The WHO programme for virus diseases in relation to recent trends in medical virology

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The Third International Congress of Virology held in Madrid in 1975 highlighted the considerable progress which has recently been made in molecular virology in understanding the organization of the viral genome, the functions of genes and the regulation of their expression. This progress strongly contrasts with the weight that viral diseases still impose on mankind. For example, the fear of an influenza pandemic is just as prevalent in 1976 as it was in 1918. Poliomyelitis causes an increasingly heavy toll of infantile paralysis in the developing world. It is between these two extremes that WHO has to intervene. One of WHO's functions in medical virology is to apply scientific progress to the solution of public health problems with the assistance of experts and advisers who periodically meet for planning appropriate activities.

In this short exposé, I should like to give you a few examples of what WHO does in medical virology and how it is linked to recent progress in this field. I will describe in more detail such fields as influenza, viral hepatitis, smallpox and, briefly, a few other diseases.

In the implementation of the WHO programme in virology, the network of collaborating centres for reference and research plays an important role. The network consists of 55 laboratories officially designated by WHO in 22 countries. The collaborating centres provide national virus laboratories with reference services, reagents, advice and training. They organize collaborative studies, often on a world-wide scale, for example to study, in comparable conditions with standardized reagents and techniques, the incidence of a disease in different climatic and socio-economic settings. Another field of collaborative activities is the study of prototype strains and immune preparations in view of their adoption as international reference reagents. The WHO programme in virology has still other aspects: establishment of contracts for technical services for research, provision of fellowships for training, emergency aid to countries facing an epidemic or a disaster, epidemiological studies, direct assistance to national laboratories, exchange of information, etc.

Influenza

In February 1976 the discovery at Fort-Dix, New Jersey, USA, that an outbreak of influenza was due to man-to-man transmission of swine influenza-like virus has provoked some fear that the world may again experience a pandemic similar to that of 1918. Is such fear justified? There is the possibility that the Fort-Dix epidemic was only an unique event in a population of military recruits. Nonetheless, at a meeting of WHO consultants on 7 April 1976 it was recommended that surveillance should be increased by the WHO network of national influenza centres in order to detect the possible spread of A/New Jersey-like strains in humans and swine[1].

Since 1947, WHO has established a network of 96 National Influenza Centres in 69 countries and two WHO Collaborating Centres, one in London and the other in Atlanta, Georgia, USA. Each year national influenza centres isolate strains of influenza virus during epidemics. If a strain is found which is somewhat different from previously circulating viruses, the national centres send it to one of the collaborating centres, either London or Atlanta, for further characterization. When a new influenza subtype is characterized, reagents are prepared and distributed to the national centres and the new virus is recommended for the formulation of the vaccine. National centres also carry out serological surveys to assess the level of immunity in the population and so predict to some extent the possibilities of the new strain spreading.

The New Jersey virus has so far not been isolated anywhere else since the episode at Fort-Dix. Strains at present causing epidemics in the southern hemisphere (winter May–September) are identical to the virus A/Victoria/75 subtype which was predominant last year and is expected to be prevalent next winter (1976–77) in the northern hemisphere. The serological survey results which are known at present have confirmed that almost 90% of the population over 50 years of age has antibodies against swine influenza-like viruses. In the majority of cases, the level of antibodies is higher with swine influenza-like antigens than with antigens prepared with more recently prevalent strains. This is caused by the reinforcement of original immunity by further successive infections and this phenomenon is called the 'original antigenic sin'. The swine influenza-like virus circulated from 1918 to 1932 and because of this positive results are also found in persons who were infected at that time. No activity of the virus was found in the younger age groups, except in the Mid-West of the USA. In this region there are positive sera indicating some cases of transmission of swine influenza virus to man, but apparently none succeeded in starting an epidemic as in Fort-Dix. It is noteworthy that the 1918 influenza virus which infected swine in the Mid-West of the USA has survived undetected until now and has not spread.

The constantly changing antigenic pattern of influenza viruses raises interesting points for research. Strains isolated by the network of WHO influenza centres provide adequate material for such studies, and thus WHO contributes to a large extent to progress in this field. Influenza viruses types A and B have two surface antigens, the haemagglutinin (H) and the neuraminidase (N) which can be used to characterize serological subtypes. Influenza A and B surface antigens can undergo minor changes which are called 'antigenic drifts'. Strains modified in this way are variants of a given subtype. In addition, every 10 to 20 years, influenza A surface antigens undergo a major change called a 'shift', which results in a change of subtype. 'Drift' is interpreted as a small mutation which enables the virus to overcome the mounting level of immunity in the population which caused the previously prevailing strain to disappear. After a drift, the new variant keeps cross-relationships with older strains derived from the same subtype, they are all members of the same family. As a result, epidemics caused by a new variant are only moderately severe. Since 1968 different variants have been circulating which are all related to the Hong Kong/68 subtype. The last known, of a rather long series, are A/Victoria/3/75 and A/England/864/75. However, the more recent the variants the more remote the relationship with their parent strain. Furthermore, for the last 5 years variants have appeared more and more frequently, in fact almost every year. It is thought that these two phenomena might announce a new shift which will cause a pandemic.

The first influenza A virus isolated from man in 1933 was a subtype later characterized by its haemagglutinin HO and neuraminidase N1[2]. In 1947 a shift occurred in the haemagglutinin only and the new subtype family was called H1N1. In 1957 a new subtype appeared, known as the 'Asian Flu', and characterized by a change in both antigens which became H2N2. This double shift resulted in a rapid propagation of the new virus. In 1968, only the haemagglutinin shifted thus opening the present H3N2 era with a series of variants of the Hong Kong/68 virus, the Fort-Dix episode, which is of the subtype Hsw1N1, being an exception. Shifts result in pandemics because the population has no cross-related antibodies against the new subtype.

There is, as yet, no generally accepted explanation for the mechanism of shifts. One theory put forward in the past holds that antigenic shift is the result of a direct mutation of human strains. This mechanism can be considered as a further extension of the drift which we have just described. However, LAVER[3] has shown that the polypeptide chains of the haemagglutinin of A/Singapore/57 and A/Hong Kong/68 differ so greatly that the latter could not have derived from the former by mutation.

Animal influenza A viruses have been found in swine, horses and domestic and wild birds. Their H and N antigens generally differ from human antigens. However, LAVER also showed that two strains of influenza virus, isolated from horses and ducks in 1963, possessed haemagglutinin subunits that cross reacted with those of human influenza virus A/Hong Kong/68[3]. This confirmed the hypothesis which he and WEBSTER[4,5] had raised that Hong Kong/68 virus was a recombinant resulting from the mixed infection of an animal or bird with an animal or avian influenza virus which provided the H antigen and a human influenza virus which provided the N antigen[4]. Furthermore, WEBSTER showed that by introducing into a herd of piglets or a flock of turkeys, animals infected with two different subtypes of animal influenza virus it was possible to obtain in vivo a recombinant different from the two original viruses[5]. Thus it may well be that animals, and particularly birds, are at the origin of new pandemic strains. There are still many unknown factors and intensive studies into the ecology of animal influenza viruses are supported by WHO. Interest is concentrated on China and the Kamtchaka peninsula in the USSR where it seems that the new strains of 1957 and 1968 originated. The ecology in these regions seems favourable to permit what has been reproduced in artificial conditions. No less puzzling is the fact that B influenza virus has not yet been isolated from animals. However, the fact that it undergoes only drifts and no shifts is consistent with the recombinant theory.

If we anticipate the logical development of the shifts, the next event after the H3N2 era will be the sudden appearance, probably in the Far East, of one of the subtypes H4N2, H4N3 or H3N3, the most dangerous being H4N3 with a change in both antigens which would cause rapid spread of the virus. The severity of the disease or the virulence of the virus is unpredictable. The situation concerning the treatment of pulmonary bacterial contaminations has greatly improved with antibiotics, but there is still no therapy for viral pneumonia.

The recent occurrence of the Fort-Dix episode would seem to reactivate the recycling theory. According to this theory, the number of human influenza subtypes is finite and they reappear in the same order every 50 to 60 years when the herd immunity has disappeared. Arguments come from what is called 'serological archaeology'. As has already been mentioned, persons infected by influenza virus in 1918 still have antibodies against swine influenza-like viruses. From

[1] World Health Organization, Wkly Epidem. Rec. No. 16–15, April 1976, p. 123.
[2] World Health Organization, Bull. Wld Hlth Org. *45*, 119 (1971).
[3] W. G. LAVER, Virology *51*, 383 (1973).
[4] R. G. WEBSTER and W. G. LAVER, Bull. Wld Hlth Org. *47*, 449 (1972).
[5] R. G. WEBSTER, C. H. CAMPBELL and A. GRANOFF, Virology *51*, 149 (1973).

serological tests performed on older sera it can be deduced that in the late 1800's an era of Asian-like strains preceded an era of A/Hong Kong/68-like strains[6]. In 1973, the swine influenza-like virus was predicted by MASUREL[7] to recur by 1985–1990. It is difficult to deny these facts. However, they are not compatible with what has already been mentioned concerning the wide differences in the genetic composition of subtypes and the evidence of common antigens between human and animal influenza viruses, the variety of which is explained by the recombination mechanism. In the USA it is evident that sporadic human cases of swine influenza have been observed from time to time which are related to the persisting reservoir of viruses in swine. The recent ability of the virus to pass from man to man in Fort-Dix can be explained by a recombination in man of A swine influenza virus and A/Victoria/75 virus which was then epidemic in the camp. These suppositions prove that there is still a great need for research in this field.

Viral hepatitis

A second field in which WHO has been directly involved is viral hepatitis. In recent years considerable progress has been rapidly made and WHO Scientific Groups were convened almost yearly to keep pace with the most recent advances and assess their value[8,9].

In 1967, the finding of Australia antigen, now known as hepatitis B surface antigen (HBsAg), represented a considerable breakthrough in viral hepatitis. Several techniques were soon developed for the detection of HBsAg and their different sensitivities resulted in discrepancies in prevalence studies. WHO contributed to the standardization of techniques and reagents through collaborative studies. Most investigations are now carried out with reversed passive haemagglutination tests and radioimmunoassay, which belong to the so-called third generation and are the most sensitive techniques. The prevalence of HBsAg in tropical areas, in correlation with climatic and socio-economic factors, is being investigated in a WHO collaborative study in which 25 laboratories are participating with standardized methods and standard reagents.

Several categories of personnel are at a high risk of infection with hepatitis B virus: patients receiving blood transfusions or certain blood products, staff accidentally exposed to HBsAg in hospitals and laboratories, dental surgeons, patients and staff of institutions for the mentally retarded and of haemodialysis and transplant units, infants of women who acquire the disease in late pregnancy and spouses of HBsAg carriers. These groups constitute a priority for protection either by passive or active immunization.

In the United States, very recent trials have compared the efficacy of standard immunoglobulin (SIg) and specific hyperimmune globulin (HB Ig) in pre- or post-exposure treatment of groups at high risk[10] of infection with hepatitis B. The trial showed that 5 ml of SIg given intramuscularly, which in the States has had a titre of approximately 1:100 since 1972, can protect against accidental ingestion or accidental needle puncture with a small virus inoculum. After exposure to a large dose of virus, hepatitis B hyperimmune globulin (HB Ig), with a titre of 1:200,000 to 1:500,000, provided better protection. During the first 4 months after exposure, HB Ig provided better protection than SIg, but after this period cases of hepatitis B were observed in the HB Ig group more frequently than in the SIg group, possibly because a lower dose of Ig permitted the passive-active immunization process to act. The use of HB Ig in persons under continuous exposure would necessitate repeated injections, possibly inducing allergy. Dental surgeons, for example, should receive 4 doses a year from the beginning of their studies. HB Ig will therefore soon be licensed in the USA but only for specific uses[11].

Vaccination would be a better solution to protect high risk groups. We are facing here an unusual problem, and the fact that a tissue culture system has not yet been developed for cultivation of hepatitis B virus means that we have to use a product of human origin as immunogen. A crude immunogenic preparation of heat-inactivated serum was used in 1970 by KRUGMAN[12] which prevented or modified type B hepatitis in 69% of recipients. Such material may contain Dane particles which may escape heat inactivation.

A purified inactivated vaccine containing only 22 nm surface antigen particles has been prepared by isopycnic banding in CsCl and rate zonal centrifugation followed by inactivation with formalin by two different groups in the USA[13,14]. Trials in chimpanzees proved the efficacy and safety of such a vaccine. However, in the absence of a tissue culture system for safety tests, it is difficult to demonstrate whether inactivated HBsAg particles are free from infectious nucleic acid. Furthermore, the polypeptide, glycoprotein and lipid composition of the HBs antigen is now known. There is a possibility that lipoproteins in HBs antigen may be derived from liver cell membranes and from normal plasma proteins or represent abnormal modified host cellular components, the synthesis of which may be derepressed by the action of hepatitis B virus. Such material could induce cell-mediated immunity to liver specific lipoproteins and thus produce an autoimmune reaction that might lead to active chronic hepatitis[15].

A third type of vaccine is under consideration which would use purified subunits consisting of immunogenic polypeptides which fail to induce any detectable cell mediated immunity to cellular antigens of host origin. Because of their low molecular weight, these polypeptides would have to be coupled to a carrier protein. This approach could even lead to the synthesis in a laboratory of antigenic amino-acid-moieties.

After thorough investigations in chimpanzees, the purified 22 nm particle vaccine will have to be tried for safety in an isolated environment, such as cloistered monks or nuns. Then it will be necessary to extend the trial to a group large enough to be significant from a public health point of view. Thereafter efficacy trials will be possible in high risk groups such as those defined above. These considerations underline how cautious the approach to the use of a vaccine against hepatitis B should be in the present conditions. This will be one of the major considerations for the next WHO Expert Committee in October 1976.

The elimination of commercial blood and testing of blood by third generation techniques, such as radioimmunoassay and reverse passive haemagglutination has reduced the incidence of post-transfusion hepatitis by approximately 60%. Recent progress in the detection of hepatitis A antibodies has indicated that when a population of voluntary donors pretested by RIA is used, approximately 90% of residual hepatitis is serologically unrelated to either type-A or type-B viruses[16]. There is also evidence that cytomegalovirus and Epstein-Barr virus are not implicated, thus indicating the existence of non-A/non-B viral hepatitis which will now have to be studied.

Smallpox

When the WHO global programme of smallpox eradication started in 1967, smallpox was endemic in 37 countries in Africa, Asia and South-America. In early 1976, continuing transmission was limited to four provinces in Ethiopia. Such an achievement was only possible with a high degree of international cooperation.

In Africa, an unexpected finding of the eradication campaign was the discovery of cases of natural transmission of monkeypox virus to man. At the end of 1975, 21 cases had been diagnosed in Zaïre, Liberia, Nigeria, Ivory Coast and Sierra Leone. Although clinically similar to variola, monkeypox is far less easily transmitted from man to man and a secondary case has occurred only on two occasions in spite of a large number of susceptible contacts. Monkeypox virus can be differentiated from variola through laboratory tests. GISPEN found monkeypox antibodies in wild monkeys of Africa.

Furthermore, two whitepox viruses have been isolated from the kidneys of monkeys captured in Zaïre. The isolates resemble the two whitepox viruses which were isolated in Utrecht from monkeys in captivity. These four whitepox viruses have been intensively studied in various laboratories, but all tests so far have failed to distinguish them from variola virus. There are no epidemiological grounds, however, for identifying them as variola. Whitepox viruses originated from areas where smallpox had not been reported for a considerable length of time and has not re-emerged since. Monkeypox and whitepox viruses do not, at present, appear to pose a threat to the smallpox eradication programme. Nevertheless, WHO has undertaken a wide programme of intensive surveillance in Africa.

The last cases of smallpox in South-East Asia were reported in India in May 1975 and in Bangladesh in October 1975. In May 1976 no cases were found in the world, apart from 34 remote villages located in mountainous and desert areas of Ethiopia with fewer than 45 active cases. More than 1000 Ethiopian and international health workers were necessary to achieve this result. In all the countries recently freed from smallpox, an intensive 2-year period of surveillance is carried out – sometimes with continuing routine vaccination – before an international commission authenticates the eradication of the virus after a careful visit to several areas in the region.

The eradication of smallpox has several implications, which will be studied by an international conference, among which three are most obvious. The first one concerns vaccination. Should it be continued? In addition to the cost of mandatory vaccination, in some countries there have been more neurologic complications than cases or risk of cases. During the Assembly in May 1976, WHO urged Member States to restrict their requests for international certificates of smallpox vaccination to travellers who have visited a smallpox infected country within 14 days, i.e. at present only Ethiopia. The vaccination being stopped, all the world's population will rapidly become receptive to smallpox as the duration of a strong protective effect of the vaccine is approximately 3 years; newborn babies being particularly susceptible as they are no longer vaccinated. The second implication is that a reserve supply of vaccine should be available 'in the event of unforeseen emergencies'. WHO will manage to handle a reserve of vaccine for 200 to 300 million people. A third implication is that in view of the well known risk of contamination of laboratory personnel handling the virus, only a few laboratories should be authorized to maintain strains of the wild virus and

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[15] J. L. MELNICK, G. R. DREESMAN and F. B. HOLLINGER, J. infect Dis. *133*, 210 (1976).
[16] H. J. ALTER, P. V. HOLLAND and R. H. PURCELL, Am. J. med. Sci. *270*, 329 (1975).

WHO is preparing a list of these laboratories in consultation with governments. This presents a unique situation of a disease which has been artificially eradicated, but is still potentially dangerous should the virus escape.

Other diseases

The WHO programme for virus diseases extends also to other activities where progress has recently been made or needs to be stimulated.

Poliomyelitis and its vaccination are now neglected but all problems have not been solved. After the extraordinary success of mass vaccination in developed countries, the present lack of enthusiasm for vaccination creates an increasing lack of immunity in some populations. In developing countries the number of cases of infantile paralysis is increasing and vaccination becomes more frequently necessary, raising logistic problems which WHO helps to solve through its Expanded Programme for Immunization. The live poliomyelitis vaccine needs constant monitoring to safeguard against its reversion to neurovirulence. Here again WHO has taken steps to provide safe seed-lots from SABIN's original strains.

The discovery of rotaviruses has given a new insight into infantile diarrhoeas. A vaccine appears possible, but will it be necessary to vaccinate children? This question might not be so easy to answer and rapid dehydration might be just what is needed. What is the importance of rotaviruses in warm climates? This is still unknown and a collaborative study has been undertaken by WHO to study this question. In fact the study will be extended further in order to elucidate the possible association of gastro-enteritis with other viral particles, such as the astroviruses, caliciviruses and coronaviruses. Several authors have described virus-like objects they have observed in the stools and at present it is not clear whether the varying findings by different laboratories reflect differences in viruses or variations in the techniques used in looking for them. As a first step, aliquots of the same stools will be examined in different laboratories using their own electron microscopy technique and results of findings will be compared.

Turning to a different field, insect-borne viral diseases cause dengue-like fevers, haemorrhagic fevers and encephalitides which are now more and more frequent in countries with warm climates where rapid urbanization and insufficient sanitation favours the proliferation of mosquitoes. Dengue haemorrhagic fever in South East Asia is among these.

During the last two decades, great progress has been achieved in the inventory of viruses responsible for the above-mentioned syndromes. This is mainly due to the contribution by WHO Collaborating Centres for Arbovirus Reference and Research – (the arthropod-borne viruses). More than 370 arboviruses have now been characterized and registered in a Catalogue, thanks to the assistance provided by the American Committee on Arthropod-Borne Viruses. 80 of these viruses are known as pathogenic for man. The others have been isolated from different animal reservoirs or different hematophagous insects and their capability of causing disease in man is still unknown. The pathogenic mechanism of haemorrhagic fevers caused by arboviruses is not well known. As dengue haemorrhagic fever is a public health problem of great importance in the South-East Asian, Western Pacific and Carribean areas, research in this field has been supported and co-ordinated by WHO. In one outbreak in Bangkok in 1962 it caused in a few months approximately 8,000 cases in children aged 5 months to 12 years and 800 of these died. In a WHO collaborative study, Thai physicians together with American researchers have shown that the rapid hypovolhemic shock which aggravates the haemorrhagic symptoms is caused by the antigen-antibody complexes which are formed during the 3rd to 5th day after the onset of the disease. Complexes activate both complement pathways and this results in the release of anaphylatoxin which increases the vascular permeability[17]. If plasma or saline physiological solution can be infused in the proper quantity and time, the death rate may be reduced to 3%.

Lassa fever and Marburg disease are well known diseases from the publicity which has been given to them when cases have been diagnosed in temperate climates. The original focus for these two viruses is in tropical Africa. The reservoir of Marburg virus is not known but for Lassa virus it has been shown that an African rodent is the source of contamination mainly excreting the virus through urine. These two viruses are also dangerous, because they can be transmitted from man to man through infected excreta or through droplets. Infected patients with Lassa virus have already travelled on commercial flights but so far no secondary cases have been reported. The epidemiological distribution of Lassa fever is now under study in endemic foci which have been found in West Africa. The pathogeny of Lassa fever, as well as that of Bolivian and Argentine fever, which are caused by related viruses, is under study and first results have been reported in an international seminar held jointly by WHO and the Center for Disease Control in Atlanta, Georgia, USA[18].

The foregoing are some examples of research in virology to which WHO is associated in order to help solve problems of public health interest. The choice has been limited to comply with the time factor of this Assembly and in fact the scope of WHO's activities in medical virology is wider. If you wish to establish contact with us, my colleagues and I will be happy to reply to your requests.

[17] World Health Organization, Bull. Wld Hlth Org. *48*, 117 (1973).
[18] World Health Organization, Bull. Wld Hlth Org., in press (1976).