The Lewis Cameron Undergraduate Prize Essay

Epidemic Influenza: Its Causation and Prevention

Thomas M. Kennedy,
IMPORTANCE OF INFLUENZA.

Any account of scientific work should be prefaced by the reasons for which it has been done. This is a wise discipline for the most isolated and independent worker, for it keeps his purpose ever in his mind, but where research is costly and financed by the state, or by public organisations, it becomes the investigator's duty to state his object and pursue it constantly. This essay on causation and prevention of epidemic influenza must, therefore, start with an attempt to assess the status of the disease in the ailments of human society, and to prove that its importance is such as to justify the work aimed at its prevention.

The morbidity of epidemic influenza is high. Attack rates are not easily obtained except from such selected groups as schools and colleges, for, in general practice, doctors are usually only called upon to deal with complications of this normally self-limiting disease. Most epidemics, however, seem to have attack rates of 5 - 10% of the population. The typical case involves two or three days in bed, off work, and in addition several days of impaired efficiency at work, so a severe epidemic might easily cost Great Britain a million working days. On this count, it would seem a sound proposition to invest £1,000,000 in influenza research, if prevention of future epidemics could be guaranteed. In addition, epidemics cause great disruption of public and domestic life; schools, transport, factories and even hospitals are disorganised, and home life is often upset by the simultaneous illness of several members of a family, with great attendant misery.

The mortality of influenza is normally very low, and it is not an important cause of death; in 1936 - 37, for example, a fairly large epidemic caused 2% of all deaths in Great Britain. But it should be remembered that complicated cases of influenza may be
reported not as influenza but as, for example, bronchopneumonia or cardiac failure. The death rate from all causes is always higher in epidemic years than in the years between, the "extra" deaths being those of old people. It is probable that many of the old people dying from influenza in an epidemic would have died in the next three or four years, on the basis of life expectancy. The "years of life lost by death" are much fewer in influenza than in, say, coronary heart disease. But the aged population is always growing, and it becomes more and more important to protect old people from a disease which, though a passing inconvenience to younger generations, is to them potentially fatal.

Perhaps the greatest spur to research is the story of the 1918 - 19 pandemic. Here influenza suddenly developed high virulence and caused high mortality, particularly in young adults. In the three waves which passed round the world, the total death roll was at least 25,000,000. In European countries the death rate was about 5 per 1,000, but some non-European populations had higher rates. In some small communities the whole population was affected simultaneously, and nearly 50% died. The reason for this pandemic's severity is as yet obscure, and will never be known with certainty. So there is no reason known why a similar disaster should not break out any year: all influenza workers are concerned about this very real possibility.

Very often good medical research throws some light on established biological principles, or even reveals previously undiscovered ones. Influenza research has increased our understanding not only of the characteristics of influenza virus, but also of other subjects such as virus epidemiology, immunology, genetics and multiplication, and even of the biological role of ribonucleic acid (RNA). These are useful side-products of influenza research, but the primary aim must remain the prevention of epidemic influenza.
HISTORICAL SURVEY.

Influenza has waxed and waned in importance in recent centuries. A great epidemic in the 1840's was followed by a period of very little influenza which ended in 1889 with the 1889-90 pandemic. Since that time, there have been many epidemics and two pandemics (1918-19 and 1957), but the mean mortality-rate has gradually fallen, whether because of improved treatment and resistance, or because of a real decrease in the virulence of the disease. It has not, however, reached the low level which obtained before 1890.

Modern influenza research may be said to start in 1932 when Smith, Laidlaw and Andrewes produced "influenza" in ferrets by intranasal inoculation of the filtered throat washings of a human patient. The patient was, in fact, one of themselves - yet another bacteriologist victim of the disease he studied! The ferrets developed fever, nasal discharge and attacks of sneezing, and lost interest in their surroundings, but recovered in about ten days.

It was found also that convalescent serum could protect ferrets from contracting influenza, i.e. this serum contained antibody to the pathogenic organism, which was rightly thought to be a filter-passing virus.

But ferrets protected from this virus were not immune to virus from the next epidemic. It soon became clear that there were many different strains of influenza virus, and that immunity to one strain did not confer immunity to all. This offered an explanation of the short-lived immunity to the disease, each epidemic being caused by a slightly different strain.

Soon, many strains had been isolated and characterised immunologically, using ferrets, mice and hamsters for cross-immunity and protection tests. The strains diverged progressively from the original 1933 WS strain, which disappeared completely from the field, so that it was convenient to group them round a new strain PR8,
isolated in Puerto Rico in 1934.

In 1940, Francis discovered an influenza virus similar to those already reported, but sharing no antigens with them. This was called the B virus, and the strains already isolated were collectively termed the A virus group. The B virus was soon shown to consist of a similar group of antigenically related viruses. Its pathogenicity is similar, and it is responsible for a significant proportion of major epidemics, but it has not been studied as much as the more important A virus group.

Two important advances in laboratory technique followed in 1940 and 41, which made influenza research much less difficult and costly to perform, and so enabled much more work to be done. The first was the discovery by Burnet in 1940 that the virus can be grown fairly easily in the amniotic cavity of the 14 day chick embryo. It was later found that amnion-adapted virus can be grown in the allantoic cavity. This is an easier technique, and gives a rich yield.

The second advance was the discovery by Hirst and Hare independently, in 1941, that a suspension of virus can agglutinate fowl and mammalian red cells, the agglutination being inhibited by antisera to the specific strain of virus. These two new techniques are the basis of modern laboratory work, and without them much of our present knowledge would not have been gained.

Strains continued to change. New strains more distant from PR8 appeared and, in 1946, a new strain CAM, or A-prime (A') was recovered in Australia and its related strains held sway for some years. The "Asian 'flu" virus (FE) may be another such "master" strain.

A 'C' virus has been reported, but is of little epidemiological significance.
Since 1933, all aspects of influenza have received much attention, but, broadly speaking, advances have been made on two wide fronts:

(a) Field studies have been made of many epidemics. Workers have used the classical methods of epidemiology, investigating space and time relations of spread, incidence in different sections of the community, and other possible aetiological factors. Together with these investigations, serological studies of antibody constitution have been made in individuals and communities both during and between epidemics, in conjunction with analysis of the antigenic properties of the viruses present in epidemics.

(b) Side by side with this approach, there has been intensive study of the biological characteristics of the influenza viruses, made possible in 1940 by the discovery that the virus could grow in the developing chick embryo. Since then, attempts have been made to describe the virus in terms of physical dimensions, chemical composition, viability and genetic and other behaviour.

In both these fields many more or less significant observations have been made. It will be our task to determine which are most significant, and to attempt a synthesis between theories developed in immunological and biological fields.
A. Epidemiological and Immunological Observations.

Some degree of standardisation has been achieved in the investigation of epidemics. The establishment of Influenza Centres in many countries by WHO has done much to promote international communication between workers, and it is to be hoped that standardisation will soon enable scientists all over the world to compare and interpret each other's results. The problem of influenza will not be solved by one country alone - the virus knows no frontiers.

It is usual when an epidemic comes under observation:

(a) To isolate and characterise the virus strain or strains of the epidemic.

(b) To assess the population's immunity before and after the epidemic.

Isolation of the Virus.

The virus is usually isolated from a patient in the acute phase of the disease. He gargles with 20 mls. of saline and the mouth washings are filtered and treated with antibiotics to kill contaminating bacteria. 0.2 ml. are injected into the amniotic cavity of a fertile hen egg that has been incubated at 38 - 39 °C for 11 days. The eggs are incubated at 36 °C for 48 hours, when the virus is harvested by drawing off the amniotic fluid. If this fluid agglutinates fowl cells, further passage may be allantoic inoculation: if not, the process of amniotic inoculation has to be repeated. The virus is titrated against reference sera by haemagglutination-inhibition tests; thus its antigenic composition is determined. For preservation, virus suspensions survive indefinitely at the temperature of dry ice (-80 °C).
Haemagglutination-Inhibition Test.

Saline suspensions of influenza virus have the property of agglutinating the red cells of certain species: those most commonly used are fowl cells and human O group cells. This agglutination can be observed naked-eye in test-tube experiments. In the absence of virus, the cells in a saline suspension settle at the bottom of the tube, forming a "button". If active virus is present, the cells form a "shield" covering all the curved surface of the bottom of the tube:

- no agglutination
- agglutination

By serial dilution, the smallest amount of virus giving haemagglutination under certain defined conditions can be determined. This is called a haemagglutination unit (H.U.

In the presence of specific antibody to the virus, agglutination does not take place, because the antibody coats the virus particle and prevents it from adhering to the red cells and joining them together. Some mucoprotein constituents of human serum also inhibit haemagglutination, but they can be prevented from acting by adding *Vibrio cholerae* filtrate which contains an enzyme, R.D.E. (receptor-destroying enzyme). RDE is said to block some of the receptors on the red cell which
the virus attacks: it is difficult to see how it prevents non-specific but allows specific (antibody) inhibition.

The principle of haemagglutination-inhibition may be applied in two ways. Given standard virus strains, the antibody pattern of sera can be evaluated: given a set of standard antisera - produced by infection of experimental animals - freshly isolated strains of virus can be typed. Antibody levels are measured in H.I. (Haemagglutination-Inhibition) units. As most people have had several attacks of influenza and consequently carry some antibody in their sera, diagnosis of influenza by this method depends on the demonstration of a rising titre. A fourfold rise in HI titre between the acute phase and convalescence is diagnostic of infection with the virus under consideration.

Two forms of complement-fixation test are also used. One uses the infective particle as antigen, and is said to be more specific than the haemagglutination-inhibition test. The other is used to distinguish between infections with A & B viruses. The antigen is a group-specific soluble antigen known as CFA (complement-fixing antigen), prepared from an emulsion of infected cells. For most purposes the haemagglutination-inhibition test is satisfactory, and, being a simpler procedure, it is most widely used.

It is by these means, as well as by orthodox epidemiological methods that influenza is now being studied. We will now consider some of the important features of the disease that have been revealed in 25 years of modern influenza research.

It is common knowledge that one can "catch 'flu" by contact with a person who has the disease. Even before it was proved that influenza is caused by a microorganism, epidemiologists
had assumed that this was so, and that transmission from case to case was by droplet spray. This approach is useful on the family level or when the spread through districts, or even countries, is being studied. But when it is applied to the epidemiology of continents, difficulties are encountered which make it necessary to postulate that many epidemics are preceded by a widespread infection with the virus in a non-pathogenic form which is later enabled to cause disease by some alteration in the host population.

A hint that the virus might exist in a latent form was given by the natural history of the disease known as "hog 'flu", a virus disease of pigs. The disease first appeared in pigs on show at the Iowa State Fair in 1918, at the same time as the 1918-19 pandemic was affecting America. It had the same kind of clinical features as the human disease, and was naturally thought to have some connection with it: later findings suggested that the viruses of hog 'flu and the pandemic were closely related, for the sera of those who had experienced the pandemic contained antibodies to the hog 'flu virus. This view is supported by the discovery, in connection with a 1937 epidemic, that pigs fed on garbage developed evidence of infection with virus of the current human type.

The disease has been extensively investigated by Dr. Richard Shope, and his findings are of great interest. The virus is carried by earthworms, and pigs are infected by eating these worms. But infection with the virus does not automatically cause hog 'flu: on the contrary, most pigs in the endemic area carry the virus without showing any sign of disease. It has been shown that some kind of stress must be imposed upon them before they show clinical signs. Hog 'flu has been produced experimentally by injection of killed
H. influenzae suis bacilli, and the disease has been hailed as being caused by synergistic action of this bacillus and the virus, synergism, I suppose, implying some fundamental relationship. But under natural conditions the disease has followed other, non-specific, stresses. Simultaneous outbreaks may occur at widely scattered isolated farms following a single very cold night. Outbreaks have occurred when a pigsty door has been left open causing a draught which chilled the inmates. So it seems as if non-specific stresses may also precipitate the disease.

Hog 'flu is important because it shows that:

(i) There is a precedent for the existence in a population of a latent influenza virus.
(ii) "Stress" might be an important factor in 'unmasking' such a latent virus.
(iii) The possibility of an animal reservoir is not to be lightly passed over.

In the epidemiology of human influenza, there is much support for the theory that the virus can remain latent in a community for quite long periods. Høygaaard5 has described an epidemic at Angmagssalik in Greenland in which infection had seemingly been introduced two months before the actual outbreak of the disease. In Denmark and Sweden in Summer 1950, there were local outbreaks of influenza, which soon died away, without causing an epidemic. But in October the virus reappeared and produced a considerable epidemic affecting most of North-West Europe. Between the Summer "flurry" and the Winter epidemic, the virus must have remained latent in the communities of these two countries at least. The small outbreaks of influenza in the Winters between epidemics may be examples of the same principle. Sometimes, too, epidemic influenza breaks out almost simultaneously in several communities, so that case-to-case spread is very unlikely: and again, when epidemics seem to spread, it
is often not by travel routes but regardless of them, and across geographical obstacles to travel, that it seems to progress. These peculiarities could be explained by the theory of a latent virus widely seeded in the population, activated by a factor which affects distant places simultaneously and affects countries (geographically) rather than their populations. This factor would obviously be weather - probably cold, wet weather: this is in line with the accepted concept of influenza as a Winter disease. Influenza epidemic spread, then, might sometimes be shown better on a weather chart than on a road map! There is no doubt, however, that, when families rather than countries are concerned, case to case transmission is the only important means of spread.

Some epidemics, however, seem to spread simply from case to case, and as an extension of this, along lines of human communication, showing none of the features which we have associated with a preceding latent infection. The march of such epidemics is orderly, and there are few or no anomalies of spread: they are not confined to Winter months, but sometimes spread through the Summer.

These epidemics have a higher attack rate than usual: and when the virus strains of the epidemics are typed antigenically they are found to be markedly different from the strains of previous epidemics, much more different from them than they are from each other. This suggests to us that antigenic novelty is a factor which influences the pathogenicity of a new strain, and that the resistance of a population to a new strain depends on the types of strain previously experienced. There is a great deal of evidence for this theory, a fundamental one to the epidemiology of influenza.
First, there is, as we have seen, the extreme pathogenicity of antigenically novel viruses: the best example here is supplied by the recent Asian pandemic. The virus (A/Asian/57, otherwise 'FE') was first noted in Spring 1957 in China, and during the next six or eight months it spread through Asia, Europe and all the world, causing a high rate of morbidity in all the populations it encountered. This virus was found to be of very individual antigenic composition, being as far removed from the current A' strains as A' is from the original WS strain. The epidemic spread actively throughout the Summer: presumably it was pathogenic enough to overcome the higher resistance encountered in Summer and thus did not need to "go underground" until Winter.

Again, studies of epidemics show that it is almost unknown for a widespread epidemic to be followed by another with the same causal virus: there is nearly always some antigenic variation from epidemic to epidemic. It seems as if variation might be a necessity if new epidemics are to occur: certainly, the epidemiology of the disease would be much changed if the virus ceased to show such variation. We can get some idea of the possible effects from the epidemiology of small isolated communities. It is characteristic of such insulated communities that as long as they remain apart from the world they are free from infectious diseases. Tristan da Cunha in the South Atlantic has a community of about 180, who are by no means well nourished, have bad sanitation and endure cold, wet weather, but are extremely healthy. They are free from colds but only as long as they remain isolated: when a boat calls there is usually a severe outbreak of colds, and the islanders are then cold-free until the next boat comes.

The same seems to be true of influenza. The disease seems to disappear completely in the absence of infection from the outside. When such infection is introduced, the consequent
epidemic shows a high attack rate. This is well demonstrated by the story of the Ocean Island epidemics.

It had been noticed that epidemics of influenza often followed the introduction of Chinese coolies into the labour force of this small Pacific island. Arrangements were made to study the events following the arrival on 3rd October, 1948, of a ship from Hong Kong with 803 labourers aboard. The voyage had lasted 3 weeks: there had been some coughing among the men, but no clinical influenza. These men were observed in quarantine for 24 hours and then allowed to mix with the local population, about 400 Polynesians from the Gilbert and Ellice Islands. Ten days later the first case of influenza was seen: in the next 14 days, 271 cases were reported. Of these, 259 were Polynesians - 60% of all the Polynesians. 12 were Europeans, of a possible 90, i.e. 13%. There was not one case among the Chinese. A virus was isolated, and Chinese sera were shown to have high titres of antibody against it. The Chinese were obviously subclinical carriers of the virus.

This piece of epidemiology illustrates the high attack rate associated with the introduction of a virus which is new to the population. It also leads us to consider another principle, that immunity to influenza is determined not only by the types of virus previously encountered, but also by the amount of infection to which a community has been exposed - if there has been little influenza in previous years, an epidemic may be expected to have a high attack rate. The 1890-91 pandemic, for example, broke out on a world which had almost forgotten influenza, and its high attack rate and severity may have been caused by this lack of previous experience of the disease.

Immunological studies suggest that the immunity of a population can be assessed by the antibody levels of the blood of its members. Davenport, Stuart-Harris and Hennessey, in 1953,
compared sera and viruses from Michigan, U.S.A. with those from Sheffield, England. They found that the same strains were prevalent at the same time on both sides of the Atlantic, but the levels of antibody to these strains were higher in America than in Britain. The British population had a higher attack rate than the American, and it was suggested that this relative lack of antibody had been a contributory cause. Where not populations but individuals are concerned, there is a strong but not absolute correlation between antibody levels against the epidemic virus and immunity in epidemics.

Antibody levels are high immediately after epidemics, and fall off over a few years to quite low levels. Burnet believes that immunity to one virus strain would take about six years to run down, and that after that time the same virus might cause another epidemic. But there is a record of the same strain causing a second outbreak in a school after two years. This community, however, was perhaps more susceptible than the community at large.

This decline in immunity has been incorporated into theories to account for the cyclic recurrence of epidemics. Constant antigenic variation is a fundamental property of the virus, and it is supposed that every three or four years the population's immunity is low enough to permit a variant strain to cause an epidemic. This undoubtedly represents part of the truth, but some other factors, which we will discuss in relation to the properties of the virus and the initiation of clinical disease, must also be considered.
B. Biological Characteristics of the Influenza Virus.

1). Physical Dimensions.

The infective particle has been photographed under the electron microscope, on laked red cells. Two forms have been observed. The commoner is a spherical form, of diameter 80 - 120 mu. The other is a filamentous form of the same diameter, but of considerable length, so that it can be observed under the light microscope. The spheres seem to have a "nucleus" but the filaments on section have no detectable internal structure.

There is also a "soluble antigen" (CFA) - a small particle of diameter 10 mu. It is not infective, does not agglutinate red cells, and can be detected by a complement-fixation test.

2). Chemical Composition.

Ada & Perry\textsuperscript{3} have found the composition of virus particles to be much the same as that of animal protoplasm. In terms of dry weight, it is :-

<table>
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<tr>
<th>Component</th>
<th>Percentage</th>
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<tbody>
<tr>
<td>Lipid</td>
<td>20 - 30%</td>
</tr>
<tr>
<td>Protein</td>
<td>60%</td>
</tr>
<tr>
<td>CHO</td>
<td>10%</td>
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<tr>
<td>Ribonucleic acid (RNA)</td>
<td>0.8%</td>
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The protein has been shown immunologically to be partly derived from the host cell.

3). Multiplication.

Most of the work on influenza virus multiplication and genetics has been done at the Hall Institute of Medical Research, Melbourne, under the direction of Sir Macfarlane...
Burnet, so this account will be largely based on his monograph of 1956, to which an essay of this size cannot do justice.

When a virus particle infects a cell in the allantoic membrane, and presumably in the respiratory epithelium, it attaches itself to the cell membrane in the same way as it adheres to red cells in the H.I. test; an enzyme, mucinase, present in the outer coat of the infective particle, attacks receptor sites on the mucoprotein and mucopolysaccharide meshwork of the cell membrane. The particle then enters the cell cytoplasm by dissolving the membrane on which it has alighted.

After infection, no virus particles can be seen inside or outside the cell until new particles begin to be liberated 3 or 4 hours later. The entry of the virus into the cell must start some process which results in the release of a new generation of particles after that time, and the nature of this process inside the cell is at present the subject of much conjecture and research.

Burnet argues strongly that the RNA of the virus is the genetic material ("genome") responsible for replication of protein specific to the virus - antigens, and other reactive groups including enzymes. He suggests that the RNA is carried on the CFA. Key findings in this connection are:

(i) CFA contains RNA. The CFA of virus A differs from that of virus B, and the RNA of A virus differs from B virus in adenine : uracil ratio.

(ii) Staining for RNA and DNA in infected cells shows that RNA accumulates in large quantities in the cytoplasm; but
there are no changes in DNA staining, and virus multiplication is not affected by agents which destroy DNA, such as nitrogen mustard and heavy X-radiation.

(iii) CFA is detectable in infected cells before any haemagglutinating or infective activity can be demonstrated.

(iv) When a cell is infected by several particles at once, new particles are formed which, though they have an outer coat and can agglutinate red cells by means of the enzymes on it, cannot successfully infect other cells. They contain less RNA than a normal infective particle. These deficient particles are termed incomplete.

We can imagine a multiplying pool in the infected cell, in which fresh RNA and specific protein is being synthesised for the new generation of virus particles. It is in line with other biological findings to regard RNA as a template for protein synthesis. The CFA, in Burnet's view, is a protein - RNA complex which can, under different conditions, synthesise either RNA or specific protein. Multiple infection might cause more surface protein than CFA to be synthesised.

The specific protein molecules somehow collect at the cell membrane and are linked together to form the outer coat of new infective particles, when these are released. Sections of infected cells have been cut which on electron microscopy show tiny evaginations of the cell membrane, never present in
normal cells, which probably represent new virus particles being budded off. If this is so, then it is obvious that some host cytoplasm must be caught up in the infective particle, and this offers an explanation for the presence of host protein in the infective particle. For the particle to be infective, it has to be assumed that some CFA, the RNA-containing genetic material, must be incorporated into it, but this process, as we have seen, may fail, resulting in the release of an empty shell of a virus particle. The filamentous form does not appear to have a "nucleus" and it is tempting to regard it as an incomplete particle, but further investigation is necessary before this can be proved or disproved.

We can now try to visualise the structure of the virus particle. Burnet has suggested a structure of this nature:

![Diagram of virus particle]

- virus-specific surface protein including antigens and mucinase.
- host lipid?
- casually accumulated host cytoplasm, containing a "nucleus" of CFA protein-RNA units.

We are led to think that the CFA is the 'real' virus, actively reproducing itself inside the infected cell, and that the infective particle represents a "resting phase", a capsule in which several CFA's are conveyed from cell to cell, whose only purpose is to attack the surface of new cells to be infected. It is perhaps unlikely that antigens as such confer any advantage on the virus: more probably antibody stimulation is the price to be paid for the use of protein
as a structural material. Faced with this handicap, the 
virus is forced to change its surface protein regularly.

4). Mutation and Recombination.

The virus was found in the field to be very labile. In the laboratory, too, it rapidly changes its serological and other characteristics. The commonest change is the 0 – D change, by which new virus strains are "tamed" for the laboratory. The original "wild" strain (0) is pathogenic to man and does not grow in the allantoic cavity or agglutinate fowl red cells. After one or two passages in the amniotic cavity, there is a change to a D strain which is not pathogenic to man, clumps fowl cells, and grows readily in the allantoic cavity.

It is important to know whether such changes are "Lamarckian" responses to environmental change or to a genetic change resulting from a replacement of the O population by descendants of a mutant, D, which has survival advantage over it. The matter has been settled by passaging O virus fluid at so high dilution that the inoculum contains only one infective particle (ID50). When this is done the virus can be passaged in the O form for as many as 24 passages. This procedure prevents the small proportion of D mutants from exercising their survival advantage in subsequent passages, as they do under normal conditions.

Many examples of mutation in the laboratory could be quoted. The virus's extreme plasticity is shown by the fact that virus passed in two lines through mice gave rise to strains antigenically different from each other and from the parent strain. It is interesting that antigenic mutation increases in rate in the presence of antibodies against the strain.
Antigenic mutations are not the only ones. Mutations occur in qualities unconnected with antigenic structure: of these, we are particularly interested in virulence. The best-known example of a mutation in virulence was the appearance (in Stuart-Harris's Laboratory) of a variant of WS which was neurotropic to mice and caused in them a kind of meningitis. A similar strain was isolated from an epidemic in 1951 by Wilson Smith. We must not rule out the possibility of a virus neurotropic to man. The peculiarities of some epidemics, i.e. the lethal nature of the 1918 virus, and perhaps the Liverpool strain in 1949 - 50 may be due to such changes in virulence.

Another type of genetic change is recombination. It has been shown by Burnet and Lind that if cells are infected by two different strains in delicately adjusted proportions, recombinant strains occur, which inherit some of the qualities of both parent strains. "Antigenic" recombinants are unstable, perhaps because of defects in their "patchwork" surface coat, but recombinants with non-antigenic qualities such as virulence are quite stable and can be passaged many times. This may represent another type of variation by the virus in the field, but I think that plain mutation is much more likely, and is the only method deserving serious attention.

5). P - Q - R Variation. This is another property of the influenza virus which may be relevant to our object. Viruses isolated during epidemics are usually active and readily neutralised by homologous antiserum: they are said to be in the P phase. In the laboratory they can be made to change their character by passage in eggs in presence of gradually increasing amounts of homologous antiserum: the virus changes to the
Q phase in which it is poorly inhibited even by homologous antiserum. An R phase is also described which reacts readily with antisera to itself and to P & Q phases:

<table>
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<tr>
<th>Virus</th>
<th>Inhibition by Immune Serum</th>
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<tr>
<td></td>
<td>anti-P</td>
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<tr>
<td>P</td>
<td>++++</td>
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<tr>
<td>Q</td>
<td>+</td>
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<td>R</td>
<td>+++++</td>
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Table showing "avidities" of P, Q & R phases

It has been suggested that the P & Q forms have the same antigenic composition but that in the Q phase the antigenic groups are not on the surface of the virus particle. Such a configuration would be of advantage to a virus in a host which has formed antibody against it, and this might be the form adopted by latent viruses. In the 1950 - 51 Scandinavian outbreak the earliest strains isolated were in the Q phase, and the P phase virus only appeared later.

Let us now consider the possibilities when one human is exposed to the influenza virus. The only significant way of infection is by droplet spray. Little seems to be known about the viability of virus in droplets; the only relevant fact I have found is that mouth-washings at ordinary temperatures lose their infectivity to chick-embryos rapidly, over a few hours. Some mucinous substances present in respiratory tract secretions inactivate the virus in vitro, and they may, if present in droplets, help to reduce the viability of virus in droplet spray. This suggests that fairly close contact with a
carrier would be required to produce infection, as in crowded public transport, factories and offices or in visiting or nursing a case.

The inhaled virus particles come to rest on the layer of mucus which covers the pharyngeal and respiratory mucosa. This mucus has virostatic properties: another important action is in mechanically preventing the virus from reaching its target cells in the upper respiratory tract and in this it is aided by ciliary action which moves the mucus towards the mouth, away from the vulnerable cells.

It is probably the extent to which the epithelial cells are infected that determines the severity of the attack. Here we are concerned with two factors:

1). The amount of virus reaching the epithelial cells. This is, in effect, the dose inhaled minus the amount of virus inactivated by mucus and/or "cleared" by ciliary action. The dose inhaled is determined by the degree of contact with clinical or subclinical cases: the "respiratory resistance" is probably influenced by many factors such as weather — chilling depresses ciliary activity, autonomic tone, general health and nutrition, and the health or diseased state of the respiratory tract.

2). The immunological resistance to the strain of virus. This depends on:

(a) The amount of previous infection with influenza virus, and in particular the amount of recent infection.
(b) The relation of strains which have caused such previous infection to the present virus.

It has been shown that the response to a vaccine of any strain is a production of antibody not only against that strain but also against all the strains the subject has encountered, particularly the earliest strains encountered in childhood. Perhaps antibody to all these strains is present in small quantities in the plasma, and is reinforced on any infection. If Mulder\(^6\) is correct, people aged 70 and more were able to combat the FE virus of 1957 with antibody against a similar virus which caused the 1890 - 91 pandemic.

The best immunity is conferred by a recent infection with the same strain. At the other extreme, a B virus infection offers no protection at all against an A virus. Among the A viruses there is cross-immunity to an extent determined by the number of antigens common to both strains.

In experimental animals the virus has been shown to stick to the cells of the epithelial lining of the respiratory tract just as in the allantoic cavity\(^1\)\(^7\). The cytopathogenic effect in the first cells infected probably causes dilation of the mucosal vessels, allowing the formation of an exudate: the cells are thus bathed in a fluid containing antibodies. There is no record of virus being cultured from the blood, so the battle is probably confined to the respiratory epithelium. The lymphatic system is probably involved in antibody formation: the cervical glands are often enlarged.\(^1\)

It has been calculated from egg-inoculation work that 100 - 1,000 infective particles may be liberated from an infected cell; from this we may guess that the disease is in the balance after the first one or two generations of virus - a defence which
cannot deal with a thousand infective particles is unlikely to defeat a million. It is at this point too that the virus probably reaps the benefit of its capacity for antigenic variation - Burnet\textsuperscript{15} has pointed out that antigenic plasticity is much more advantageous to organisms like the influenza virus, whose contact with the bloodstream, and the antibodies it contains, is indirect and slight than to an organism which multiplies in the blood: a minor antigenic modification in the first case may be enough to overcome the few antibody molecules likely to be encountered, but would not confer any advantage on a microbe which has to meet the full force of the antibodies in the blood.

The virulence of the virus, as distinct, perhaps, from its antigenic composition, is another factor to be considered. A virus with increased capacity to kill cells when it multiplies in them, and thus to intoxicate the host's metabolism and lower his resistance, is at an advantage.

The outcome of the interaction of all these factors may be:

(i) Elimination of the virus by what I have called "respiratory resistance".

(ii) A mild "immunising infection", with a rise in antibody titre.

(iii) A clinical case of influenza.

(iv) The virus may, perhaps, be carried in a latent form. Enough is not known about carriers. What kind of people, and what percentage, carry the virus? How long do they carry it? In what form (P or Q) is the virus carried? These are important questions which should be faced.
We can now speculate on the way in which an epidemic is produced. If we exclude the possibility of animal vectors we can accept as fundamental that whatever may be the contributory causes, the disease is ultimately caused by a virus which, as far as we know, only multiplies in the cells of the human respiratory tract. It follows that the only means of spread of the germ is by droplet infection from one human to another, by fairly close contact. The possibility of latent infection has already been accepted. An epidemic or pandemic must therefore start with the transformation of latent infections to overt disease. Factors which might precipitate this "break-through" are:

(i) Antigenic mutation of the virus.

(ii) Mutation (or recombination) increasing the virulence of the virus, with or without antigenic change.

(iii) Lowered general resistance of the host, especially "respiratory resistance".

(iv) Lowered immunological resistance of the host.

An eruption might be caused by one of these factors alone, but it is more likely to be caused by the combined action of two, three or all four. With present knowledge their importance relative to each other can be only roughly assessed.

Once a "break-through" has occurred, a fifth factor comes into operation, the amount of virus-laden droplets available to the population. In a community of low general and immunological resistance, a person receiving a large dose of virus from a case of influenza is likely to succumb: he in his turn will add to the amount of virus that others will inhale, enabling the overt disease to maintain itself and increase its rate and extent of spread. It is easy to see how the disease can be aided in this
by crowding in public places, and by the refusal of "walking cases" to go to bed.

1). **Antigenic change.**

It seems as if a large antigenic change may allow the virus to spread through populations of fairly high resistance, as in the recent "Asian 'flu" pandemic. But a slightly altered virus may require the population's resistance to be lowered for it to "break through". Thus the Asian virus was able to cause influenza all through the Summer of 1957, but normal epidemics, caused by only slight variants, require a population of low resistance and usually occur in Winter. An intermediate type of epidemic can be distinguished where the disease only just breaks through in Summer and causes a small outbreak. Going "underground" again, it waits for Winter, when it produces the epidemic proper.

2). **Increased virulence.**

This factor, by increasing the cytopathogenic effect in the respiratory tract, could lower resistance and precipitate clinical influenza. It is not known how important virulence changes are, but they should not be ignored.

3). **Lowered general resistance**

Here, weather is most important, though nutritional deficiencies might play a part. It is significant that most epidemics occur in January and February in the Northern hemisphere.

4). **Lowered immunological resistance**

The fairly regular incidence of epidemics in time - every three or four years - suggests a
cyclic rise and fall in the herd immunity. Immunity may fall gradually after one epidemic until it is low enough for an antigenic mutant to break through and cause another one.

To sum up, we may represent the genesis of influenza epidemics graphically, thus:

Epidemics and Pandemics.

There is no fundamental difference between influenza epidemics and pandemics. When influenza is studied on a world-wide scale, it is realised that the virus of one epidemic here may cause other epidemics in the Southern hemisphere in six months' time and may break out again in North America next Winter. Epidemics are, in fact, episodes in a slowly moving pandemic whose spread is not obvious because it is slower than that of a 'real' pandemic and because it involves dissemination of a latent virus. Any strain of virus will spread as far as possible in either a latent or an overt form, until it can find no more populations whose resistance to it is low.
PREVENTION OF EPIDEMIC INFLUENZA.

Much of what will be said about prevention of epidemics follows logically from our consideration of their causes, which we have listed as:

(i) Antigenic mutation of the virus.
(ii) Mutation increasing virulence.
(iii) Exposure to droplet spray.
(iv) Lowered general resistance and "respiratory resistance" of the population.
(v) Lowered immunological resistance.

Mutation is a fundamental property of the virus, and the only way of preventing it would be to exterminate the virus. We have as yet no inkling as to how that could be done. Even if it were possible to kill the virus in human throats, it might be dangerous to attempt its total extermination: such an attempt would almost certainly fail, and in a few years a population with no immunological resistance might be exposed to a highly virulent virus, and the resulting pandemic might rival or surpass the 1918-19 one in severity. It would be much better to learn to live with the virus than to have this happen.

No known antibiotic can kill the virus or even prevent its multiplication. This would probably require an agent which can interfere with the RNA metabolism of the virus while leaving the host cell's RNA unharmed. It is unlikely that such an agent will be produced by scientific research for a long time yet, and probably just as unlikely that such an agent will "turn up" as some antibiotics have done.
It is possible, however, to reduce the amount of virus-containing droplet spray present in the population. This is largely a problem in public health. The amount of air infection is increased by crowding together of clinical and subclinical carriers. This occurs in such situations as public transport, offices, factories, large stores, dance halls, cinemas and sporting events. The public should be educated to avoid such situations during epidemics, and in particular to "give in" easily to the attack of 'flu. Public opinion should be turned against people who turn up for work with streaming eyes and running noses, incapable of carrying out their work, but highly capable of increasing infection in others. Education in the use of handkerchiefs might also help. "Smog masks" might be useful, but a great deal of persuasion would be needed before they were accepted by the public. Such public education should be effected with well-designed posters and advertisements, and by television broadcasts. All general practitioners should help in this education.

This problem should also be tackled by encouraging the use of adequate well-designed ventilation in all public buildings.

Clinical cases should be nursed in a room apart from the rest of the family, and visited only by one person.

Increasing General Resistance.

Several measures are desirable for the prevention of disease in general and respiratory disease in particular, and can play a part in the prevention of influenza. They are :-

(i) Intake of a well-balanced diet.
(ii) Wearing suitable clothing.18
(iii) Suitable heating and ventilation.
(iv) Prevention of atmospheric pollution.
(v) Proper physical exercise.
Increasing "Respiratory Resistance".

The factors listed in the preceding paragraph also have a bearing on "respiratory resistance". Some other means of increasing resistance to the entry of the germ have been sought. Burnet & Stone have shown that RDE can remove receptors from normally susceptible cells in the chick embryo and the mouse, and prevent them from developing signs of disease following infection. But this immunity is only temporary, for the receptors are replaced by regeneration within 48 hours, and the animal is again susceptible. The mucopolysaccharide and mucoprotein inhibitors present in human respiratory secretion have also been studied in the hope of finding some particularly active substance or chemical grouping which will inactivate the virus by preventing it from attacking cells.

Increasing Immunological Resistance.

This is done by administration of antigens to increase the immunity of individuals. What form of antigen should be used? The first choice is between living and dead virus. Living attenuated virus has been used in Russia but not in this country. Promising results have been obtained by spraying this virus into the nasal passages. Its advantage is probably that it gives higher antibody titres than killed virus: it will cause the formation of antibody to CFA, unlike killed virus. Most Western workers use virus from infected eggs, purified, concentrated and inactivated with formalin. They are injected subcutaneously.

The use of killed virus means that in effect only surface antigens are administered - no antibody to CFA is formed. Yet CFA is common to all group A viruses, and an immunity based on it would obviously be desirable. CFA is not on the surface of the virus particle, so antibody against it in the blood would probably be ineffective. But it might be possible to make the immunity a property of the cells in which CFA is active, the epithelial cells of the airway. The immunology of tuberculosis and phenomena such
as allergy and anaphylaxis suggest that immunity can be a cellular as well as a humoral property. Could a cellular immunity be produced in this case? Some indication might be obtained by seeing if a first infection of human respiratory tract cells in tissue culture confers any immunity to a second infection with the same strain. If this were the case it might be worth while attempting to immunise animals against influenza by giving CFA intranasally.

Surface antigens have been adopted for use, and the problem is which strain or strains to use for the vaccine. American workers have favoured a polyvalent vaccine containing several important strains. New strains are incorporated into the mixture as soon as they are isolated. This policy was based on the hypothesis that the virus had a limited number (about 18) of antigens, each of which was in turn quantitatively exalted over the others, variation in this view being a ringing of the changes of a definite number of antigens.

The British view is that a vaccine to be effective must be prepared from the strain against which it is to be used. Its protagonists point to the fact that most of the old strains are disappearing - WS and PR8 have gone, and CAM and FMI seem also to be on the way out: it is therefore useless to prepare a vaccine from them. This view, that old strains will never reappear, has recently been challenged by Mulder's suggestion that the FE strain of Asian pandemic may be related to the strain that caused the 1890 - 91 pandemic, which also started in the Far East. He found that the sera of people of 70 years or more could neutralise FE virus in H.I. tests. An objection has been raised that this neutralisation is non-specific and results from the antibodies to antigens which are common to the Asian strain and the many strains that these old people have encountered. But if this were so, one would see a gradual increase with age of the percentage of people whose sera can inhibit
the FE virus, instead of the sharp rise reported by Mulder. In fact when antibody production is stimulated by a polyvalent A vaccine, many sera do come to inhibit it, but the age distribution is different from that obtained by Mulder from straightforward samples of serum, as can be seen by comparing these graphs constructed from Mulder's figures.

![Graphs showing age distribution of HI positive sera](image)

(a) From casual sampling  
(b) After stimulation with a polyvalent A vaccine

It seems that Mulder's suggestion might be right, and we cannot rule out the possibility of reappearance of old strains. It might be worth while to investigate influenza trends in China, for the virus might be latent in a reservoir (perhaps animal?) in that country.

Results from field trials with vaccines, however, indicate that in practice an ad hoc vaccine is preferable to a polyvalent vaccine which does not contain the strain of the epidemic. Vaccines against PR8 were effective between 1943 and 1945 in America, but the same vaccines were completely ineffective in 1947 when FM1 was appearing on the scene. In field trials in Autumn 1957, it was found that vaccines prepared with FE virus were effective against the Asian pandemic, giving a protection rate of about 60%, but that polyvalent A virus and B virus vaccines gave no protection.
Against this, it may be said that the FE virus is far removed from previous strains: perhaps a vaccine might protect against minor variants of the strain from which it was prepared. There may be a chance to test this if the FE-strain produces a minor variant: but experiences with vaccination in America,\(^5\) where many field trials have been carried out, suggest that the vaccine at present in use will offer little protection against any such variant.

If vaccination is to be carried out on a large scale, it is important to know how the killed virus can be most economically used to attain immunity. If we accept antibody level as a criterion of immunity, it has been shown that two injections of vaccine separated by 3 weeks give much higher immunity than one injection.\(^2^1\) A single injection of a vaccine, of strength 14,000 HU, gave titres averaging 9.8 HI units. When an injection of 7,000 HU was repeated after 3 weeks, an average titre of 56.5 HI units was attained. This "booster" method has not in fact been given a field trial in an epidemic, but it will certainly be much better than the single-injection method, and should be used, when time permits.

One of the drawbacks of early vaccines was the production of some severe allergic reactions to the egg protein in the injection. Though this has been largely overcome by concentrating the virus on to aluminium phosphate, vaccine should still be given with care to allergic individuals.

To increase the antibody response to a vaccine, adjuvants are used. Salk\(^5\) has experimented with vaccines of virus emulsified in Arlacel A, a lipid compound, and has found that by this method only 5 - 10% of the virus used for saline vaccines is necessary to produce the same levels of antibody. Severe "chemical abscesses" were at first sometimes encountered, but this hazard has now been overcome.
It seems, then, that it will soon be possible to produce a cheap, safe and effective virus - given time. Several weeks must elapse between the isolation of a new strain and the mass-production of a vaccine, and in a fast-spreading pandemic this delay can ill be afforded. To minimise it, it is necessary to maintain:-

(i) An efficient international organisation to spot new strains as soon as possible and assess their significance. This is done by the World Influenza Centre of the WHO.

(ii) A vaccine-producing plant with trained workers ready to mass-produce a new vaccine at short notice. The maintenance of such a plant would be too expensive for a private firm, and it would have to be subsidised. It would be advantageous to have international co-operation in vaccine production under the guidance of the World Influenza Centre.

It is not yet possible to vaccinate more than a fraction of the population against a new strain. The policy adopted has been to protect debilitated and elderly people, particularly those with chronic respiratory disease, and key personnel - doctors and nurses to tend the sick, and those people essential for the running of public services and the basic industries.

Will it ever be possible, by efficient quarantine and mass vaccination with a quickly prepared vaccine to stop an epidemic in its early stages? Or would the virus become latent, soon to reappear in other, unprotected populations? We cannot yet tell; but, by the measures which have been indicated, the worst effects of epidemic influenza, high death rates and civic disruption, can be prevented.
Epidemic influenza is important because:

1). It is the cause of much civic and domestic disruption, and a source of economic loss to the country.

2). It carries a risk of death, particularly in old people.

3). An epidemic as severe as the 1918 - 19 pandemic, might, as far as we know, occur any year.

It is therefore justifiable to try to find a way to prevent it.

An epidemic occurs when a population's combined general and immunological resistance is overcome by the infectivity and virulence of the influenza virus. The antigenic variation of the virus is a very important factor in bringing this about.

An epidemic can be limited by:

1). Steps to prevent spread of infection in the community; in particular the early isolation of cases.

2). A high general resistance, and good respiratory health, in the population.

3). Vaccination with an up-to-date vaccine, prepared from the virus causing the attack, or one very closely allied to it.

It is not yet possible, and perhaps never will be possible, to prevent an epidemic from spreading, but by these measures its worst effects, civic disruption and the deaths from influenza, can be prevented.
IMPORTANT REFERENCES

Where original papers were not available or were not consulted, references to them will be found in the works quoted:--

5. WHO (1954) 'Influenza: A Review of Current Research'. By various authors.