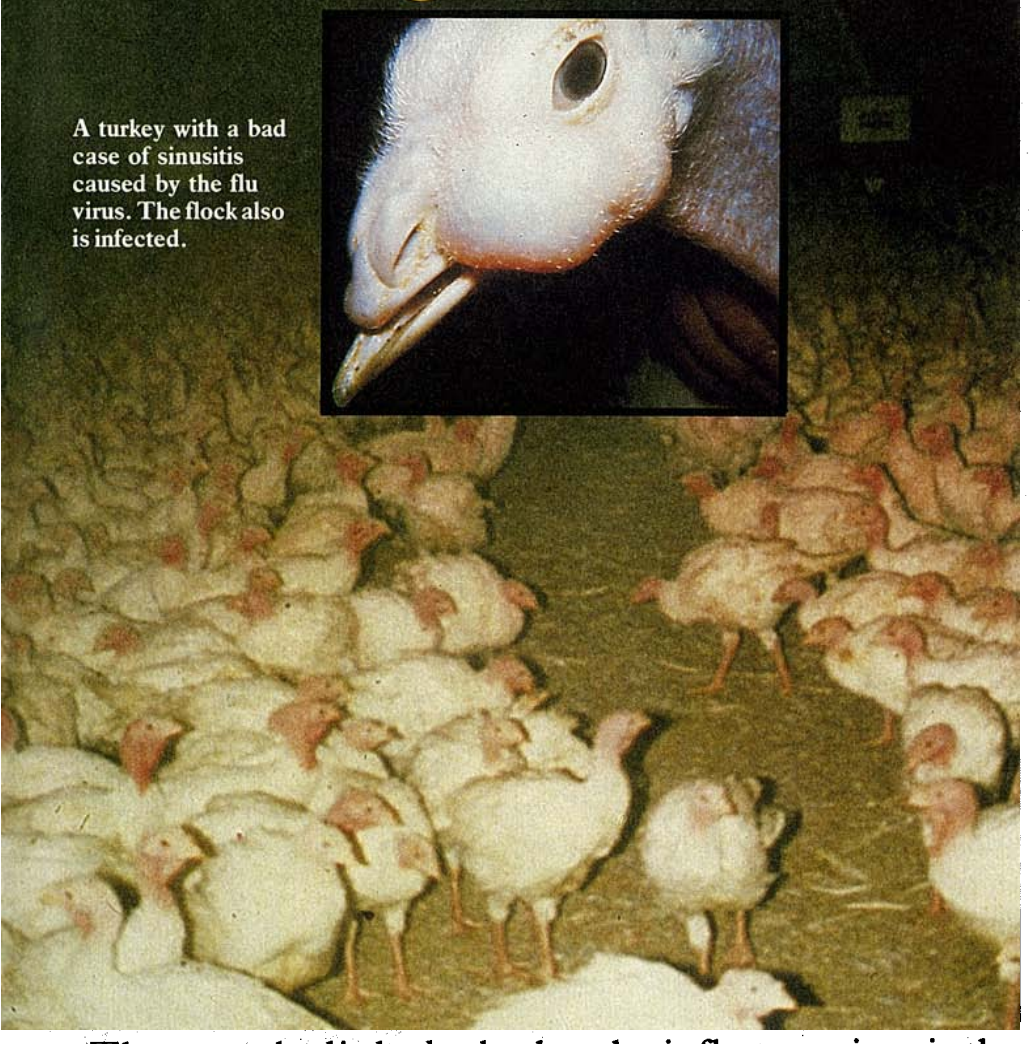
Confronting the changing flu virus



A turkey with a bad case of sinusitis caused by the flu virus. The flock also is infected.

There can be little doubt that the influenza virus is the most successful virus on this planet. It has been causing misery and death for all mankind, at least since the beginning of recorded history and probably for much longer. To date, all attempts to control it have failed, and it has been aptly described as 'the last great plague'.

The unpredictable onset of influenza within countries and cities and its rapid spread defied explanation for many years. In fact, the name 'influenza' is derived from one early explanatory attempt: 14th Century historians in the Italian town of Florence attributed the outbreak of the disease to an unusual conjugation of the planets — or planetary 'influence' — and this name has persisted over the centuries.

The controversy over the cause of fresh outbreaks of the flu persists to this day. Recently the old 'planetary influence' theory re-surfaced in a new form with the quite serious suggestion by Professor Fred Hoyle and a collaborator, Professor Chandra Wickramasinghe, that meteor showers introduce new genetic material from deep space into the flu virus. However, as will be shown in the following pages, recent research in a number of countries, including Australia, suggests strongly that the interaction between man and his most persistent virus is a modern example of evolution in action, and the erratic nature and occurrence of the virus has a very down-to-earth explanation.

Cause and course of the disease

Only in 1933 was a virus identified as the cause. Soon afterwards, scientists found that it could be grown on the embryo sac of fertile hens' eggs, and the ease of this technique, combined with the importance of the disease, has made the influenza virus a most intensively studied organism.

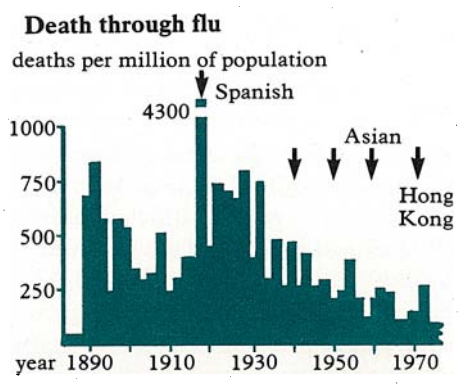
The virus, spread through sneezing and coughing, enters the nasal passages and penetrates the cells of the respiratory tract. Within a day or two the afflicted individual begins to experience the first hint of the disease. In about two-thirds of cases the onset is sudden, the first symptoms being a headache, chills or fevers, and a general feeling of illness. In the space of a few hours the body temperature rises to 39°C and sometimes higher, and a short, dry cough — one of the most regular features — begins.

About 50% of sufferers experience sneezing, nasal blockage and discharge, and a sore throat, although these symptoms are seldom prominent. Many have muscle aches and painful joints, and often painful and watery eyes. Occasionally nausea and vomiting occur, but diarrhoea is rare.

In its classic form, the fever lasts for 3 days, but duration may vary from 1 to 6 days. But that is not the end — the cough, lassitude, and a depressed feeling may linger on for weeks. If the high temperature persists it probably indicates secondary complications.

Migratory terns also suffer from the flu and can spread it on their travels.

A typical flu epidemic may afflict anywhere between 10% and 40% of the population with these symptoms. But the people who don't become ill don't necessarily escape infection — they may be infected, without becoming ill or suffering more than minor inconvenience. Surveys indicate that these subclinical infections commonly number about the same as the clinical cases. The reason why they occur remains uncertain, but it is thought that some people have a partial immunity while others don't receive an effective dose of the virus; such apparently innocuous infections are particularly important because they help spread the disease.



Deaths in England and Wales from influenza and influenza-associated diseases. Arrows mark the times when antigenic shift occurred.

Influenza has a marked economic impact on our societies.

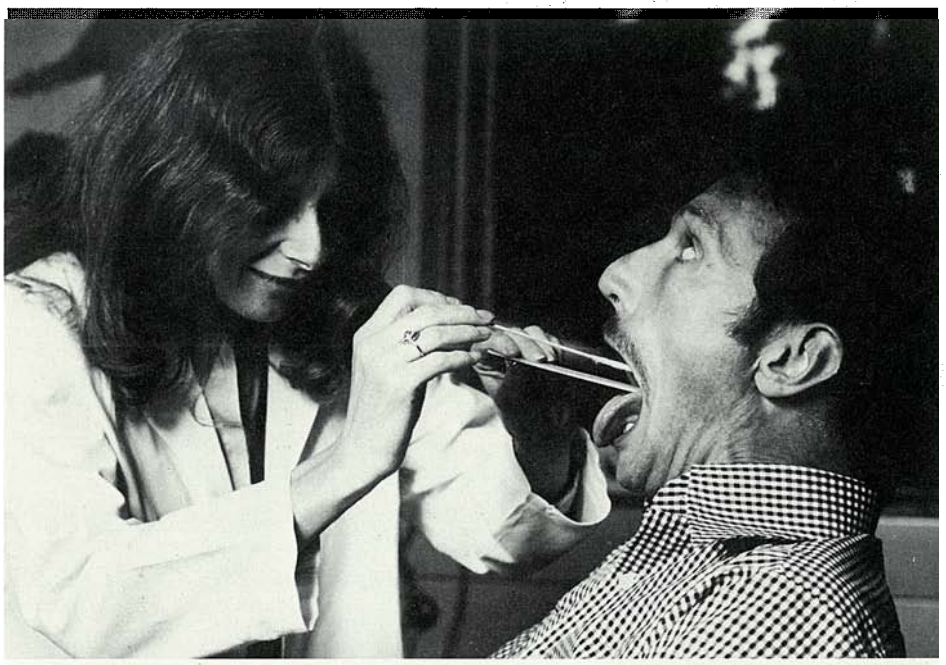
Death and dollars

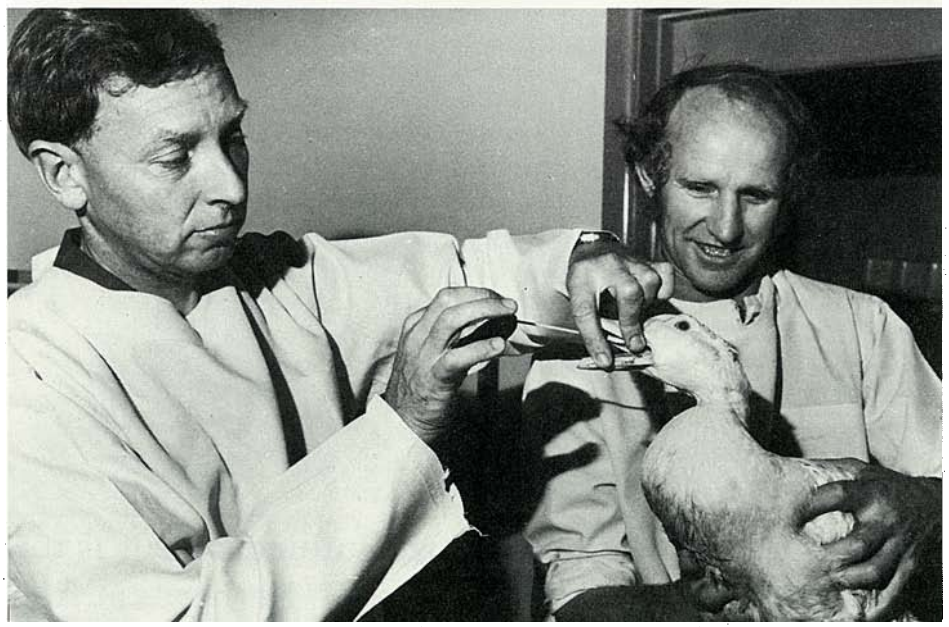
While the great majority of people quickly recover from their bout with the influenza virus, a small percentage (anywhere between 5 and 10%) develop bronchitis or pneumonia. Sometimes the virus can cause pneumonia directly, but this is extremely rare and the lung problems that arise are usually secondary phenomena due to infectious bacteria following in the path of the virus.

Modern antibiotics and competent hospitalization soon clear up these secondary complications, but in earlier days the appearance of pneumonia, particularly in the very young or old, often meant a death sentence. Afflicted individuals literally drowned in the fluids that congested their infected lungs.

And the death rate could be staggering. The 1918–19 influenza outbreak was one of the most destructive epidemics in history, ranking alongside the Black Death of the 17th Century. Although most of those infected suffered only the usual 3–5 days' illness, about 20% of cases developed primary viral pneumonia and half of these died World-wide, at least 20 million people perished during this outbreak and some estimates place the number of deaths at 40 million. In comparison, only 8·5 million soldiers died during World War I.

Yet, even though modern medicine has reduced the fatal consequences of secondary complications, influenza still has a marked economic impact on our societies. Apart from the disruption of government, industry, and commerce, hospital and medical costs can be very high. During the 1968 outbreak of the Hong Kong flu, the United States government spent $225 million just in providing Medicare aid to old people suffering from influenza. In total, the Department of Health, Education and Welfare estimated that the one flu epidemic cost the American economy $4600 million.





The flu virus can be collected by throat swabs from both humans and birds and then grown-on in hen's eggs.

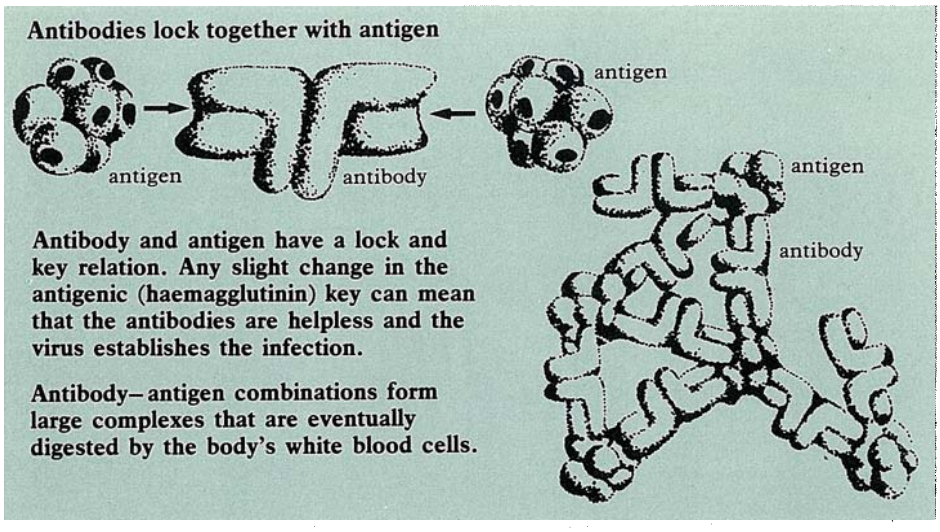
Antibody and antigen

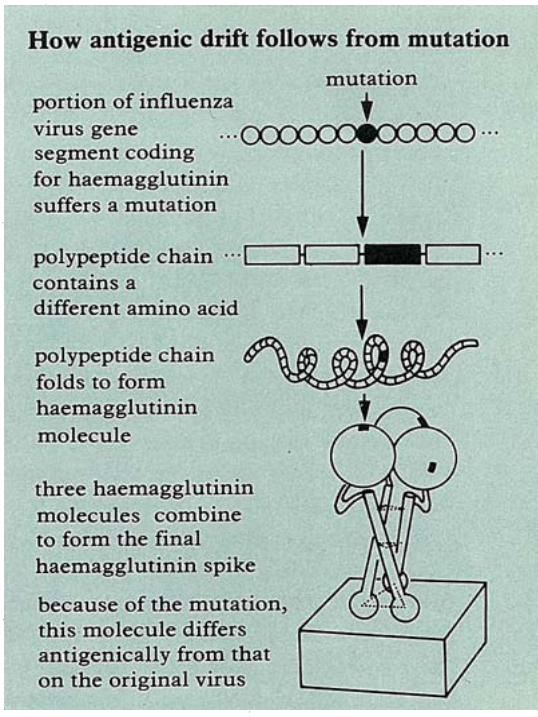
The immune system, developed during the course of evolution, protects the body from invasion by foreign agents — whether living or non-living. As a crucial part of its role, it must distinguish between self and not-self and achieves this through the white blood cells constantly surveying the molecular characteristics of the many chemicals found in the body.

When these cells find something, such as a virus, that is not part of a person's normal array, they mobilize the resources of the immune system to produce antibodies that inactivate and then help to expel the virus.

But it takes time — at least several days — before the body can accumulate sufficient antibody to free itself of the virus. Afterwards, antibody levels subside; but the memory lingers on in special cells that are always ready to spring into action to produce fresh antibody and quickly suppress any new attack by that particular virus.

The chemicals of the virus or microbe that provoke the antibody response are termed antigens. These are generally proteins found on the surface of the invading organism, and the flu virus carries two such proteins. One, an enzyme called





neuraminidase, prevents the virus particles from clumping together and also helps release them from infected cells and may be involved in the first stages of infection. The other protein — haemagglutinin — attaches the virus particle to the host cell wall and mediates cell infection.

The haemagglutinin antigen provokes the major response; the antibodies formed latch onto the haemagglutinin molecule and block the attachment or receptor site and, soon after, the virus is absorbed and digested by scavenging cells of the immune system. Detailed studies on a range of flu types have shown that the changes in the structure of the haemagglutinin molecule are largely responsible for the regular emergence and success of new flu viruses.

Shift and drift

Other viruses that infect man, like mumps and measles, rarely change and will probably never again trouble any individual who has passed through the discomfort of the initial infection. In these cases the body's immune system has a permanent record of the viruses' antigenic 'fingerprints'. When the body encounters the mumps or measles virus again, new antibody is rapidly synthesized, and the virus is quickly expelled.

Unfortunately, the flu virus is a little bit more adaptable than the body's immune system; its antigenic fingerprint changes regularly. The immune system treats an encounter with a flu virus containing different antigens as a completely new experience, and another bout of influenza begins.

In 1918 (Spanish flu), 1957 (the Asian flu), and 1968 (Hong Kong flu), new influenza virus sub-types appeared that had radically different antigenic properties. People who had experienced Asian flu had absolutely no antibodies effective against the Hong Kong flu and succumbed to the disease.

The origin of these radically different strains remains uncertain. There is some evidence that they may arise through some recombination of the different types of flu infecting man and animals (see the box on page 16).

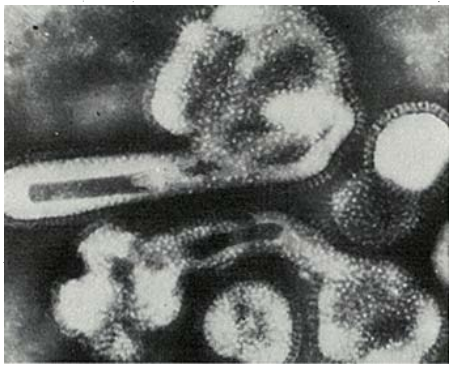
In between these major shifts in the virus's antigenic properties, however, the flu virus may still manage to overcome the body's immune system. Soon after the appearance of a new sub-type, virologists are able to detect small but significant changes in its antigenicity. These arise through mutations that result in the virus carrying slightly different haemagglutinin molecules. Sometimes such viruses can evade the body's immune defences, and bring on another flu attack (see the diagram).

These slight changes, termed antigenic drift, mean that over a period of time the antibodies formed during infection with a new sub-type become less and less effective. People who were infected with the original Hong Kong sub-type of 1968 and had developed antibodies to this virus, for example, were virtually unprotected against infection by later strains of the Hong Kong sub-type such as the Port Chalmers virus of 1973, the Victoria virus of 1975, the Texas virus of 1977, or the Bangkok virus of 1979. Antigenic drift is the reason why regular mini-epidemics occur between the major epidemics that follow major changes to the virus, as shown in the chart.

Electron micrographs of the flu virus, with the haemagglutinin and neuraminidase projections just visible. The virus is very variable in size and structure because its coat is actually the membrane from its host cell, which is more flexible than the protein coats of most viruses.

The haemagglutinin molecule

Both antigenic drift and the appearance of new sub-types result from the changes that occur in the haemagglutinin molecule.

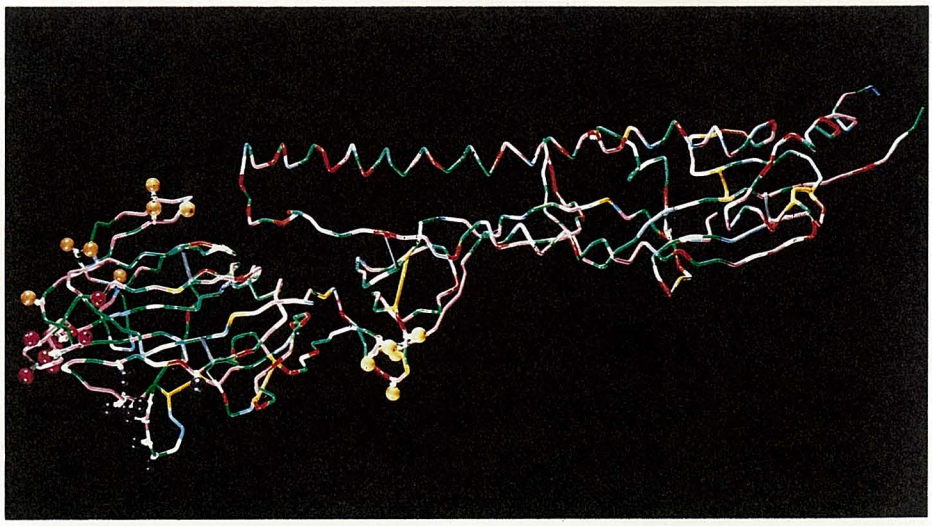


Electron micrographs of the flu virus, with the haemagglutinin and neuraminidase projections just visible. The virus is very variable in size and structure because its coat is actually the membrane from its host cell, which is more flexible than the protein coats of most viruses.

Although crude details of the shape of the molecule — it is a spike that protrudes from the virus — can be determined in electron micrographs, much finer detail is necessary for a complete understanding of how it changes and functions. Within Australia, scientists such as Dr Colin Ward and Dr Theo Dopheide, from the CSIRO Division of Protein Chemistry in Melbourne, and Dr Gillian Air and Dr Graeme Laver, from the John Curtin School of Medical Research at the Australian National University in Canberra, have been studying the structure of the whole molecule using protein-sequencing techniques.

In another approach, Dr Gerry Both, Dr Merilyn Sleigh, and Dr Berny Moss, from the CSIRO Molecular and Cellular Biology Unit in Sydney, have analysed the nucleic acid sequences in the virus that code for the haemagglutinin, as well as analysing small sections of the molecule itself, to help identify areas most subject to change.

Early in the protein-sequencing studies, a comparison of Australian results with those obtained by Dr Mike Waterfield of the Imperial Cancer Research Fund Laboratories, in London, showed that approximately half the amino acids in the haemagglutinin molecule had been altered in the shift from the Asian to the Hong Kong sub-type. This massive change could be attributed to the recombination of different strains described in the box, but tells us little about the subtle changes occurring during antigenic drift.



A wire model of the haemagglutinin molecule from the Hong Kong flu virus, showing the complicated 3-D folding. Different amino acid groups are represented by the various colours, while the coloured balls indicate where all the changes occurred during antigenic drift from 1968 to 1979.

Dr Ward and Dr Dopheide concentrated their attention on the Hong Kong sub-type and, through a long series of sophisticated analyses, determined virtually the complete order of arrangement of the amino acids in virtually all of the haemagglutinin protein.

The only portion whose amino acid sequence they couldn't determine was its tail section, composed of approximately 25 amino acids, which serves to anchor the haemagglutinin to the virus. This section of the protein, once it is released from the virus, clumps together and cannot be analysed; but, since it is hidden and plays no real role in determining antigenic properties, this is not important. All the

Cold or flu?

Many people confuse the symptoms of colds and flu and, possibly to attract more sympathy, will quickly attribute a severe cold to the flu virus. Certainly the symptoms do often overlap and a mild form of influenza is virtually indistinguishable from a common cold. But colds rarely kill, and people suffering the symptoms described in the main text can be fairly certain that they are infected with the flu virus.

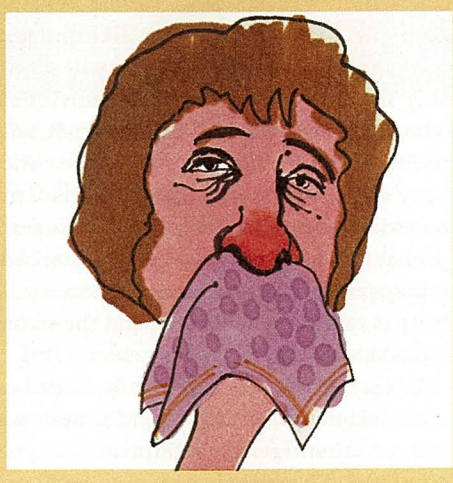
At the molecular level, flu and the common cold are quite distinct. Influenza is caused by a single type of virus — a myxovirus — while the common cold is caused by a wide range of types that vary even more than influenza virus.

The first successful isolation of a virus causing cold-like symptoms occurred in 1943 as an accidental by-product of the massive studies then beginning on poliomyelitis. Not unnaturally this was a polio-like virus termed an enterovirus, and subsequently a range of other enteroviruses, some adenoviruses, and some influenza-like paramyxoviruses were isolated and shown to cause mild colds. However, these viruses were only occasionally detected in patients suffering from real colds. The great majority of colds are not caused by these viruses.

It was not until 1960 that researchers, at the Common Cold Research Unit at Salisbury in England, isolated the main culprits. They found that these viruses — rhinoviruses ('rhino-' = 'nose') — had defied detection for so long because conditions in the nose, with a temperature of 30°C and a pH of 6·9, were very different from the usual 37°C and pH of 7·6 used in tissue cultures. When the conditions were altered to match those found in noses, the virus grew readily in their cell cultures.

Rhinoviruses probably cause about 40% of the common cold infections, and vaccination against them is virtually impossible because of their great antigenic diversity. So far more than 100 different strains have been isolated, and probably many others are floating around in the population.

As well as those mentioned above, other



viruses that cause colds have been discovered and probably many more await discovery. Already, more than 120 different causative agents from five major virus groups have been identified and, given the diversity found in a single virus, such as influenza, it would be a brave man who'd bet on the chances of developing a cure for the common cold. Perhaps colds will eventually take over, from flu, the title 'the last great plague'.

Unfortunately, the flu virus is a little bit more adaptable than the body's immune system.

antigen–antibody action and all the changes take place higher up on the molecule.

The scientists found that, having established the amino acid sequence of the haemagglutinin from one strain of the virus, they could determine the structure of the haemagglutinin from closely related strains relatively easily.

They did these sorts of analyses on another major epidemic strain of the Hong Kong sub-type and, in collaboration with Dr Laver and Dr Air, obtained partial sequences for seven other Hong Kong strains that appeared between 1968 and 1977. Their results made some of the changes that were occurring quite obvious. Often strains differed from one another — in their antigenicity and their effects on man — simply because one or two amino acids out of the total of 549 in the haemagglutinin molecule had changed.

Looking at their genes

In the other approach, Dr Both, Dr Sleigh, and Dr Moss had a close look at the genetic structure of the various strains of Hong Kong flu. Since the arrangement of nucleotides in the virus's genes determines the arrangement of the amino acids in its proteins, they were able to use techniques developed in genetic engineering to study the changes that had occurred in the Hong Kong sub-type since 1968.

Separating out the gene that codes for haemagglutinin, they incorporated it into *Escherischia coli* bacteria and cultured large quantities of these. After separating and purifying the bacterial DNA (deoxyribonucleic acids), they could isolate the section that coded for the viral haemagglutinin.

In this manner Dr Both and his colleagues determined the complete nucleotide sequences of the haemagglutinin gene from four of the strains of the Hong Kong sub-type that appeared between 1968 and 1979, as well as partial sequences for four other strains. Their results meshed in very well with the protein-sequencing studies.

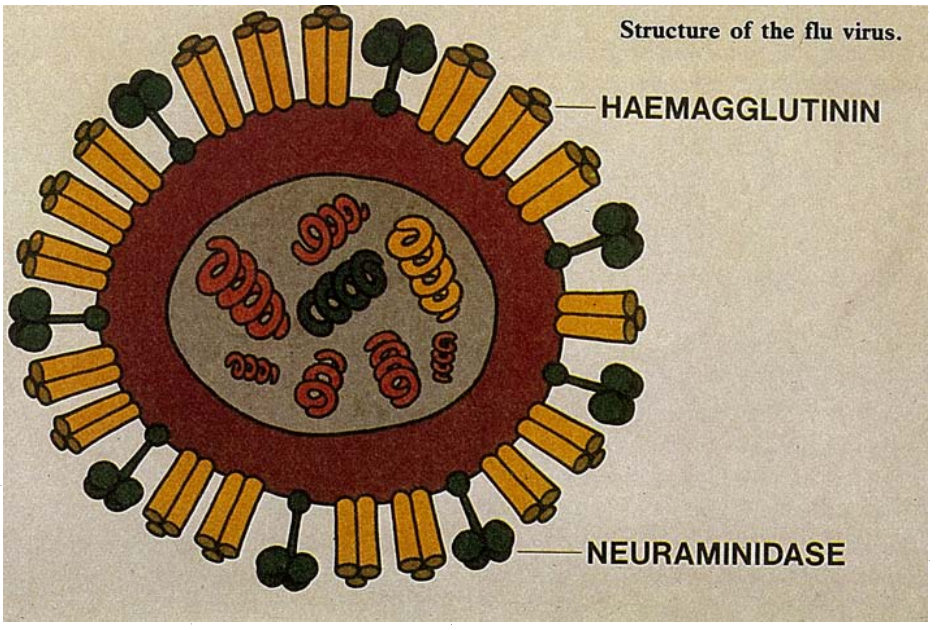
Each group noted that most of the changes in the haemagglutinin molecule occurred in one of four distinct parts. Moreover, they occurred in steps: a strain isolated soon after the appearance of the Hong Kong sub-type would have one or two amino acids changed, a few more changes would occur the next year, and so on. By 1979, approximately 10% of the amino acids found in the haemagglutinin of the original Hong Kong sub-type had changed.

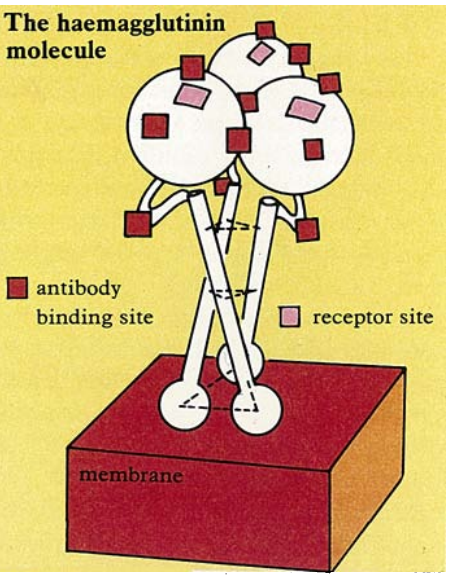
Most of these changes were in the antigenically important parts of the molecule. The virus, through mutation, was creating antigenically different haemagglutinins, and so it could beat the antibodies formed during infections with earlier strains of the Hong Kong flu.

The 3-D picture and antigenicity

The nature of the haemagglutinin molecule and its antigenic sites became a lot clearer with the publication, early in 1981, of the first three-dimensional description of the protein. The group responsible for this breakthrough — Dr Ian Wilson and Dr Don Wiley, from Harvard University in the United States, and Dr John Skehel from the National Institute for Medical Research in the United Kingdom — combined protein-sequencing data they obtained from Australia with data from a Belgian group led by Dr Walter Fiers and X-ray crystallography to build their picture of the haemagglutinin molecule.

In between major shifts, the flu virus may still manage to overcome the body's immune system.





A simple depiction of the haemagglutinin structure, showing the sites where the molecule attaches to the host cell, and where the host's antibodies attach to the haemagglutinin.

Although this picture is very complex, a simplified diagram can be constructed and is given above. This clearly indicates where all the action occurs during a flu infection.

As could be expected, three of the four sites that the CSIRO groups had identified as being antigenically important and subject to the most change occur at the top of the spike, where they are most exposed to antibody attack. The fourth occurs lower down the molecule.

The three sites at the top of the haemagglutinin molecule are grouped around a deep pocket-like depression that involves about 25–30 amino acids. The amino acids in this pocket don't appear to change as easily as the surrounding amino acids, and it is proposed that this is where the virus attaches to the human cell wall.

The origin of new flu sub-types

Dramatic shifts in the antigenic character of the flu virus, such as that occurring between the Asian sub-type of 1957 and the Hong Kong sub-type of 1968, happen regularly. It has been estimated that over the past 250 years at least 10, and perhaps as many as 20, influenza pandemics have swept the globe after the appearance of a new sub-type.

Four explanations for the sudden appearance of new sub-types have been put forward. One is that the existing strains in man undergo rapid multiple mutations that produce radical shifts in the antigenic character. The second suggests that an influenza virus of lower animals is transmitted to man, while the third suggestion is that the human influenza virus recombines with an animal influenza virus and acquires a completely new coat protein while retaining the capacity to cause disease in man. The final suggestion is that influenza virus may be recycled: lying dormant after its initial appearance and only re-emerging when the immunity of the general population has declined.

According to Dr Laver and Dr Robert Webster from St Jude Children's Research Hospital in Memphis, Tennessee, there is considerable evidence — both experimental and circumstantial — for the last three suggestions, but little in support of the first.

It has long been known that human influenza will infect other animals. For example, the Hong Kong flu caused natural infection of pigs in many countries soon after that sub-type appeared. It has also been shown to infect dogs, chickens, and calves, and, as it drifted and changed through the '70s, its new strains have also been isolated from animals.

While nobody has yet demonstrated that animal influenza will transmit to man, a number of historical accounts of the disease mention the interesting coincidence of influenza-like diseases in animals, particularly horses, immediately preceding or accompanying influenza epidemics.

A particularly intriguing observation is that the first definitely observed cluster of human influenza cases in the 1918 outbreak was recorded at a United States army camp that drew most of its recruits from farm-workers in the mid-west.

There is also experimental evidence that flu viruses infecting man and those of animals can mix and recombine to produce radical shifts in their antigenic character. The unusual nature of the flu virus genetic material aids this process. Since its genes are in eight separate packages, infecting one host cell with two different types of flu may result in 2^8 (or 256) different, and new, strains of flu.

In one project at the Plum Island Animal Disease Centre, in New York State, researchers infected a single pig in a herd with the human Hong Kong flu and another with the common strain of pig influenza. Within a week, other members of the herd were found to be suffering from infections with a varied array of recombinant flu viruses.

Similar scenes occurred when flu infections were exchanged between turkeys and pigs and among turkeys. In one case a serious 'mini-epidemic' raged among the turkeys, killing several, even though the original strains were not particularly virulent.

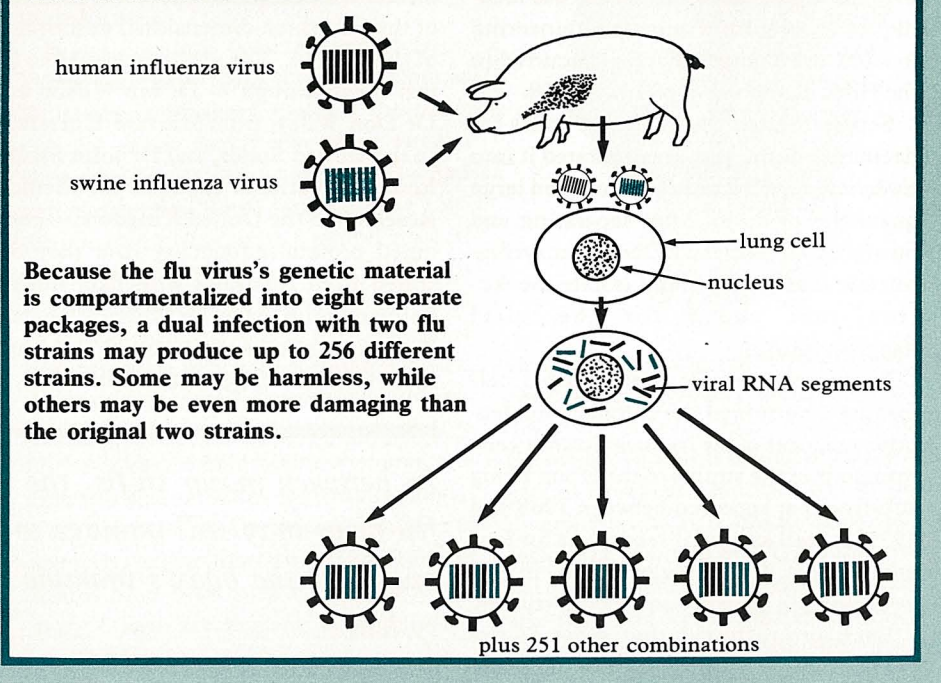
Recombination of flu viruses has only recently been observed in the wild, wide world, and good circumstantial evidence suggests that the Hong Kong flu sub-type of 1968 may have arisen through the mixing of a strain originally isolated from ducks in 1963 and the old Asian flu of 1957. This idea recently received further support when Dr Ward and Dr Dopheide, as well as the Belgian group led by Dr Walter Fiers, determined the protein sequence of the original duck strain and showed that its haemagglutinin was virtually identical to that found in the first human Hong Kong variant.

It is also known that the flu virus is capable of being recycled. For example, the strain that caused the 1951 epidemic got lost for 26 years before suddenly reappearing in China and Russia, then world-wide, during 1977.

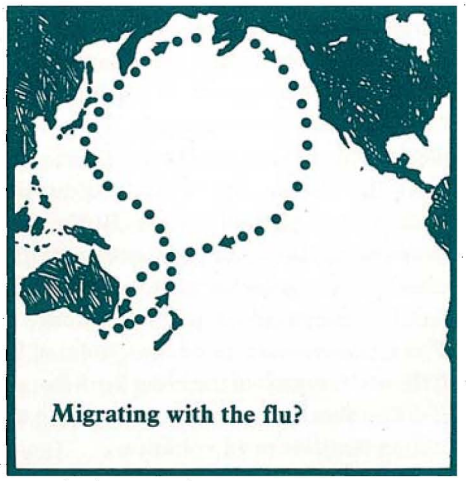
Other evidence suggests that migratory birds — such as ducks, terns, and shearwaters — not only provide a reservoir of new flu types but also spread the flu virus in their travels around the globe. Although flu transmission between wild and domestic birds and then on to man appears, at first glance, to be unlikely, Dr Webster has shown that many of the influenza viruses of ducks are shed into water along with the faeces. These viruses remain viable for 4 weeks at 4°C and for about 5 days in water at room temperature, and so it is likely that the flu virus is water- as well as airborne.

Collectively, these studies emphasize the great versatility of the flu virus and indicate why it enjoys continuing success among both men and beasts.

How flu viruses recombine



Because the flu virus's genetic material is compartmentalized into eight separate packages, a dual infection with two flu strains may produce up to 256 different strains. Some may be harmless, while others may be even more damaging than the original two strains.



The migratory path of the short-tailed shearwater. The migratory route of the closely related Pacific shearwater is unknown, but it is of interest that a flu strain isolated from this species on the Great Barrier Reef subsequently appeared in chickens in Hong Kong, in turkeys in California, and in wild ducks in Delaware.

Obviously maintaining the integrity of this site is of crucial importance to the success of any flu strain: if the virus can't attach to the cell, it can't penetrate and multiply and, in evolutionary terms, it is effectively dead; this is why so few changes occur in that area.

Neuraminidase — the second antigen

Antigenic variation is also observed in the other surface protein of the virus, the neuraminidase. Work similar to that described for the haemagglutinin is now in progress with Dr Air and Dr Janet Blok, at the John Curtin School of Medical Research, characterizing a variety of different influenza variants by sequencing the neuraminidase gene.

At the CSIRO Division of Protein Chemistry, Dr Ahmed Azad, Dr Tom Elleman, and Dr Ward are determining the nucleotide gene sequence of the neuraminidase from the first Asian variant that appeared in 1957 and the protein sequence of a later (1967) Asian strain. Dr Peter Colman, also with the Division, is determining the three-dimensional structure of the neuraminidase from both those strains using protein provided by Dr Laver. These studies will provide a full description of the structural changes associated with antigenic variation in the flu virus.

Targeting vaccines

Injecting people with killed or altered viruses, which are incapable of causing clinical symptoms, was a major advance in medical science. The body's response to this vaccination — through the production of antibodies — gives the individual lifelong protection against viruses such as those causing polio or smallpox.

But, as we have seen, the flu virus is constantly changing, staying one step ahead of the antibodies produced by the immune system.

Certainly, vaccines can be produced against strains of flu, and these were used extensively in the late 1960s after doctors realized how severe the new Hong Kong flu was. However, as the flu evolves, the vaccine becomes less and less effective, and producing a vaccine proves to be a very frustrating exercise: by the time the machinery for producing it has been set up, a new flu strain is likely to be circulating among the population.

Some researchers, including Professor Fazekas de St Groth, formerly of the Molecular and Cellular Biology Unit, have tried to anticipate the changes that may occur in the flu virus by growing strains in the presence of antibodies and selecting out the mutants that survive. Vaccines have then been developed against the mutant strains and some of these have been used successfully in Europe. However, this evolution on the lab bench doesn't always produce the same changes as those seen in the wild and, from the complex changes revealed by the sequencing data, it is easy to see why.

It may be possible to produce a flu vaccine that will confer lifelong protection against all strains.

The large number of amino acids involved in determining antigenicity, with changes occurring singly or in groups, means that the potential for change is so great that attempting to anticipate it is a fairly optimistic exercise that is unlikely to succeed. But all is not lost; it still may be possible to produce a truly effective flu vaccine — one that will confer lifelong protection against all strains of the flu virus. This will happen if vaccines that are active against the large stretches of the haemagglutinin that remain unchanged can be developed.

If this is the case, vaccines targeted against these sites, produced by genetic engineering or direct chemical synthesis, may provide lifelong protection against all the strains of flu we are ever likely to encounter. Even if this broad goal is not reached, since we know that a large part of the protein remains constant within sub-types such as the Hong Kong flu, a vaccine targeted against such sites could provide protection for the 7–20 years elapsing before the emergence of each new sub-type.

Scientists are currently exploring these possibilities. The first steps in the process — the cloning of the haemagglutinin gene and the production of haemagglutinin by genetically engineered bacteria plus chemical synthesis of parts of the molecule — have just been completed and, if developments follow the course outlined above, the flu virus may well be consigned to the history books.

Wayne Ralph

More about the topic

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