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GENETIC VARIATION AMONG INFLUENZA VIRUSES

edited by

DEBI P. NAYAK

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PREFACE

Influenza is still a major threat to the health of mankind because unlike smallpox, polio, mumps, measles, or rubella it is not yet subject to either an effective prophylaxis or therapy. Year after year influenza inflicts enormous human suffering in the form of high morbidity and increased mortality. In addition, the economic loss resulting from the loss of man hours runs into billions of dollars every year. It is no wonder that influenza has been the subject of intense study by clinicians, virologists, epidemiologists, molecular biologists, immunologists, geneticists, public health officials, and others over the last four decades.

This ICN-UCLA symposium was organized to bring together people from different disciplines working with the common objective of reducing the ravages of influenza and to expose them to the totality of the problem of influenza. The arrangement of the conference provided a setting for both formal (plenary session) and informal (poster session, group discussion) exchange of ideas. The number of participants (approximately 120) was optimum for both formal and informal gatherings without any further subgrouping, and thus enabled the participants to join each session without missing another.

This volume documents the proceedings of this major international conference on genetic variation among influenza viruses held at Salt Lake City, Utah from March 8-13, 1981. It includes papers presented by the speakers of the plenary sessions as well as the keynote speaker, Sir Charles Stuart-Harris. It also includes the selected papers presented in the poster sessions. Also included by popular demand are two poems on influenza by Dr. Edwin Kilbourne, which were presented at the conference banquet. The quality of the papers (including the poems) reflects the high standard of the meeting. As stressed in organizational communication to the speakers, most papers contain information not published before.

The papers presented at the meeting included nearly all major aspects of influenza in which important advances are being made. Because of recombinant DNA technology and rapid DNA sequencing, a number of genes of influenza virus from a number of strains have been either completely or partially sequenced. Among these, the gene coding for hemagglutinin (HA) has been most intensively studied and the HA of one or more strains from each subtype (H^1, H^2, H^3) has been completely sequenced. The question of drift and shift at the genomic level was discussed by a number of speakers (Brownlee, Davis, Fields, and Fiers). It became clear that these nucleotide changes are not limited to HA only but occur among other genes as well (Air, Blok, Palese, and van Wyke). More important, these clones are now being successfully used to express viral proteins in both prokaryotes (Davis) and eukaryotes (Hartman and Lai). It is expected that this area will be the subject of intense study in the next few years because of the biological significance of the expression product of cloned genes either for use in prophylaxis or for studying their function. Also, the complete sequence of a DI RNA was presented, locating the point of deletion in the progenitor (PI) gene (Nayak).

The three-dimensional structure of the hemagglutinin gene which has been recently completed was presented by Ian Wilson (it is unfortunate that this important paper was not submitted for publication in this volume). The topological significance of epitopes and other functional domains of HA molecules which have been proposed by either sequencing proteins, nucleic acids, or using monoclonal antibodies or cyanogen bromide cleaved fragments were presented by Laver, Webster, Gerhard, Sleigh, and Jackson. Interesting discussion followed to correlate a direct structural relationship of a sequence(s) to its proposed function(s).

The role of the capped host mRNA is the process of initiation of transcription was further defined by Krug, and the regulation of viral transcription was discussed by Mahy. Lamb's original observation that the NS RNA segment can code for multiple mRNAs and proteins was extended to the RNA coding for M protein(s) (Lamb and Palese) as well as to the RNA coding for NS proteins of influenza B virus (Lamb). Newer information on the biosythesis of hemagglutinin and nature of carbohydrate determinants was presented by Klenk, Compans, Brown, Basak, and Meier-Ewert.

Viral pathogenesis is a complex process involving interaction between virus and host and possibly other environmental factors. Sugiura and Schulman discussed studies conducted toward defining viral gene clusters involved in virulence. Scholtissek presented his studies on suppressor recombinants and suppressor mutants. Schulze and Carroll attempted to define the host receptors for influenza virus while Small discussed the complex host defense mechanism involved in viral pathogensis. Kilbourne discussed complex processes involved in the adapatation of influenza virus in human population. Occurrence of virus variants in human population and its epidemiological signicance was discussed by Kendal, Cox, and Six.

For influenza virologists, the greatest challenge is the control of influenza using an effective prophylaxis. Couch stressed the significance of serum IgG neutralizing antibodies, while Anders, Ennis, and Stein-Streilein discussed T cell-mediated cytotoxicity and its effectiveness in combating influenza viral infections. Dowdle discussed the limitation of present immune prophylaxis and suggested the potential use of concentrated viral vaccine which may be obtained by using newer technology. Murphy and Maassab proposed a number of newer avenues, including the possible use of host range, cold-adapted and temperature-sensitive mutants as candidates for live virus vaccine(s). Only further studies will reveal whether any of these proposed methods, i.e., concentrated antigens (Dowdle), live virus (Murphy and Maassab), or a combination of both (Kilbourne), will be successful in providing an effective immune prophylaxis against influenza. Finally, whichever method is successful in this war against influenza, a lot more understanding of the strategy that influenza virus uses in nature will be required. As Sir Charles Stuart-Harris states in his keynote address, "The present situation calls for redoubling of efforts in order that the quest may become successful and that future generations may have no more fear of influenza than they will have of smallpox.

Funding to defray a portion of the travel expenses incurred by the invited speakers was generously awarded by NIH Contract Number 263-81-0166 (jointly sponsored by the National Institute of Allergy and Infectious Diseases, Center of Disease Control, Bureau of Biologics, FDA, and Fogarty International Center), Beckman Instruments, Endo Laboratories, Merck Sharp and Dohme, and Parke-Davis.

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INFLUENZA VIRAL GENETICS AND THE FUTURE

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ABSTRACT

Knowledge gained upon the genetic mechanisms of the influenza viruses is believed to carry implications for the interpretation of the epidemiology of the disease and for its control. Present problems in understanding the epidemiology and in immunization against influenza are described in the light of this thesis.

INTRODUCTION

It was in 1952 that Wilson Smith wrote,"influenza viruses are plastic organisms undergoing constant changes in structure to produce newer forms with changed antigenic constitution, modified architecture and different biological behaviour". But already, influenza viral genetics was being studied experimentally. Burnet and Bull (1943) investigated the haemagglutinin during early passages of human influenza virus A in embryonated eggs by measuring the relative titre for guinea pig and chicken red blood cells. The change on passage in this relation was called the O-D variation. Using the limiting dilution technique to ensure that cultures were derived from minimal infective doses of virus, Burnet et al (1949) showed that it was thus possible to maintain the original 'O' form with its greater avidity for the guinea-pig than chicken red cells. Conversion to the 'D' or derived form with greater avidity for chick cells on further cultivation was explained genetically as a selective survival of mutants. But the recombination of two strains with different characteristics replicating in the same milieu by Burnet and Lind (1949; 1951) first established genetic studies on a firm basis. Henle and Liu (1951) also showed that multiplicity reactivation of partly inactivated virus, was possible, thus providing a further analogy with bacteriophage (Delbrück and Bailey, 1946; Hershey and Rotman, 1948; Luria, 1952).

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The contribution of genetic recombination, now more correctly described as genetic reassortment, to theoretical and practical considerations has been immense. It is widely accepted that its mechanism lies in the segmented structure of influenza RNA revealed by Duesberg and also Pons and Hirst in 1968. Even before knowledge of this structure however, the prophetic words of Burnet in 1951 must be remembered. He wrote that "as a result of some interaction with cell constituents, the virus particle disaggregates (after entry of virus into the cell) and gives rise to a certain number of genetic units". The current intensive study of the genes of the virus by molecular techniques has afforded ample proof of the truth of these remarks.

Problems in the Epidemiology of Influenza

My role in this Conference is to look at and discuss the impact of genetic research on present day problems of human influenza. Let us start upon the epidemiology of human influenza by examining its periodicity.

The most striking feature of influenza is its irregular periodicity whereby it is apparently absent in some years and a cause of large epidemics in others. When many individual countries in one hemisphere suffer epidemics in a relatively short period of time, the occurrence is called pandemic influenza. Formerly, it was argued (Commission on Acute Respiratory Diseases, 1948), that community experience and the wide age-range of influenza suggested that epidemics first caused infection in those most susceptible in whom it produced an immunity which was relatively short-lived. Prevalences were renewed as numbers of susceptibles including those born since the last epidemic, built up in the community. The theory did not explain why epidemics ceased even when as many as half the susceptible persons in the community had escaped infection. However, it was favoured by the finding that between epidemics localised outbreaks and sporadic cases could be located, thus suggesting that virus was persisting in the community. Latterly, however, the view has prevailed that immunological defence of the community resulting from exposure to the surface antigens, the haemagglutinin and neuraminidase of a particular subtype, is defeated by variation, particularly of the haemagglutinin as a result of antigenic drift. Such antigenic variation was originally described by Magill and Francis (1936) and Smith and Andrewes (1938).

The mechanism of antigenic drift was shown by passage of virus in mice partly protected by inoculating a small amount of antibody or by immunization. Virus variants arose whose antigens did not fit the antibodies and which were selectively favoured (Archetti and Horsfall, 1950; Magill, 1955).

To see how closely antigenic drift and epidemicity coincide the experience of a particular country must be examined over a long period of time. Taking mortality from influenza as the index of virus activity, the experience in England and Wales from 1940 to 1970 is that over a whole period in spite of the ageing population, annual mortality has tended to decline. Yet large numbers of deaths occurred in 1943, 1951, 1957-1958 and 1970. The epidemic in 1943 was caused by HoN1 viruses which, as typified by the Weiss strain (Salk et al, 1944), drifted appreciably from WS, PR8 and the 1937 viruses. Secondly, the 1951 epidemic caused great mortality in Liverpool but relatively much less elsewhere and the period from 1946 to 1956 was otherwise marked by relatively smaller prevalences. The 1951 viruses, however, excited much interest because though they had drifted away from the H1N1 prototype of 1946 and 1947, they exhibited two major variants with differing avidity for red cells and Isaacs and Andrewes (1951) were able to follow their separate geographic spread in Europe.

The appearance of the Asian virus (H2N2) in 1957 represented the first antigenic shift of the influenza virus era with alterations in both the H and N antigens and in consequence a large pandemic spread throughout the world in the unprotected population. In fact, this was the largest epidemic of the whole 40 years and, apart from 1958 to 1960, the years from 1957 to 1961 had recurrent prevalences and mortalities without any significant drift of the antigens of the viruses. The last of the Asian (H2N2) epidemics occurred in 1967-68 but soon paled into insignificance when a new pandemic started in 1968 and lasted until 1971 as the H3 haemagglutinin of the A/Hong Kong/68 virus replaced that of Asian strains. Antigenic drift after 1972 of the H3N2 viruses has been closely watched throughout the world. Figure 1 shows the first ten years of the H3N2 sub-type epidemics in England and Wales. It compares the deaths from influenza, influenzal pneumonia and bronchitis with the monthly number of cases of clinical influenza notified to the Royal College of General Practitioners by a panel of 60 G.P's observing a scattered community of 150,000 persons. This index of influenza was used by Clifford et al (1977) in their study of excess mortality because it

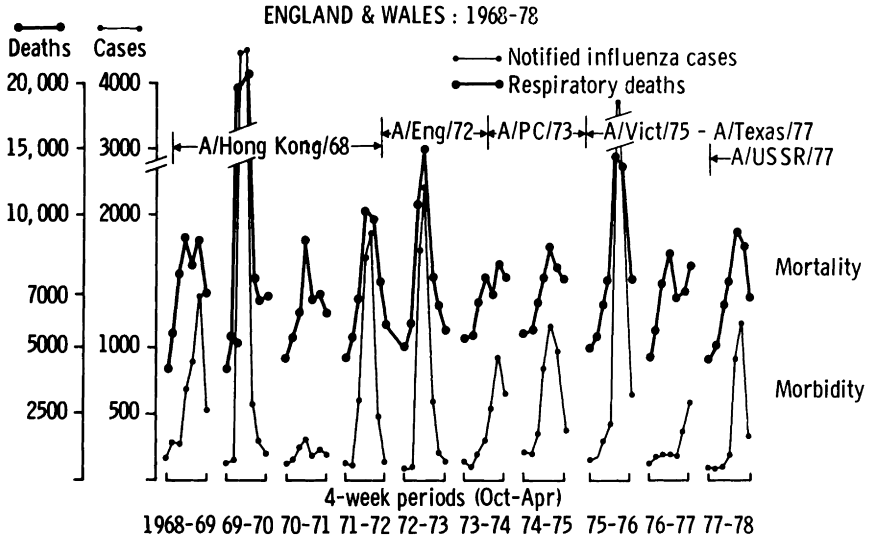

Fig. 1 Antigenic Drift and Influenza Epidemics. England and Wales, 1968-1978

correlated so well with laboratory evidence of influenza including isolation of viruses. The figure also shows the antigenic variance of the H3N2 viruses up to 1978. The A/England/72 virus appeared briefly at the end of the third wave of A/Hong Kong/68 viruses. It then returned to cause a relatively sharp outbreak in the winter of 1972-1973. Then came the A/Port Chalmers virus which caused relatively feeble outbreaks of 1973-74 and 1974-75. It was succeeded by A/Victoria/75 which everywhere caused a sharp epidemic. This strain was antigenically more remote from A/Hong Kong/68 than its predecessors but the Port Chalmers experience shows that antigenic diversity is not the only factor determining the ability of any particular virus to involve the community. In fact the way in which viral and host factors are intertwined has been well shown by epidemiological experience

since 1977.

The reappearance of the H1N1 subtype of viruses in 1977 in China, then spreading to the USSR and eventually reaching all over the Northern hemisphere was a totally unexpected phenomenon. It is true that a new antigenic shift had been anticipated round about 1978 but reappearance of a former subtype dormant since 1957 was a new experience. The 1977 A/USSR/77 (H1N1) virus was soon recognised to be antigenically close to the 1950-51 viruses (Nakajima et al, 1978; Kendal et al, 1978) which were the variants of the prototype H1N1 virus of 1946-47 (Isaacs and Andrewes, 1951).

The human reaction to the new virus was surprisingly predictable. Serological monitoring had, before the epidemic, shown that persons in the community born since 1957 and aged 20 or less at the time of arrival of the H1N1 virus were without antibodies to this subtype. Infection by H1N1 strains in the USA was confined almost entirely to young adults and children below the age of 26 both in 1977-78 and 1978-79 (Kendal et al, 1979a) and experiences elsewhere were similar. The morbidity and mortality was low in 1977-78 winter but greater in 1978-79 when the epidemic resembled in size the A/Port Chalmers epidemic of 1974-75. Mortality in both these years occurred predominantly in adults particularly in those over 60 and influenza in adults over 25 years of age was produced almost entirely by the continuing prevalence of H3N2 viruses, then the A/Texas/77 variety. What was much more surprising was the escape from H1N1 infection by adults even though these had in many cases relatively low serum HI titres.

Apart from sharp outbreaks of H1N1 influenza from 1977 to 1980 in residential school and other communities, the infection in children and young adults was comparatively mild. This may have been due to the thermal sensitivity (ts) property of the H1N1 viruses found by Oxford et al (1980) which should have rendered the viruses at least partly attenuated. Genetic analysis by oligonucleotide mapping (Young and Palese, 1979) and by competitive RNA hybridization (Bean et al, 1980) has revealed also that the H1N1 viruses recovered in 1978 in California and Brazil, with general circulation in 1979, differed from the A/USSR/77 virus.

Genetic research on antigenic drift and shift

It is uncanny to see the speed with which individual chemical resemblances and differences between the RNA of different strains of influenza virus can now be shown. Recombinant DNA techniques have had considerable success in the elucidation of antigenically-drifted virus. Thus DNA copies

of haemagglutinin genes from viruses of the A/Hong Kong/68 era have revealed nucleotide sequence differences which foreshadow amino-acid differences in the individual haemagglutinins (Verhoeyen et al, 1980), Min Jou et al, 1980). Laver et al,(1980)have also shown alterations of even single amino-acids in the polypeptide sequences of haemagglutinin particularly of the HA1 portion. The picture fits that of a series of point mutations but the precise relationship between amino-acid changes and antigenic variation is so far obscure. What is worse perhaps is the detection of antigenic differences by monoclonal antibodies which appear to have no epidemiological significance (Gerhard and Webster, 1978). Sir Christopher Andrewes drew attention in 1956 to the likelihood that genetic variation would probably throw up differing virus strains, many of which would not be able to spread or even to persist in the partly immune human population. What the clinician and the epidemiologist seek is how to predict the precise antigenic variant which is likely to occur by genetic mutation in the haemagglutinin in the future and which will be epidemiologically successful. This is a tough and perhaps impossible question to answer. Yet without this forecast, the selection of a particular strain or strains of influenza viruses to form the seed for inactivated vaccine for a succeeding year becomes subject to nature's whims and the vaccine may fail to match events. The origin of antigenic shift was formerly explained either on the basis of genetic mutation, now clearly unlikely on grounds of the haemagglutinin structure (Gething et al, 1980), or by persistence of viruses perhaps as a genetic anlage even though inapparent after ten or more years of activity. Reappearance of the H1N1 subtype in 1977 was the first formal evidence that a human virus dormant for 20 years can somehow persist. True, it may have been in a frozen state or it may have been latent in man (Hope-Simpson, 1979) or some other reservoir.

Recycling of former epidemic viruses was first envisaged by Francis et al (1953) and Mulder and Masurel (1958) and the evidence was based on the presence of antibodies to former viruses in sera from persons living during the era when the subtype was prevalent. On this basis also Shope (1944) believed that the A/Swine/31 virus was the survivor in swine of the 1918 virus from man. When the A/Hong Kong/68 (H3N2) virus appeared in 1968 antibodies to its H3 antigen were present in abundance in persons who were alive at the time of the 1890 or earlier pandemics (Masurel, 1969). But this H3 haemagglutinin also had peptide links similar to those of the equine A/Equi 2/63 and duck Ukraine/63 viruses and the meaning of these links was unknown (Laver and Webster, 1973).

Belief in an animal reservoir of human viruses began to be strengthened when it appeared that the A/Hong Kong virus passed relatively rapidly into the animal kingdom and most obviously to domestic pigs after its human debut (Harkness et al 1972).

Latterly the possibility of other animal reservoirs for viruses with human haemagglutinins has been enhanced by work on ducks and other Avian species. Webster et al (1975) showed that antigenic links existed between the Asian virus H2 haemagglutinin and those of two duck viruses isolated in 1972 and 1973 some 4 or 5 years after disappearance of the Asian virus from the human scene. Shortridge (1980) recovered five strains of an H2N2 virus from ducks in Hong Kong in 1978 and in 1979 Shortridge et al found serological evidence in Hong Kong that infection by H1N1 viruses in domestic poultry had occurred in 1975 and 1976, two or three years before the H1N1 virus reappeared in man. Thus, the probability exists that a virus equipped with the genes of former human virus H and N antigens may be sheltering in wild birds and moving from this to man involves the questions 'how' and 'when'. Whether genetic recombination between a human and a duck or other animal virus occurs before the latter can acquire potential for human infectivity and pathogenicity is still unknown. Memory of the failure of the A/New Jersey/76 (Hswl.N1) virus of Port Dix to establish itself as an epidemic strain is too recent for anyone to think that mere possession of the right surface antigens is the only pre-requisite for a virus to change its host species. However, genetic recombination is a regular phenomenon among the viruses of a single host species such as ducks (Gardner and Shortridge, 1979) and obviously occurs among human viruses. The hunt to uncover the precise mechanism of antigenic shift is clearly on and there could be many surprises ahead.

In the meantime it must be remembered that the goal is not just that of understanding mechanisms but the anticipation of future antigens. Only once has the laboratory permitted the anticipation of a human antigenic variant and that was when Fazekas de St. Groth and Hannoun (1973), derived from A/Hong Kong/68 virus a strain with similarity to the A/England/72 virus which had yet to appear in human infections. But this triumph was short-lived because later variants resembling A/Port Chalmers/73 or A/Victoria/75 did not subsequently appear in the laboratory. Clearly genetic research has a long way to go before it can answer the problem of the source of antigenic shift in the future. However, genetic analysis of the haemagglutinins of animal and human strains should be rewarding, remembering that Scholtissek et al established in 1978 RNA homology between the haemagglutins of Duck Ukraine/

63 virus and A/Hong Kong/68 virus. Also Porter et al (1979) have sequenced the nucleotides of the haemagglutinin gene of fowl plague virus. Knowledge of the sequences of avian and human haemagglutinins though permitting a comparison of structure and of the chemical equivalence of antigens, is still very far from complete. The question remains open as to whether such knowledge will provide evidence of interspecies movement of the genes of antigens such as has been proposed as the basis of antigenic shift. Surely it is necessary also that such a transfer to man of a recombinant from an animal host should be 'caught in the act' before accepting theory as fact. It is a fact that transfer from man to animal kingdom does occur. But when transfer does occur in the reverse direction as for instance of swine influenza virus to man, which occurred sporadically before and after the Fort Dix epidemic of 1976,(Smith et al, 1976; Thompson et al, 1976), the end result may not be a widespread epidemic and the conditions for adaptation to man are not yet understood. The problem of prevention of influenza by immunization. Experiments with influenza vaccines have been conducted for more than 40 years yet a satisfactory basis for their use is still lacking. Inactivated whole virus, split virus or surface antigen preparations are the only varieties available for routine use. The recommendation that immunization should be offered to persons at special risk of dying from influenza because of pre-existing chronic organic disease, still has a shaky foundation. Controlled clinical trials in healthy persons have also given conflicting results dependent in part on diagnostic confusion of influenza with other respiratory virus illnesses. Consequently carefully studied small groups of persons immunized with inactivated vaccine and later challenged with attenuated live virus have been substituted of recent years.

Two major sources of difficulty have become recognised in the use of inactivated vaccine. The first arises from antigenic drift and the realisation that unless the vaccine is produced from a strain of virus antigenically close to that of the circulating virus against which protection is sought, the latter will be relatively less effective even against the comparatively weak challenge of attenuated live virus (Potter et al, 1977). But there is a second problem arising from the fact that the humoral response to inactivated influenza virus is bound by the "original antigenic sin" acquired by the first exposure to the subtype of virus concerned (Francis et al, 1953; Davenport et al, 1953). When antigenic drift occurs, the haemagglutinin of the drifted virus given as vaccine, will in many persons effectively reinforce the antibodies to the prototype of the subtype but may fail to induce a protective level of antibodies to the drifted antigen (Oxford et al, 1979). Cross re-acting heterologous antibody directed against the prototype subtype virus has been shown to be less

protective than that which is specifically directed against the antigen in the vaccine (Couch et al, 1979). Hence even when the closeness of the virus in the vaccine to that in the field has been assured, the vaccine cannot be guaranteed to provide protective efficacy once antigenic drift has occurred.

These twin circumstances of drift and inability to create specific protective antibodies probably explain why inactivated vaccine may fail when given annually to groups of persons exposed to a high risk of infection. Such an experience of inactivated vaccine as that recorded by Hoskins et al (1979) may mean that immunized persons acquire less protection from vaccine than from an attack of influenza by a previous virus of the same subtype antigenically heterologous to the virus causing later challenge. When antigenic shift has occurred, immunization in persons not previously exposed to infection by the new subtype is far less effective serologically than at interpandemic times. Two doses of inactivated vaccine are required and even then antibody levels are lower than after one dose of vaccine in persons who were infected by the same sub-type of virus years before (Nicholson et al, 1979). Nevertheless in spite of all these drawbacks, it has to be realised that inactivated influenza vaccine is still the only immunological weapon licensed and available for use against this unpredictable group of viruses

The picture presented by live attenuated virus vaccine today presents a paradox. On the one hand millions of doses of live vaccine were at one time used in the USSR and in some European countries without apparent harm but with doubtful efficacy. Yet epidemics have continued to occur in the USSR as in other countries so that control of influenza has not been obtained. But in the USA, the UK and Belgium experience in the attempted production of seed virus strains, which are both attenuated and still infective, have been pursued in the past several years with results which are at times encouraging and at other times frustrating. There have been several reviews of these attempts but from the point of view of this Conference, it is important to pinpoint the essential goals and to perceive the role of genetic research in past and future attempts to obtain the ideal attenuated virus for general use as a vaccine.

The desirable properties of the ideal seed virus for such a vaccine were spelt out by Murphy et al in 1976. Of these the essential requirements are attenuation for seronegative persons with retention of infectivity, antibody formation and resistance to challenge infection. But of equal importance is the retention of attenuation by virus discharged to the environment by the vaccinated person and this implies genetic stability of the vaccine virus. The genetic basis of the attenuation of virulence is now recog-

nised as polygenic and this holds for fowl plague virus (Rott et al, 1979; Rott, 1980), for the human ts viruses of Chanock and the cold-adapted viruses of Maassab. The gene segments which contribute to attenuation differ in the two groups of human viruses (Murphy et al, 1980 (a); Massicot et al, 1980; Kendal et al, 1979 (b)). Genetic instability is an apparent weakness of ts viruses when used in wholly sero-negative children and the possible mechanism of this has been described by Murphy et al (1980) (b). The search for a more stable attenuated parent by genetic engineering is in progress (Chanock and Murphy, 1980). The sequencing of RNA segments (Lai et al, 1980), the magic of restriction enzymes used on DNA copies of the RNA and much hope are all thrown into the quest which is of such great importance. But there is no magic concerning the future requirements for live virus vaccines. As in the case of inactivated vaccines progress with live vaccines also depends upon the monitoring of the dance of the H and N antigens of the human viruses throughout the world. Also periodic change in the composition of the vaccines is required and it is this above everything else that suggests a future emphasis upon or at least a parallel role for chemotherapy rather than for vaccines. The pursuit of antiviral chemotherapy. In spite of many attempts to produce compounds which selectively inhibit influenza virus replication in experimental systems without provoking harmful effects on the cells and organs of mammalian hosts, few candidates have emerged. Of those with activity in infected animals fewer still have been worthy of test in man and the results in human influenza have sometimes conflicted with those in experimental studies. Amantadine, 1-adamantanamine hydrochloride, has been endorsed after many trials, for wider use in man. Its chemoprophylactic power against influenza A viruses, irrespective of antigenic subtype, is greater than its therapeutic action once illness has begun. Even so its usefulness in treatment is measurable and required greater exploitation in complicated illnesses. Absence of action against influenza B is a major handicap, however, and may be one reason for the cautious attitude of clinicians.

The recent progress in nucleic acid chemistry has been an enormous stimulus to the discovery of inhibitory antiviral compounds. Nucleosides and nucleotides whose sequences may mimic strategic sequences of viral genes or which can actively interfere with key viral enzymes have become known. One such, the nucleotide Ribavirin or Virazole (1-B-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) is active against both influenza virus A and B in vitro (Huffman et al, 1973) in mice (Khare et al, 1973) and ferrets infected with influenza virus (Schofield et al, 1977). In man negative results outweigh the occasional success and the controlled trial by Smith et al (1980) is young adults ill

with influenza due to A/Brazil/78 (HlNl) showed no diminution in symptoms or fever or the presence of virus in nasopharyngeal secretions after oral Ribavirin. This is a toxic compound and its negative result in man may be due to the difference in host metabolism in man compared with that in mice and ferrets.

Other attempts have been made to interrupt viral nucleic acid replication. Oxford and Perrin (1977) have found a number of compounds active against influenza RNA transcriptase. Zamecnik and Stephenson (1978) found a tridecamer polynucleotide with activity against Rous sarcoma virus and the polynucleotides certainly offer antiviral possibilities as indicated by Stebbing (1979) in his extensive review. But inhibitory effects are not limited to the bases as shown by the antiviral action of oligopeptides with aminoacid sequences resembling the N-termini of viral polypeptides and described by Dr. Choppin in this Conference. It is impossible not to believe that the pursuit for successful therapy by rational means will not lead to a blind alley and it is important that no clues should be ignored. The human need for help is very great and the control of influenza by specific vaccines is so hedged around with difficulties due to genetic virus variation that attempts to develop chemical antagonists are of enormous importance. Conclusion. This survey has been an attempt to perceive the contribution thus far made by knowledge of the biological and biochemical mechanisms of influenza virus towards solution of the existing problems in the attempted prevention and treatment of human influenza. It is apparent that immunization has often failed because of inability to perceive the direction of genetic variation in the human viruses of the antigens which determine future epidemics and pandemics. Biochemical research has now begun to discover the chemical basis of antigenic variation in the glycoproteins but it is too early for an improvement in the forecast of future mutational changes in the viruses of next year or the year after. There is evidence of the potential for changes in base sequences and aminoacid translations of viral RNA, some apparently irrelevant and some of antigenic significance. It is the latter which are of epidemiological significance and perhaps an experimental approach is now needed to change the emphasis from past experience to future possibilities.

As I look back however, on the past forty and more years I am impressed by the panorama of the laboratory and the world concept of influenza which has been unfolded in my medical lifetime. Many gaps in knowledge have been successively filled. When the first successful experiments on protection by influenza vaccine were made by the late Thomas Francis and the Commission on Influenza (1944), he was firmly of the opinion that the possible number of antigenic sub-

types of influenza virus A was limited. The alternative view of almost limitless variation was too awful to comprehend. Which view is correct? In my judgement we still do not know. Perhaps our colleagues of the animal influenza field may now understand our concern over the discovery of the many haemagglutinins existing among the avian viruses, if indeed birds are the reservoir of the genes of future human viruses. The message surely to the laboratory is that it is necessary to press on with the genetic, chemical and immunological analysis of the surface antigens of these viruses. As the surveillance of human experiences is still lamentably incomplete, these studies must continue to be regarded as of equal importance.

If I were a young man about to enter the field of virus research, I would take heart from the failure of previous generations. Influenza virus and human influenza continue to present a challenge to the inquisitive and to frustrate clinicians and epidemiologists. Moreover, the threat of the unexpected has not been lifted from those of us concerned with the control of infection by this unpredictable virus. The present situation calls for a redoubling of efforts in order that the quest may be successful and that future generations may have no more fear of influenza than they will have of smallpox.

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