as they evolve to effect their rapid "adaptation" to the vaccine-producing host system; 3) repeated recombination perhaps to reveal "new" antigens. As envelope polypeptides coded by the reassorted genes are put together in differing context, new avidity, reactivity, or even new antigenicity may emerge. Whether or not recombination is the mechanism of evolution of new strains in nature, the same trick can be turned in the laboratory to the detriment of influenza and the benefit of man.

Session V

The Role of Antineuraminidase Antibody in Immunity to Influenza Virus Infection

by

Jerome L. Schulman; New York, New York, U.S.A.

Immunity in influenza has generally been attributed to the presence of antibody to viral hemagglutinin in sera or respiratory secretions of previously infected or immunized subjects, and changes in antigenic structure have been assessed primarily by similarities and differences of hemagglutinin antigens in hemagglutination-inhibitions tests.

However, influenza virus possesses a second virus-coded protein antigen, neuraminidase, in its envelope, and following infection or parenteral immunization of man and of laboratory animals, antibody specific for viral neuraminidase is produced. Although the protective effects of antineuraminidase antibody have not been measured systematically in man, antibody specific for neuraminidase has been shown to inhibit virus replication and to prevent lung lesions in immunized mice challenged with a virus containing an antigenically similar enzyme.

Antigenic variations of the two virus-coded envelope proteins occur independently in nature, resulting in new viruses which differ from older strains more with respect to one antigen than the other. In particular, the Hong Kong/68 virus possesses a hemagglutinin antigen markedly different from antigens of previous A2 strains, whereas its neuraminidase has been shown in enzyme inhibition and plaque size reduction (PSR) assays to be indistinguishable from the enzyme antigens of 1967-1968 A2 viruses and to be antigenically related, but not identical to, the enzyme of A2/Japan/305/57 virus.

The influence of these antigenic relationships on immunity in mice challenged with Hong Kong virus was investigated by using recombinant viruses in which hemagglutinin and neuraminidase antigens of Hong Kong virus, and two previously isolated strains of influenza A2 virus were segregated by recombination with influenza A0/NWS virus. Immunization with inactivated A2/Japan/305/57 and A2/England/67 viruses provided no more protection against Hong Kong virus challenge than immunization with hybrid viruses possessing the enzymes but not the hemagglutinin antigens of the two A2 strains, and no protection was observed in mice immunized with the parent A2 viruses challenged with a hybrid virus possessing the Hong Kong virus hemagglutinin and the enzyme of A0/NWS (Hong Kong e).

In addition, in studies of the effects of immunity on transmission of influenza virus infection in mice, antineuraminidase antibody reduced the capacity to transmit infection but did not affect susceptibility to the initiation of infection, whereas antihemagglutinin antibody reduced susceptibility to the initiation of infection but did not influence the capacity for transmission.

Two implications which derive from these observations are: 1) the taxonomy of influenza viruses should include antigenic analyses of both surface antigens; 2) the protective effects and duration of antibody to neuraminidase must be investigated systematically in man, and the results of such studies should be reflected in the choice of strains and the standardization of virus pools employed in vaccines.

Finally, a purified neuraminidase vaccine might provide a useful epidemiologic tool. Mass immunization with such a vaccine might protect individual vaccinees and through its effects on transmission induce hard immunity without accelerating the emergence of new viruses with antigenically novel hemagglutinins.

Session V

New Criteria for the Selection of Vaccine Strains

by

S. Fazekas de St. Groth; Sydney, Australia

Serological crossing among influenza viruses is usually asymmetric: an antiserum to virus X will neutralize virus Y relatively better than an anti-Y serum neutralizes virus X. This relationship may be described by calling virus X *senior* to virus Y. The phenomenon is based on the fact that although the bulk of a senior antibody population participates in cross-reactions, only a small fraction of junior antibody populations can cross react.

When a junior virus is grown in the presence of homologous antibody, mutants are selected, all of whom are senior to their parent. Such an experiment mimics the evolution of epidemic strains, since the historical sequence within each subtype of influenza A parallels the order of seniority.

Cross-reactions between subtypes are distant—less than 5% of the respective populations cross react—and may be interpreted as occurring between antigens which differ by a double mutation. Such mutants can be selected, rarely, from senior viruses grown under homologous antibody. Towards the end of the reign of a subtype "bridging antigens" may be isolated in the field: these are the only members of that subtype which give close cross-reactions also with the next subtype.

When an animal immune to a junior virus is vaccinated with a senior antigen, it responds by producing antibody that reacts better with the junior antigen than with the vaccinating senior antigen. This is a manifestation of Original Antigenic Sin in its classical form. When basic immunity against a senior antigen is boosted with a related antigen, the response is usually at least as good against the boosting antigen as it is against the primary antigen. Thus Original Antigenic Sin is seen here in a modified, venial form.

On this evidence the use of *prospective vaccines* will be proposed. Such vaccines would contain a mixture of senior mutants selected under antibody pressure from the last epidemic strain. By this means evolutionary antigenic changes might be anticipated and countered, and the vaccination itself would not establish an Original Antigenic Sin that, based on a junior antigen, is all but irredeemable.

ABSTRACTS

Session VI

CHEMOTHERAPY AND CHEMOPROPHYLAXSIS OF INFLUENZA

THURSDAY, OCTOBER 16, 1969

10:45 A.M.—12:30 P.M.

The Antiviral Activity of the Isoquinolines Famotine (UK 2054) and Memotine (UK 2371) in Respiratory Infections in Man

by

G. M. Williamson and D. Jackson; Sandwich, England

The isoquinolines famotine—1-(p-chlorophenoxy-methyl)-3,4-dihydroisoquinoline hydrochloride (UK 2054)—and memotine-1-(p-methoxyphenoxymethyl)—3,4-dihydroisoquinoline hydrochloride (UK 2371)—exhibit antiviral activity in tissue culture at a concentration of 20 μg or less /ml against a range of viruses causing respiratory disease in man. These compounds have a direct inactivating effect upon the myxovirus and paramyxovirus particle, whereas the effect upon other respiratory viruses is during the viral replicative cycle in tissue culture. The activity towards myxovirus but not rhinovirus has been confirmed in organ culture. Both compounds reduced the yield of virus shed from the cultures and protected the epithelium from damage by the influenza virus. In vivo activity in mice infected with influenza A/PR8, although not consistently reproducible, was significant ($p = 0.05$) in approximately 50% of replicate experiments. These findings, coupled with the low toxicity of these compounds, encouraged their further study in man.

Studies in man have embraced challenge, prophylactic, and therapeutic studies with both oral (1.0-2.0 g per day) and local (nasal instillation of a solution containing 4 or 15 mg/ml) administration of the compounds.

Challenge studies with several strains of influenza A2 and B have demonstrated a significant reduction in the rate of infection with influenza B, i.e., famotine against B/England/13/65 and memotine against B/England/101/62 and a lowered rate of infection with influenza A2, i.e., memotine against A2/Leningrad/4/65. In a challenge study with para-influenza type 2 (strain DT) no effect with the compound tested, i.e., memotine, was demonstrable. Although in challenge experiments with rhinovirus the oral administration of famotine, the more active of the two compounds against this virus, had no effect, the nasal instillation of the compound did reduce the incidence of clinical symptoms but not of serological responses following challenge with rhinovirus type 2.

In the absence of any major outbreak of acute viral respiratory illness during the winters of 1967/68 and 1968/69, studies designed to assess the value of the oral administration of either compound for prophylaxis have been inconclusive. However, the nasal instillation of famotine reduced somewhat the incidence of symptoms in a small institutional outbreak due to the Hong Kong influenza virus.

Neither compound has, as yet, been shown to be effective in the therapeutic treatment of acute respiratory illness.

The Evaluation of Amantadine Hydrochloride in the Treatment of Influenza

by

R. B. Hornick, Y. Togo, V. Felitti, and M. Kaufman; Baltimore, Maryland, U.S.A., S. Mahler and D. Iezzoni; Wilmington, Delaware, U.S.A.

Amantadine hydrochloride is the first drug to show promise as a practical anti-influenza agent. Several studies demonstrating a prophylactic effect in volunteers with induced A2 disease as well as in patients during A2 outbreaks created impetus for therapeutic trials. The widespread A2 influenza epidemic that occurred early in 1968 provided the opportunity for therapeutic evaluation.

Closed population groups were selected because of the expected short-lived high attack rate, ease of clinical management, and ample facilities for collecting serological and virological specimens. One hundred and ninety seven inmates (103 drug group and 94 placebo group) of five prisons in the United States agreed to participate in the 10-day, double-blind evaluation of amantadine (100 mgm bid). All were proven to have A2 influenza disease by significant serological response and/or repeated isolation of the causative virus from the pharynx. Onset of therapy was approximately 20 hours after the first subjective awareness of illness. Assessment of drug effectiveness was based on the rapidity of the resolution of illness. There were significantly more drug-treated patients than placebo patients in the "rapid resolver" group, whereas individuals receiving placebo dominated the "slow resolver" group. Criteria for inclusion in these groups will be presented in detail. Analysis of febrile responses indicated amantadine-treated patients had significantly more rapid defervescence. Virus isolation studies revealed etiologically related virus in 90% of all volunteers during the first 5 days of therapy. It was apparent that clinical improvement was not correlated with disappearance of the virus. Nevertheless, the trials conducted during the influenza season of early 1968 indicated the therapeutic effect of amantadine hydrochloride. The mechanisms involved need elucidation. Administration of 100 mgm bid for 10 days did not cause any adverse effects.

The Prophylactic Effectiveness of Amantadine in Volunteers Challenged with A2 Viruses and in Populations Experiencing an A2/Hong Kong Influenza Epidemic

by

A. A. Smorodintsev, G. I. Karpuchin, E. G. Shvetsova, A. M. Malysheva, and L. Y. Tyros; Leningrad, U.S.S.R.

The prophylactic activity and safety of amantadine hydrochloride were determined in extensive studies with volunteers and in epidemiological field trials during an outbreak of A2/Hong Kong influenza in Leningrad in January and February 1969.

Investigations carried out in 1967-1968 on 591 healthy volunteers (medical students) in a double blind study showed amantadine hydrochloride to be an effective prophylactic drug when administered in daily doses of 100 to 200 mg for 11 days beginning 24 hours before challenge with aerosols of live influenza A2 virus suspensions containing either the A2/21/1965, A2/133/1967, or the A2/Hong Kong/1968 strain.

In the placebo group 80% of the susceptible subjects responded to the live influenza vaccine challenge with general constitutional and catarrhal clinical symptoms of various intensity. Amantadine was found to prevent clinical illness in 55% of the volunteers and to diminish the severity and duration of the symptoms in the subjects who became ill, especially in the more immune groups. A more pronounced increase in the efficacy of amantadine was observed when it was preceded by live influenza vaccination.

Studies of the prophylactic effectiveness of the drug, carried out on 10,053 persons during an extensive outbreak of A2/Hong Kong influenza in Leningrad, have shown the regular reduction of influenza cases in eight similar institutions with a mean index of 1.95-2.15 among subjects who received a daily dose of 100 mg amantadine. Regular administration of amantadine reduced not only the incidence of influenza infection but also the severity and duration of symptoms in those subjects who became ill.

The number of complaints of various side effects (sleep disturbances and others) among persons receiving amantadine was too insignificant when compared to the number in the placebo group for any contraindication to the wide general prophylactic application of the drug to be considered.

Study of 1-Adamantanamine Hydrochloride Used Prophylactically during the Hong Kong Influenza Epidemic in the Family Environment

by

A. W. Galbraith; Macclesfield, England

The design of this study follows closely that reported at the Second Conference on Antiviral Substances held June 16, 1969, in New York City.

Seventy-two family doctors throughout Great Britain volunteered to take part in the investigation of an influenzal epidemic occurring during the winter of 1968/69. Each was supplied with record forms, syringes, and active or placebo Amantadine capsules in code-labelled containers.

When a doctor saw an influenza patient and considered the family suitable for inclusion in the study, a specimen of venous blood was taken from each member and sent to Sheffield Virus Laboratory in a special container.

The capsules were then distributed, and the dose taken was 100 mg every 12 hours for 10 days by adults and proportionally less by children. The index patient kept a record of his temperature and symptoms. Other members of the family who subsequently became ill did likewise.

Approximately 3 weeks after the first venipuncture, a second specimen was obtained and antibody level to Hong Kong influenza virus was measured.

Since the distribution of the outbreak in Great Britain was patchy, only 29 family doctors saw sufficient cases of influenza to contribute results. The total number of individuals included was 252, and the analysis of results will be presented during the conference.

Session VI

Observations on the Use of Interferon in the Prophylaxis of Influenza

by

V. D. Soloviev; Moscow, U.S.S.R.

The first observations on the prophylactic and therapeutic administration of interferon to human volunteers challenged with vaccinal strains of influenza A2 virus were made in 1965-66. The interferon had been prepared from human blood leucocytes subjected to treatment with Newcastle disease virus. The effectiveness of the interferon was determined on the basis of reisolation of the virus from the upper respiratory tract of the treated volunteers and from increased antibody levels in their blood. A total of 400 volunteers were observed. The observations enabled investigators to determine the minimal prophylactic dose of the preparation, to test the intranasal method of its application, and to find out that the best prophylactic effect was attained with repeated introduction of the interferon.

A production laboratory for interferon biosynthesis set up at the Gamaleya Institute for Epidemiology and Microbiology had prepared sufficient quantities of the leucocyte interferon for epidemiological investigation by specialists of the Central Institute of Epidemiology, Ministry of Public Health, U.S.S.R., and the N. F. Gamaleya Institute, the Institute of Pediactrics, and the Virology Institute, Academy of Medical Sciences, U.S.S.R.

Epidemiological observations carried out in 1967-68 involved 3,500 persons of different ages. Those in the experimental groups were given interferon; those in the control groups received a placebo. The results showed that interferon is effective and completely safe for repeated administration to individuals of all ages, including newborns.

The most extensive controlled observations were made in 1969 during the last epidemic caused by the A2/Hong Kong/68 virus. The epidemic of Hong Kong influenza took place in January-February, 1969, lasted for 1 month, and revealed no substantial differences in the clinical course of the disease from that of the epidemics of preceding years. However, virological investigations have shown an unusually high percentage of virus isolations from patients' respiratory tracts—reaching 80%—as well as some notable distinctions in properties of the isolated strains in comparison with their predecessors.

More than 14,000 people of varying ages were included in the interferon prophylaxis study, the collectives having high, medium, and low incidence of the disease. The observations were made under controlled conditions and a placebo was administered to control groups of comparable size and composition. The effectiveness of interferon prophylaxis, as shown in reduced disease rates in groups given the interferon as compared with rates in control groups, varied from 44.8 to 73.8%.

Results suggest the following conclusions: first, interferon prophylaxis of Hong Kong influenza in children and adults is undoubtedly effective; and, second, the preparation is completely safe.

We believe that the prophylactic effect is due not only to the action of the exogenous interferon itself but also to that of endogenous interferon produced by cells of the respiratory tract. Maximal prophylactic effectiveness of the artifically introduced interferon is probably attained when an individual is infected and a latent infectious process develops in the organism, under the interferon protection, with virus multiplication which, without producing symptoms of the disease, serves to induce interferon production by cells in the mucosae of the respiratory tract.

Separate studies have established that the production of endogenous interferon is dependent on the organism's individual ability. This has been shown in experiments with animals of different species as well as in man. The proportion of interferon-refractive individuals in populations varied from 12 to 30%. This finding should be taken into account in evaluating the final results obtained in the study of interferon

as an influenza prophylaxis.

Similarly, the different influenza virus strains isolated during the epidemic of 1969 did not have equal interferonogenicity. The interferon-positive (I +) and interferon-negative (I —) strains of the Hong Kong A2 virus also appeared to be different in neuraminidase activity, thermostability, and pathogenicity to volunteers.

Besides the native leucocyte interferon, a concentrated interferon preparation has been tested for the treatment of influenza patients in limited clinical observations. It has produced promising results.

The general positive estimation of interferon prophylaxis should be supplemented by the prospect of the association of interferon with amantadine. Experiments in vitro in tissue culture, in mice, and in limited observations in man suggest a favorable outlook for such combined prophylaxis.

notes

Session VI

The Effect of an Interferon Inducer on Influenza Virus

by

David A. Hill, Samuel Baron, and Robert M. Chanock; Bethesda, Maryland, U.S.A.

The synthetic double-stranded RNA composed of polyriboinosinic acid and polyribocytodylic acid (In • Cn) is a potent inducer of interferon and of in vitro and in vivo resistance to viral infections. Incubation of human embryonic kidney cells with In • Cn caused resistance to subsequent challenge with two strains of influenza A2 virus (Hong Kong/68 and Ann Arbor/60). Ten μg In • Cn per ml in maintenance media for 12 hours caused 100% protection; 1.0 μg per ml caused 50% reduction in hemadsorption. Similar levels of protection were found with other common human respiratory virus pathogens. The level of In • Cn required to induce complete inhibition of virus replication in these human cells was 50 times less than that required to induce detectable levels of interferon in the culture medium.

The effect of In • Cn on influenza virus infection in vivo was studied in mice with a strain of influenza A2 virus (Taiwan/64) which was adapted to be pathogenic for mice. The intranasal administration of 25 to 50 μg of In • Cn up to 30 hours before intranasal challenge with 2 to 10 ID_{50} of influenza virus caused a significant reduction in the incidence and extent of pneumonic lesions in the mice. The incidence of pneumonic lesions was reduced from 87% (26 of 30) in untreated controls to 28% (13 of 47) in treated mice; the mean lesion score was reduced from 2.5 to 0.3. The use of a low challenge dose of virus was critical in demonstrating a protective effect of In • Cn; when mice were challenged with more than 100 ID_{50} of virus, no significant protection was seen. Administration of In • Cn by intraperitoneal injection was not effective in protecting against intranasal challenge. The greater effectiveness of intranasal over intraperitoneal administration may be explained by differences in the distribution and duration of interferon production following the different routes of administration. After intranasal instillation of 50 μg In • Cn, a peak serum level of 1,000 units of interferon per ml was reached and decreased only to 100 units after 48 hours, whereas, after intraperitoneal injection, a peak serum level of 10,000 units was reached after 6 hours, but decreased to an undetectable level at 48 hours. The interferon measured in homogenized lung tissue reached a peak titer of 3,500 units per gram (3.5 times the serum level) 6 hours after intranasal instillation of In • Cn; a peak titer of 1,000 units (0.1 times the serum level) was reached in 6 hours and fell to an undetectable level at 48 hours.

On the basis of findings with influenza and other human respiratory virus pathogens, we are evaluating the potential use of In • Cn in the prevention and treatment of human viral diseases.

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