Viruses are heredity exemplified—at their simplest they consist of a single nucleic acid molecule, comprising as few as three genes, protected by a protein shell which is specified by part of the enclosed nucleic acid. It is not surprising, therefore, that viruses have been favoured tools for elucidating the molecular basis of heredity. Indeed, the great edifice of molecular biology, which is one of the major intellectual achievements of this century, has been very largely built on studies of a few bacterial viruses and their host, the familiar bacterium *Escherichia coli*.

The power of bacteriophage research lies to a large extent in the ease of manipulation of bacteria and our detailed knowledge of the genetics, physiology, and biochemistry of the standard bacterial host, *E. coli*. However, I shall discuss animal viruses only and not bacteriophages, particularly those on which observations have been made that are relevant to human disease. Animal viruses are much more difficult to study in the laboratory than are bacterial viruses, principally because animal cells are so much more complex, and more difficult to handle, than bacteria.

**Classification of Animal Viruses**
Over the last few years it has become possible to classify most of the 500 or so viruses infecting vertebrate animals into quite a small number of groups, either on the basis of their morphology or their nucleic acids, or a combination of these properties. Table 1 sets out the generally accepted groups of animal viruses.

**The Nucleic Acid of Animal Viruses**
In considering genetic aspects of viral diseases I shall be concerned with two genetic systems of vastly different complexity—that of the virus and that of the vertebrate host—and the interaction between these. I will start with the simpler system. In all organisms, including bacteria, the genetic information
Table 1. The Major Taxonomic Groups of Animal Viruses

| Group              | Shape                  | Diameter (nm) | Nucleic acid | Human Diseases                  |
|--------------------|------------------------|---------------|--------------|---------------------------------|
| Parvovirus         | Spherical              | 20            | D : SS : 2°  | Warts                          |
| Adeno-associated   | Spherical              | 20            | D : SS : 2   | (tumours in rodents)            |
| viruses            |                        |               | D : DS : 3-5 | Upper respiratory tract infections |
| Papovavirus        | Spherical              | 45-55         | D : DS : 20-25 | conjunctivitis (tumours in rodents) |
| Adenovirus         | Spherical              | 70-80         | D : DS : 70-100 | Varicella-zoster               |
| Herpes virus       | Spherical (env.)       | 150-200       | D : DS : 160-200 | Herpes simplex                |
| Poxvirus           | Brick-shaped           | 100 x 200 x 300 | D : DS : 3-5 | Cytomegalovirus                |
| Picornavirus       | Spherical              | 20-30         | R : SS : 2-3 | Smallpox                       |
| Togavirus          | Spherical (env.)       | 50-60         | R : SS : 3-4 | Orf                            |
| Myxovirus          | Spherical (env.)       | 80-120        | R : SS : 3 (P) | Poliomyelitis                  |
| Paramyxovirus      | Spherical (env.)       | 100-300       | R : SS : 7   | Aseptic meningitis             |
| Coronaviruses      | Spherical (env.)       | 80-120        | R : ?        | Carditis                        |
| Rhabdovirus        | Bullet-shaped (env.)   | 70 x 180      | R : SS : 5 (P) | Common Cold                    |
| Leukovirus         | Spherical (env.)       | 100-120       | R : SS : 10-12 (P) | Encephalitis                   |
| Reovirus           | Spherical              | 70-80         | R : DS : 15 (P) | Yellow fever                   |

env. = envelope derived from modified cellular membrane; D = DNA; R = RNA; SS = single-stranded; DS = double-stranded; numerals indicate molecular weight in millions of daltons; (P) = genome in several pieces.
is encoded in double-stranded DNA. The viruses are much more versatile than organisms in this respect; they utilise both double-stranded and single-stranded DNA and double-stranded and single-stranded RNA to store their genetic information.

The genetic information in the deoxyriboviruses is always contained in a single molecule per virion, differing in molecular weight in different animal viruses, from 3 to 200 million daltons. In most groups the DNA molecule is linear, but in the papovaviruses it is a supercoiled cyclic molecule.

Among the riboviruses the genetic material may occur either as a single linear molecule, varying in size between about 2.5 and 7 million daltons, or as a fairly specific aggregate of several small molecules. The latter situation has been unequivocally demonstrated with the reoviruses, in which the genetic material occurs in the virion and is transcribed and multiplies in the cell as ten separate fragments of double-stranded RNA. The viral RNA occurs as five or six pieces in the influenza viruses; the leukoviruses and the rhabdoviruses also appear to have aggregated genomes.

**Numbers of genes in viruses**

From our knowledge of the genetic code we can make a rough estimate of how many polypeptides are specified by different viruses, if we make three assumptions.

(a) Whatever the nature of the nucleic acid, the code is a non-overlapping triplet code devoted almost entirely to specifying amino acids. (There are exceptions; a small part of the DNA of herpesviruses specifies transfer RNAs.)

(b) Because of its small size, the viral genome is likely to be more economical than that of higher organisms; there is no evidence of repetitive nucleotide sequences or of ‘non-genetic’ DNA, like the satellite DNA of vertebrates.

(c) It is a working rule with higher organisms to assume that an ‘average’ polypeptide contains 200 amino acids and is therefore specified by an ‘average’ gene of 600 nucleotide pairs in double-stranded nucleic acids (or 600 nucleotides in single-stranded molecules).

On this basis, the small viruses, polyoma virus and SV40, which are the subject of so much experimental study because of their oncogenic potential, contain only seven or eight genes, human wart virus perhaps a dozen, and smallpox virus up to 400. Of course, if some of the genes specified very large polypeptides (and several viruses, e.g. adenoviruses and reoviruses, have polypeptides of about 140,000 daltons in their capsids) these calculated figures for the numbers of genes would be too high.
MUTATIONS OF ANIMAL VIRUSES
Although few detailed studies have been made, we know that mutations occur in the genetic material of viruses with about the same frequency as in organisms i.e. about once in every 100,000 to 1 million duplications. In every infection of a vertebrate animal by a virus, one or a very small number of viral particles multiply to become several hundred million particles, so that many mutations must occur during the course of every viral infection. Whether they are of any consequence depends upon three factors: (a) whether they confer a selective advantage on the mutant; (b) whether they occur early or late, and (c) whether the mutant virus is transmitted to another animal.

Virologists have utilised mutation and selection in their everyday work since virology began, for the familiar process of 'adapting' a human virus to multiply and produce recognisable symptoms in a laboratory host usually involves these processes: the production of attenuated viruses for vaccines involves deliberate selection of appropriate mutants. Of the different viral mutants used in laboratory studies one class, called conditional lethals, requires a mention. These are mutants that affect a virus so that it cannot grow under certain conditions determined by the experimenter, but yields mutant progeny under other conditions. With conditional lethals a single selective test can be used to obtain mutants in any one of many different genes. Animal virologists have studied temperature-sensitive mutants, in which the selective condition used is the high temperature of incubation of the infected cells. The defects in temperature-sensitive mutants lie in the primary structure of the polypeptide specified by the mutated gene, which ultimately produces an altered secondary or tertiary structure in the protein. Thus, a change in a single nucleotide leads to a single amino acid substitution that may, for example, so weaken internal bonding that the structure and function of the protein may be radically changed by a rise in temperature of only a few degrees. Temperature-sensitive mutants are useful for genetic mapping and for the biochemical analysis of viral functions, and since they are always less virulent than wild-type virus they may be useful for making vaccines.

ABORTIVE INFECTIONS AND DEFECTIVE VIRUSES
When an infected cell yields infective progeny we call the cell permissive and the infection productive. In many cases infections are non-productive or abortive, i.e. viral replication begins but does not go on to completion. Some particularly interesting cases occur with adenoviruses. In 1965 it was found that preparations of human adenoviruses were often contaminated with much smaller particles called adeno-associated or adeno-satellite viruses.
At first they were thought to be partial adenoviruses, but molecular hybridisation experiments show that they are genetically unrelated to adenoviruses. They appear to be absolutely defective viruses which fail to multiply in any cells except those in which an adenovirus is multiplying, i.e. they are parasitic on adenovirus functions as well as the cellular machinery. Adeno-associated viruses can spread through populations of children in epidemic waves without themselves causing any symptoms that have yet been recognised but only if there is concurrent epidemic spread of an adenovirus.

Human adenoviruses themselves are defective in monkey cells, yet much of the early work with adenoviruses was carried out in monkey kidney cell cultures. We now know that growth of human adenoviruses in these cells depends upon their simultaneous infection with certain simian viruses, of which a simian adenovirus and the papovavirus SV40 are the best characterised. SV40 used to be a very common contaminant of monkey cell cultures; indeed, it contaminated most early batches of Salk poliovirus vaccine that had been prepared in those cells. Further study of adenovirus rescue by SV40 has led to a host of novel and intriguing ‘mixed’ particles, which I shall mention again. In some, the viral coats are mixed; in some, there is, in addition, a hybrid DNA molecule.

The extreme state of adenovirus defectiveness is seen in cells transformed to the malignant state by certain adenoviruses. Here it seems that only a small portion of the viral genome is transcribed, and it persists through cellular divisions by virtue of its integration into the cellular DNA, probably as a small part of the total viral genome.

**Mixed and Multiple Infections**
Over the last decade we have gradually come to realise that latent viral infection is very common, and that frequently both intact animals and the cells used for the study of viral growth carry viruses other than those we infect them with. Mixed infections are not just a laboratory worker’s tool; they also occur commonly in nature. Two kinds of interactions between different viruses infecting the same cell can be distinguished; those involving physical exchange or union of nucleic acids, and those involving the gene products. Frequently both types occur together.

**Nucleic Acid Interactions**
Genetic recombination involves the exchange of pieces of nucleic acid between different viruses in such a way that some of the progeny contain a combination of genes not found in the parents (Table 2). The viruses involved may be closely related mutants, different wild-type strains of the same
### Table 2. Nucleic Acid Interaction: Genetic Recombination and Reactivation.

| Phenomenon                                                                 | Parent 1 | Parent 2 | Progeny | Comment                                           |
|---------------------------------------------------------------------------|----------|----------|---------|--------------------------------------------------|
| Genetic recombination                                                     | ABC      | ABC      | ABC     | Wild type                                        |
| (i) Between conditional lethal mutants of the same virus                  | ABC      | AST      | ABT     | With influenza and vaccinia viruses              |
| (ii) Between different strains of the same virus                          | ABC      | XYZ      | ABCYZ   | Note addition of nucleic acid                    |
| (iii) Between unrelated viruses (adenovirus and SV40, a special case)     | ABC      | 123      | 12AB3   | Integration of viral genes produces malignant cell |
| Cross reactivation                                                       | ABC      | AST      | ASC     | Rescue of genes from inactivated parent          |
| Between UV-inactivated virus and active virus of a different strain       |          |          |         |                                                  |
| Multiplicity reactivation                                                 | ABC      | ABC      | ABC     | Viable virus produced from inactivated parents   |
| Between virions of same virus inactivated in different genes              |          |          |         |                                                  |

A, etc. = active viral genes; 1, etc. = active cellular genes; B, etc. = mutant gene; A, etc. = inactivated gene

Recombination has been used to help construct genetic maps of bacteriophages and, more recently, of influenza virus and poliovirus. It can also be used for ‘tailor-making’ viruses, either for research purposes (this has been important in studying influenza virus neuraminidase, for example) or for vaccine production. Although recombination in animal viruses has been investigated for about twenty years little is known about the mechanism. For the large deoxyribovirus, vaccinia, it is probably closely analogous to the well-known T-even bacteriophage system. However, recombination has never been observed with the ribophages. The situation with animal riboviruses appears to include two different processes. With poliovirus, whose genome consists of a single molecule of single-stranded RNA, it is probable that recombination occurs between partially double-stranded replicative intermediate forms of the virus by the familiar ‘cross-over’ mechanism. The same mechanism may also operate with other riboviruses like reovirus and influenza virus, but with these viruses there may also be exchange of pieces of RNA during the replicative process—rather like assortative mating. The basis for this statement is the very high rate of recombination found with these
two viruses and the growing evidence that their genomes consist of and replicate as several (6–10) separate pieces of RNA.

I have already mentioned the interaction between SV40 and human adenoviruses grown in monkey kidney cells. Among the great variety of different types of particle yielded by such mixedly-infected cells are some that have an adenovirus capsid but a genome that is a single linear molecule containing DNA derived from both SV40 and adenovirus. One can envisage that a novel group of viruses could be ‘created’ by such a process.

When viruses are inactivated by methods that damage parts of their nucleic acid, mixed infection can lead to reactivation of the inactivated virus by a process of genetic recombination (Table 2). Apart from its theoretical implications if attempts were to be made to use UV-inactivated vaccines (and there is little interest in these nowadays), reactivation is of minor importance.

**Gene Product (Protein) Interactions**

I have already pointed out that cells are often latently infected with viruses. If such cells are infected with a second virus, or if cells are deliberately mixedly infected, a variety of interactions can occur. As well as recombination, which involves the viral nucleic acids, interactions can also occur between gene products. The commonest is probably interference, which may operate by a variety of mechanisms including interferon production.

A variety of productive reactions may occur (Table 3), involving the rescue of defective viruses by complementation and the production of viruses with mixed coats, due to phenotypic mixing. Complementation involves the interaction between gene products that are functionally important in viral multiplication but not necessarily part of the virion.

One example of complementation is worth a detailed description because it illustrates the variety of ways in which one observation has been interpreted as knowledge of the molecular biology of the virus has grown. In 1928, Fred Griffith, whose seminal contribution to molecular genetics was never adequately recognised during his lifetime, demonstrated pneumococcal transformation in mice. The virulence of pneumococci depends on the production of a polysaccharide capsule and there are a number of pneumococcal types that have different capsular polysaccharides. Mutants that have lost the ability to synthesise capsules are avirulent and produce rough colonies (compared with the smooth glistening colonies of capsulated organisms). Griffith showed that if mice were injected with either living rough type II pneumococci or heat-killed smooth type I organisms, the animals survived. If, however, they were injected with a mixture, some mice died and their blood contained living smooth type I organisms. Oswald Avery and his colleagues
| Phenomenon                                    | Parent 1 | Parent 2 | Progeny          | Comment                                      |
|----------------------------------------------|----------|----------|------------------|----------------------------------------------|
| Complementation                              | ABC      | ABC      | ABC and ABC      | Reciprocal; both mutants rescued             |
| (i) Between conditional lethal mutants in different genes | ↓↓↓ a b c| ↓↓↓ a b c|                  |                                              |
| (ii) Between defective virus and unrelated helper virus | ABC↓ a| BYZ↓ c | ABC and BYZ      | Defective virus is rescued by gene product 'b' of helper BYZ |
| Phenotypic mixing                             |          |          |                  |                                              |
| (i) Enveloped viruses                         | ABC↓ a  | XYZ↓ x  | ABC, XYZ         | Mixed peplomers in envelopes, genomes unaltered |
| (ii) Nonenveloped viruses (trans-capsidation) | ABC↓ a  | XYZ↓ x  | ABC, XYZ         | Heterologous capsids. Not always reciprocal  |

A, etc. = active viral gene; B = mutant gene; B = defective gene B; a, b, etc. = product of gene, A, B, etc.; ABC genome
\[
ax = \text{proteins in envelope (or capsid).}
\]
at the Rockefeller Institute went on to reproduce this change in the test-tube and then showed that transformation was due to the passage of DNA derived from the killed organism into the living bacterium and subsequent integration by recombination.

In 1936, long before Avery's studies, two American workers, G. P. Berry and his assistant, Dedrick, decided to test whether Griffith's transformation phenomenon also occurred with viruses. They used a system that was operationally analogous to Griffith's, namely, heat-killed myxoma virus, which was a highly virulent virus for rabbits, and the related benign virus of Shope's fibroma. As in Griffith's experiments, control rabbits suffered no disease, or got fibromas, but some rabbits inoculated with both viruses died of myxomatosis. This Berry-Dedrick phenomenon was long regarded as an example of viral transformation, even after Kilham, in 1957, had reproduced it in cultured cells.

My own involvement with myxomatosis research in Australia in the 1950s had made me aware of Berry's and Kilham's findings, and I had also become very interested in viral genetics because of the changes in the virulence of myxoma virus that we had found in field strains. I looked for a poxvirus that was easier to handle in the laboratory than myxoma virus, and started work with vaccinia virus and its close relative, rabbitpox. Having defined a number of genetic markers in these viruses, and shown that genetic recombination occurred between different strains, I turned with my colleague W. K. Joklik to a re-examination of the Berry-Dedrick phenomenon, using genetically characterised viruses. It soon became clear that the phenomenon occurred with these poxviruses, and that it was not a 'transformation' involving transfer of DNA, as in the pneumococcal example, but a reactivation of the heat-inactivated virus. Moreover, although genetic recombination was restricted to viruses belonging to the same subgroup of the poxvirus group, Berry-Dedrick reactivation occurred with all members of the group.

Further investigation of the mechanism of reactivation is due to Joklik and, later still, to his former student, McAuslan. Joklik showed that nitrogen-mustard inactivated virus as well as active virus would reactivate heat-killed virus, and went on to investigate the mechanism of uncoating of vaccinia virus. He proposed that uncoating of this complex virus was a two-stage process; the first stage of stripping the inner coat was performed by lysosomal enzymes, and a second step was due to a cellular enzyme induced by a viral protein released during the first-stage uncoating. With heat-inactivated virus the DNA was presumed to be intact, first-stage uncoating occurred normally, but the 'inducer protein' was inactive. However, if it were supplied by any other poxvirus, uncoating would occur normally. In 1966–67 McAuslan
began to question this hypothesis because of Dales's finding that the drug actidione inhibited uncoating at the core stage.

In a series of ingenious experiments he showed that the cellular genome was not involved in uncoating, and made the novel discovery, soon confirmed by others, that poxviruses contain within the virion a DNA-dependent RNA polymerase ('transcriptase') which transcribes some of the DNA even in the core stage, before the DNA is susceptible to DNAse. Among the early products of the transcription is the mRNA for an enzyme (still to be demonstrated biochemically) that completes the uncoating of the core. This led to the view that heat-inactivation, at the level permitting reactivation, was due to damage to the transcriptase. The Berry-Dedrick phenomenon can now be seen as another example of viral complementation.

The other sort of gene-product interaction, phenotypic mixing, involves viral structural proteins. It leads to the production of virus particles with mixed coats, or, in extreme examples, to heterologous genome and capsid. Two examples will suffice (Table 3). All animal viruses with envelopes acquire their envelopes as they bud through cellular membranes, usually the plasma membrane. In cells infected with such viruses the plasma membrane is altered by the substitution of virus-specified proteins for the normal cellular membrane proteins. When a cell is mixedly-infected with two enveloped viruses the membrane is mixedly altered by insertion of proteins specified by each virus. The enveloped virions bud off with such mixed coats, and usually the genome of one parental strain or the other. If the viruses are genetically compatible, recombination may also have occurred by a quite separate mechanism; sometimes the nucleoproteins of the genome may also be mixed.

Non-enveloped viruses may also show phenotypic mixing by the constructions of capsids containing protein subunits specified by either or both of the infecting viruses. By special laboratory tricks it is possible to encapsidate one genome entirely in another virus's coat; for example using poliovirus and Coxsackievirus it is possible to obtain particles that have Coxsackievirus coats and poliovirus genomes.

**HOST RESPONSE TO VIRAL INFECTIONS**

For physicians, viruses are important because they cause disease, which is a response of the whole organism. Genetic aspects of viral diseases therefore involve consideration not only of the genetics of viruses, but also the genetics of the host response, a much more complex topic.

Many viruses show a high degree of species specificity. Chickenpox and smallpox are viruses of man, foot-and-mouth disease virus affects cattle, myxomatosis affects rabbits. Except for a few rodent-pathogenic mutants
obtained by a laborious process of adaptation, polioviruses infect only primates, and, in cell culture, only primate cells. In this case species susceptibility depends on the viral receptors on the cell surface. Mouse cells do not adsorb poliovirus particles, whereas monkey cells do. However, mouse cells can be infected with RNA extracted from poliovirus; there is no metabolic block, only the difficulty of getting in. The same point is illustrated even more clearly by phenotypic mixing experiments in which a poliovirus genome is enclosed within the capsid of a Coxsackievirus. Mice are susceptible to Coxsackieviruses and the mixed particle attaches and releases its poliovirus genome within the mouse cell. The virions formed are pure poliovirus, and they cannot initiate a second cycle of infection in the mouse.

Apart from the all-or-none susceptibility associated with viral attachment, the host response is complex. Vertebrates have evolved in a world infested with parasites, and have developed a variety of mechanisms to enable them to adjust to parasitic invasion with a minimum of bodily disturbance. The most clearly defined defence mechanism is the immune response, but a host of other physiological factors play a role—interferon, body temperature, nutrition, hormones, etc. Studies of human subjects with various immunological defects, which are usually of genetic origin, illustrate the role of different immune mechanisms in resistance to viral infections. Agammaglobulinaemia is associated with recurrent infection by extracellular pyogenic bacteria, but recovery from viral infections is usually normal; selective IgA deficiency, on the other hand, predisposes to viral and bacterial infections of the mucous membranes of the gut and respiratory tract. Failure to recover from generalised viral infections seems to be most clearly associated with the very rare cases of defective cell-mediated immunity that sometimes occur in individuals whose gammaglobulin production is not greatly impaired.

As well as these more general genetically determined defects in the immune response, there is now good evidence that the ability to respond to particular antigens is under strict genetic control. For example, a single dominant autosomal gene determines whether or not guinea-pigs can form antibodies to hepten-poly-L-lysine conjugates, the nature of the polymeric backbone being the important factor. In mice, genetic factors exist which discriminate between tyrosine, histidine and phenylalanine in the antigenic determinant; the genetic differences being dominant-, unigenic-, quantitative- and determinant-specific. Clearly, in specific cases such genetic factors could affect the immune response to viruses, although no examples are known.

Genetic analysis of properties like resistance to infection is simpler with mice than with men. Three types of inheritance of resistance to viral infections have been observed in mice. With certain group B togaviruses the susceptibility of
mice is under single gene control with resistance dominant; in this system the yield of virus from cultures of splenic macrophages from the susceptible strain of mice is up to 1000 times greater than in cultures from resistant strains. With mouse hepatitis virus, a representative of the newly recognised coronaviruses, susceptibility is under single gene control with susceptibility dominant. This is what might be expected if the gene in question controlled the presence of the appropriate viral receptor. The most common situation is exemplified by mousepox and oncogenesis due to polyoma virus, where resistance depends on the action of two or three independent genes. Polyoma virus injected into newborn mice of most strains rapidly produces a variety of malignant tumours, hence its name. This does not occur with C57B1 and A strains of mice, yet organ explants or cultured cells from these mice can be transformed in vitro. In this case resistance clearly depends on the integrity of the body. Experiments with C57B1 mice, using neonatal thymectomy and antilymphocyte serum, have shown that this resistance is associated with the cellular immune response. The high resistance of C57B1 mice to footpad injection of virulent mousepox virus is likewise correlated with their vigorous cellular immune response. This resistance can be abolished by the injection of antilymphocyte serum and enhanced, in susceptible mice, by the transfer of sensitised lymphoid cells from a resistant donor, although the passive transfer of enormous doses of immune serum has only a marginal effect.

Although attractive on theoretical grounds, it has so far been difficult to evaluate the role of interferon in recovery from viral infections; and we know of no human or animal examples of genetic incapacity to synthesise interferon.

VIRUS-HOST INTERACTION IN POPULATIONS
This article began with a consideration of the simplest system, viruses as nucleoproteins. More complex were the virus-host interactions, as far as they have a genetic basis, at the level of the individual organism. The last consideration is at the most complex level of all—that of the real world where animal viruses spread and survive in populations of their vertebrate hosts. Discussion is limited to two examples in which we have some evidence of the importance of genetic changes in the virus, or in the virus and the host animal, namely, influenza and myxomatosis.

Influenza
Influenza is the most important viral disease of modern man. It killed an estimated twenty million people in 1918–19; the 1957–58 pandemic of Asian influenza was even more extensive but fortunately, and fortuitously, much less
lethal, and in the winter of 1969–70 there was a fairly severe epidemic of the Hong Kong variant of Asian influenza virus.

There are two common types of influenza virus which are distinguished by their internal ribonucleoprotein antigens as influenza type A and influenza type B. Influenza B is a specifically human disease; influenza A occurs as an enzootic disease of birds, swine, horses, and perhaps other animals, as well as man. It has been possible to follow the antigenic changes in influenza virus ever since its discovery in 1933. It is common for epidemiologists to make guesses, based on human serum surveys, of what happened before 1933; but this sort of ‘serological epidemiology’ is subject to so many different interpretations that I prefer to base my argument on the firmer ground of the analysis of the antigens on the viruses that have been recovered each year since 1933.

In influenza, as in other viral disease, antibodies acquired as a result of prior infection give considerable protection against reinfection with the same virus. The protective antibodies are those directed against the haemagglutinin, for the haemagglutinin subunits attach the viral particles to susceptible cells, and antibodies to the haemagglutinin prevent this attachment. Antibodies to the internal antigen have no effect on the infective process; and antibodies to the neuraminidase prevent release of the virus but do not prevent infection.

Studies of these three antigens (haemagglutinin, neuraminidase, and nucleoprotein) of strains of influenza A and influenza B isolated from man over the past thirty years have given us the following picture of secular changes in influenza viruses (Fig. 1). The type-specific internal (nucleoprotein) antigens have shown very little antigenic or chemical change over many years; peptide maps of these antigens from strains of influenza A virus isolated at intervals of thirty years are almost identical. With both influenza A and influenza B, however, there has been a regular progression of changes in the serological reactivity of the haemagglutinin antigen, and this is paralleled by changes in the peptide maps. Burnet coined the term ‘immunological drift’ to describe this secular change in the antigenic determinants of the haemagglutinin. It can be explained by supposing that previous infection of man with influenza virus gives rise to antibodies that prevent re-infection by a virus which has not altered in its antigenic pattern, but sometimes permit re-infection by a virus with slightly altered antigenic pattern in the haemagglutinin antigen. Immunological drift of the haemagglutinin is observed with both influenza A and influenza B, and there is a similar immunological drift in the antigenicity of the neuraminidase of both types of influenza virus. It is important to note that the haemagglutinin and the neuraminidase are separate polypeptides specified by separate genes. Both are subjected to strong
Since both subtype viruses of influenza A virus recovered subsequent to the neuraminidase. Like drift, i.e., parallel change, is quite different and have not changed over the whole period. Both the haemagglutinin (A: - - , and B: ---) and the neuraminidase (A: ..., and B: ----) have shown antigenic drift, i.e., roughly annual but independent changes in their antigenicity. A larger change than usual in the haemagglutinin antigen of influenza A in 1946 led to the 1947 and subsequent strains being called a new subtype, Al. However, there was no parallel change in the neuraminidase in 1946. All changes from 1933 until 1957 resulted from antigenic drift. There was a major change in the haemagglutinin of influenza B in 1962, without a parallel change in the neuraminidase, but the strain died out.

The appearance of Asian influenza virus (subtype A2) in 1957 produced a major break, since both the haemagglutinin and the neuraminidase differed greatly from those of subtype Al in antigenicity and in other ways. Asian influenza was therefore probably due to the introduction of a novel virus from an animal reservoir. Since 1957, the haemagglutinin and neuraminidase antigens of influenza A2 have themselves undergone antigenic drift. In 1968 the Hong Kong variant of A2 appeared and spread throughout the world; it differed greatly from preceding strains in the antigenicity of its haemagglutinin, but not in the neuraminidase. Like subtype Al, the Hong Kong virus resulted from antigenic drift.

Fig. 1. Diagram illustrating antigenic drift and the appearance of new human influenza viruses. 'Antigenic drift' (ordinate) represents the serological relatedness of the antigens of influenza A virus recovered from man between 1933 and 1968, and influenza B between 1940 and 1968. The internal (ribonucleoprotein) antigens (--- - -) of each virus are quite different and have not changed over the whole period. Both the haemagglutinin (A: - - , and B: ---) and the neuraminidase (A: ..., and B: ----) have shown antigenic drift, i.e., roughly annual but independent changes in their antigenicity. A larger change than usual in the haemagglutinin antigen of influenza A in 1946 led to the 1947 and subsequent strains being called a new subtype, Al. However, there was no parallel change in the neuraminidase in 1946. All changes from 1933 until 1957 resulted from antigenic drift. There was a major change in the haemagglutinin of influenza B in 1962, without a parallel change in the neuraminidase, but the strain died out.

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selection for antigenic novelty, and therefore both antigens exhibit drift, but the changes do not necessarily occur in parallel. The best examples of this were the appearance of the so-called influenza type A1 in 1946–47, and of the Hong Kong strain in 1968. In both cases there was a very pronounced change in the antigenicity of the haemagglutination (which was why Hong Kong influenza virus spread so extensively) but only a trifling comcomitant change in the neuraminidase. As far as influenza B is concerned, immunological drift in the haemagglutinin and the viral enzyme are the only changes found. A major change occurred in the haemagglutinin of influenza B in Taiwan in 1962, but the strain did not spread, however, and has now disappeared.

With influenza A, an additional rare and unpredictable but most important type of change in antigenic pattern can occur. This consists of an abrupt departure from the previous antigenic pattern in both the haemagglutinin and the neuraminidase, accompanied by other changes. This has happened only once since 1933, with the Asian influenza virus that caused a world-wide pandemic in 1957–58 (Fig.1), and has been the only type of influenza A strain found since then. As with earlier A strains, immunological drift has since occurred in the haemagglutinin and neuraminidase antigens of Asian influenza virus. The inescapable conclusion is that in 1957 Asian influenza was due to a new virus, as far as man was concerned. It spread in pandemic fashion, first, because it had the capacity to spread in man, whatever the factors are which govern this, and secondly because its haemagglutinin and neuraminidase antigens were quite different from those of the influenza A viruses that man had been exposed to for several decades, so that virtually all human beings were immunologically fully susceptible.

By relating discoveries about the molecular biology of influenza virus to its epidemiology, we are able to remove some of the guesswork from prophecies about influenza but much remains unknown. We do not know what the qualities are that govern ‘infectiousness’, nor do we know why some strains cause severe disease and others usually produce mild or unapparent infections. One consequence of our present knowledge is to underline the unpredictability of real pandemic influenza, and by this I mean the occurrence of a ‘new’ virus, like those of 1918–19 and 1957, and not just the type of epidemic that follows the occurrence of a major change by antigenic drift, as with the Hong Kong variant of 1968–69. And if new strains of influenza A arise in animal hosts—what is the mechanism? We know that antigenic drift is hardly apparent with influenza A virus in swine, where there is no strong and consistent selection for it. We also know that influenza A strains readily undergo recombination, both in the laboratory and in infections of chickens and swine. However, we
do not know if recombination is important in generating novel strains of influenza A virus that subsequently infect man.

Myxomatosis
Finally, I shall discuss the evolution of a disease of rabbits, which is probably as familiar to Britons as it is to Australians, namely myxomatosis. Myxomatosis provides a unique example of what happens when a very virulent virus spreads in a very susceptible animal, a situation in which natural selection operates very powerfully to produce rapid changes in both the virus and the host animal. In myxomatosis the skin of the infected rabbit becomes covered with lumps in which the cells are filled with virus. The disease is transmitted mechanically by arthropods which contaminate their mouthparts with virus when they probe through infected skin, and then wipe the virus off when they bite another animal. If the other animal is a man or a mouse nothing happens; if it is a rabbit that has not had the disease before, it will get this severe generalised infection.

The fact that in Australia myxomatosis is spread mainly by mosquitoes has important epidemiological consequences. Epidemics occur mainly in the summer, when mosquitoes are numerous. Since the vectors are mobile, occasionally over quite long distances, the disease can spread very rapidly during the summer and therefore small pockets of residual disease can seed vast areas of the continent. The pressure for survival of the virus is exerted most powerfully during the winter period, when mosquitoes are rare.

Rabbits were effectively introduced into Australia as a shipment of two dozen wild rabbits which arrived at Geelong in the clipper Lightning in 1859. Six years later 20,000 rabbits were killed on the property of the importer, and thereafter the wild European rabbit spread over Australia with astonishing speed, aided at first by transfers of animals from one part of the country to another by colonists anxious to recapture something of the atmosphere of their English homeland. Like rabbits, which had been intermittently introduced as domesticated animals since the First Fleet arrived in 1788, myxomatosis got away to a slow start, for the first field releases were made as early as 1942. Eventually, in 1950, after five months of arduous inoculation and seeding of this virus in several sites in the Murray Valley, started in May, it suddenly spread spontaneously from Christmas and was soon found over a vast area coinciding with the river system of south-eastern Australia. Subsequently there have been epidemics of greater or lesser severity every summer, in the mosquito season, and the areas between the rivers were completely seeded with the virus by 1954. In an area where regular counts of the rabbit population were
made, the initial mortality rate was 99.4–99.8 per cent; a year later, in the same locality, the mortality had fallen to 90 per cent.

As far as protection by previous infection is concerned myxomatosis is, like its human analogue smallpox, a monotypic virus over time and space. The virulence of myxoma virus can be measured by the ability and speed with which different strains of the virus kill rabbits. Changes in many different genes of the estimated 400 contained in myxoma virus may affect its virulence. Since the virulence of the original virus was so very high we might expect to find and recognise lowered virulence more easily than increased virulence, and indeed, within a year of the introduction of the virus, strains of lowered virulence, which killed only 90 per cent of rabbits, were found in nature, and they have been found commonly ever since. How can we explain the replacement of the original very virulent strain by other less lethal strains, a result quite the reverse of what happens in the laboratory when we passage viruses in a susceptible host animal? Natural selection for the persistence of viruses in nature operates at the level of transmission of the virus from one animal to another. The weak point in the indefinite survival of a virus that kills all rabbits about ten days after they are infected (the highly virulent myxoma virus) is the over-wintering period. A rabbit that survives in an infectious state for three or four weeks has a much greater chance of being the source of the virus transmitted to another rabbit than one that lives only five days after it becomes infectious. This selective advantage is of some importance even during epidemic spread of viruses in summer, as field experiments have clearly demonstrated; it becomes of major importance for survival between epidemics, i.e. for over-wintering. This period selected viruses that allow rabbits to live for prolonged periods in an infectious condition, i.e. viruses of reduced virulence. Having survived the winter the attenuated or weakened viruses were in competition with virulent viruses introduced in the inoculation campaigns carried out each year by rabbit control authorities. Field experiments with virulent and attenuated viruses in natural epidemics have shown that although early in the epidemic the virulent virus may cause a high mortality, if it has been introduced extensively enough and at the right time in relation to the abundance of vectors, the attenuated virus is the only one found at the end of the epidemic and the only one that survives until the next season.

In my comments on influenza I did not attempt to trace any effect of influenza on the genetic constitution of man; it is not a lethal enough disease. Myxomatosis is different. The natural host of myxoma virus is the wild rabbit of Brazil, which belongs to a different species and genus from the rabbit of Australia. In this animal ‘virulent’ myxoma virus causes merely a persistent skin tumour, and attenuated viruses cause trivial lesions, or none at all. In
genetically unselected Australian wild rabbits the same virus causes a generalised disease which is almost always lethal. If neither the virus nor the rabbit changed as a result of their interaction, the result would be local eradication of rabbits and consequent disappearance of the virus, since it has no other host. This probably happened in many localities. But natural selection for effective transmission, especially during the winter, led to the appearance of attenuated viruses which allowed 10 per cent or more of the infected rabbits to survive. This observation immediately provoked questions concerning the transmission of increased resistance to the progeny. Investigations in the field, and subsequently in the laboratory, have provided an answer. The capacity to recover from infection is indeed a genetic trait, although we do not understand how it operates at a physiological level. In a natural situation of severe annual epidemics of myxomatosis, natural selection by viruses that kill about 90 per cent of laboratory rabbits led to a change in the rabbits such that this preparation of virus was able to kill only 25 per cent of animals seven years later. Breeding and progeny-testing experiments give almost identical results and show further that the capacity to resist the attenuated virus is paralleled by an increased capacity to resist the virulent virus.

CONCLUSION
Viruses have the simplest genetic systems; vertebrates the most complex. An adequate understanding of their dynamic interaction can be obtained only if the subject is approached at the four levels of complexity at which experimental studies can be made; the molecular biology of the virion, the physiology and biochemistry of the virus-cell interaction, the pathogenesis of the infective process in the intact animal, and the epidemiology of infections in populations of animals.

This lecture was given at the Virology Conference held at the Royal College of Physicians in June 1970.

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It would be impossible to give the original references for all the statements appearing in this article. The reader is therefore referred to the following books and review articles for further information and detailed references.

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