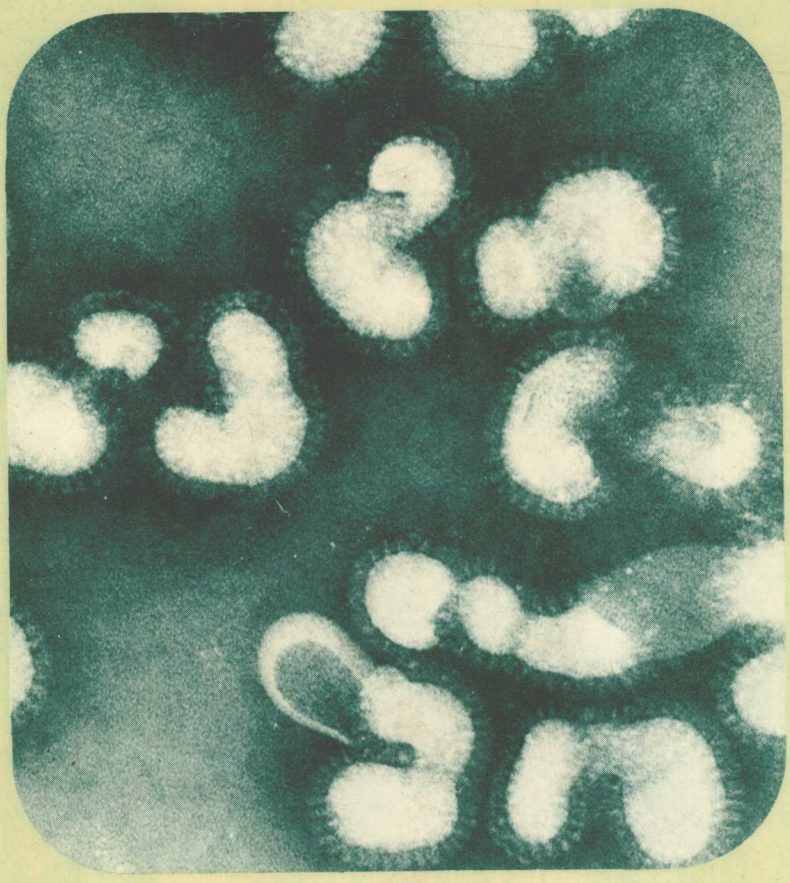
The Specific Treatment of Virus Diseases



D. J. Bauer

THE SPECIFIC TREATMENT OF VIRUS DISEASES

by

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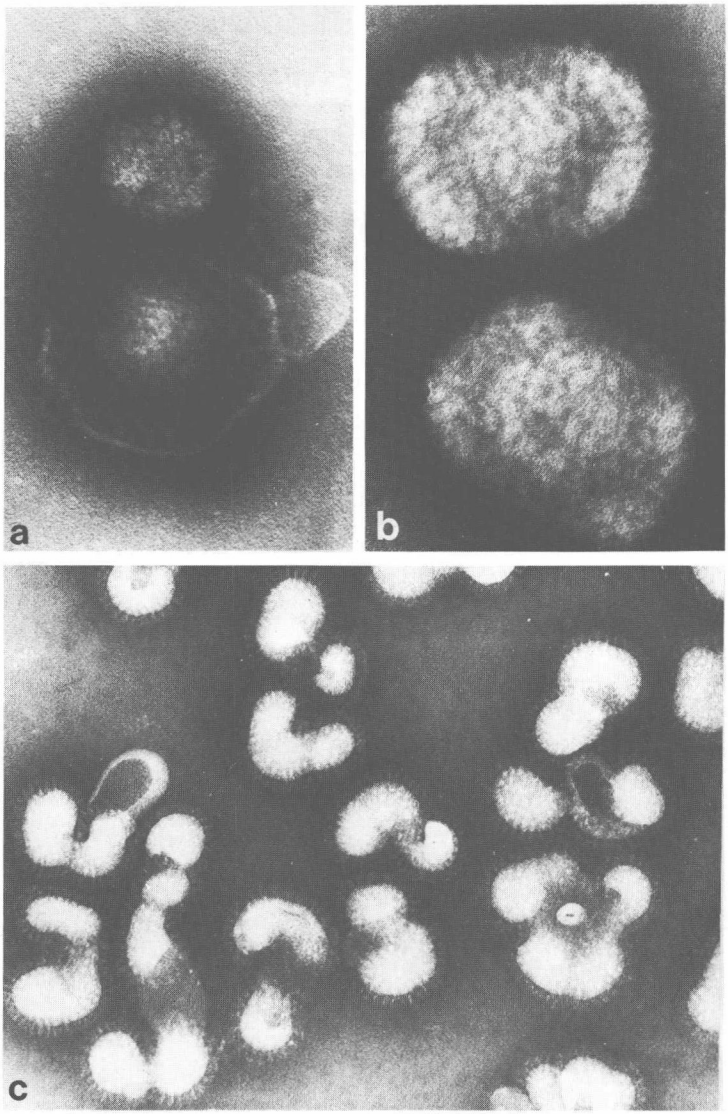


Viruses susceptible to chemotherapy (*magnification* × *180 000*). (*a*) *Herpes: the upper particle has lost its capsule and shows structural detail.* (*b*) *Vaccinia: two brick-shaped particles showing surface detail.* (*c*) *Influenza A: the projections on the surface are haemagglutinin and neuraminidase.* [*Photographs by courtesy of Dr. J. D. Almeida*].

Foreword

In comparison with antibacterial chemotherapy the clinical use of specific antiviral drugs is a fairly recent development. By the 1950s a number had been discovered, but their clinical application was delayed until 1962, when methisazone was used for the treatment of infective complications of smallpox vaccination and idoxuridine in the treatment of herpetic keratitis.

In retrospect the reasons for this slowness in development is now evident. It was due in large part to the prevailing view held at the time that virus infections could never be treated with specific agents, since the multiplication of virus is so closely integrated with the metabolic processes of the host cell that specificity of action seemed impossible of achievement. In contrast, bacteria were independent entities with metabolic systems differing widely from those of the mammalian host, which afforded points of attack by specific agents which had no action upon mammalian systems. Although the mode of action of the sulphonamides and penicillin was not known for some time, they afforded perfect examples of the success of this mode of attack.

It is now known that a number of viruses contain enzymes which are distinct from those of the host cell, and others carry the information for synthesizing virus-specific enzymes in their genetic code, thus affording points for specific attack by antiviral agents. Also, specificity is not always as essential as it may seem. For example, the synthetic nucleosides used in the treatment of infections with members of the herpesvirus group inhibit steps in the pathways of DNA synthesis of both virus and host cell, but inhibition of the multiplication of the virus can nevertheless be achieved by concentrations below those which affect the host cell. In this case specificity is lacking, but advantage can be taken of a favourable therapeutic ratio.

The way in which the subject of antiviral chemotherapy has developed inevitably means that it will be of less concern to general practitioners than to consultants in ophthalmology, dermatology and venereology. Nevertheless, an attempt has been made to present the subject on two different levels.

Firstly, as a text-book on antiviral therapeutics for medical students, who may abstract its salient points as they would a text-book of general medicine and pass over the details. For this purpose a general reading list is appended to each chapter. Secondly, as a reference work for postgraduate students preparing for the examinations of the Royal Colleges who may need to study the subject in greater detail, and also for consultants who wish to have a comprehensive assessment of the relevant literature in a single volume. For such readers the text is provided with references to the original work which are arranged alphabetically in a bibliography at the end of the work. The illustrations have been restricted in number and show clinical material where possible. Illustrations of disease conditions in untreated subjects have not been provided, since these are available in the standard texts which are quoted as general reading. Descriptions of treatment are confined to the use of antiviral agents, and it should be understood that additional measures such as antibiotics, steroids and intensive care may be necessary, and information of this kind may be obtained by consulting the relevant references.

The selection of compounds included in this book has been limited to those which are actually in clinical use as antiviral agents, and they are all to be found in the 25th edition of Martindale's Extra Pharmacopoeia. For this reason no attempt has been made to delve into the controversy as to the value of ascorbic acid in the prophylaxis of colds, since although it is extensively used for this purpose by the general public it is devoid of specific antiviral activity.

I am deeply indebted to my secretary, Mrs. B. P. Moore, for her constant encouragement and her assistance in preparing the manuscript for publication. My thanks are also due to Mr. J. W. T. Selway for assistance in preparing the illustrations, to Dr. K. Apostolov, Royal Postgraduate Medical School for supplying the photographs for Figure 5.1, and to Dr. J. D. Almeida, who kindly supplied the electron micrographs of the frontispiece.

D. J. Bauer

Contents

Chapter 1

An introduction to the viruses

Viruses are obligate intracellular parasites of man, other animals, plants and bacteria. They can only multiply within cells, and thus differ from bacteria, which can multiply in tissues in an extracellular position, and also in artificial culture media in the laboratory.

It has always been recognized that viruses are distinct from the bacteria, but originally other groups of infective agents were included among the viruses which are now known to be organisms of a different and more complex nature. These included the Chlamydiae, organisms causing diseases such as lymphogranuloma venereum and trachoma, and the Rickettsiae, the causative agents of typhus and related infections. The specific treatment of infections caused by these two groups of agents will not be considered in this work, which is restricted to the chemotherapy of infections caused by the true viruses. The Chlamydiae and Rickettsiae are complete organisms, with cell walls, which possess both types of nucleic acid, enzyme systems which function within their cytoplasm, and a number of cell organelles. The viruses, on the other hand, possess either DNA or RNA as their genetic material, but not both. In some cases they contain enzymes, but these exert their functions within the host cell after the virus particle has undergone a process of dissolution during the early stage of infection. Except in the case of the arenaviruses, a group which contains the virus of Lassa fever, the virus particles do not contain organelles. A virus is thus much more rudimentary in structure, and relies upon the host cell to provide the systems for synthesizing its components which it does not possess itself. This is the underlying reason for its obligate intracellular parasitism and inability to multiply in any other situation.

CLASSIFICATION OF THE VIRUSES

The scope of this work is limited to those viruses which cause disease in man, but in order to view them in proper perspective it is desirable to consider the place which they occupy in the classification of the animal viruses

as a whole. The viruses fall sharply into two classes: those which utilize RNA as their genetic material, and those which use DNA. As stated earlier, viruses never contain both types of nucleic acid.

Further subdivision is based on structural features, such as symmetry and properties of the component parts. The complete virus particle is known as the virion. It has an outer protein coat known as the capsid. This may be associated with the virus nucleoprotein, in which case it is referred to as the nucleocapsid, or the nucleoprotein may be present as a central core, in which case the capsid may consist of an assemblage of repeating units known as capsomeres. The virion may have an outer envelope derived from the nuclear membrane or plasma membrane of the host cell.

The virion is usually symmetrical, and the symmetry may be helical or of the type known as cubic. These features are used in the construction of the presently accepted system of classification. The system used for the DNA viruses is shown in Table 1.1, which is based on a table given by Melnick (1974). Except for the parvoviruses all groups are of importance in human medicine.

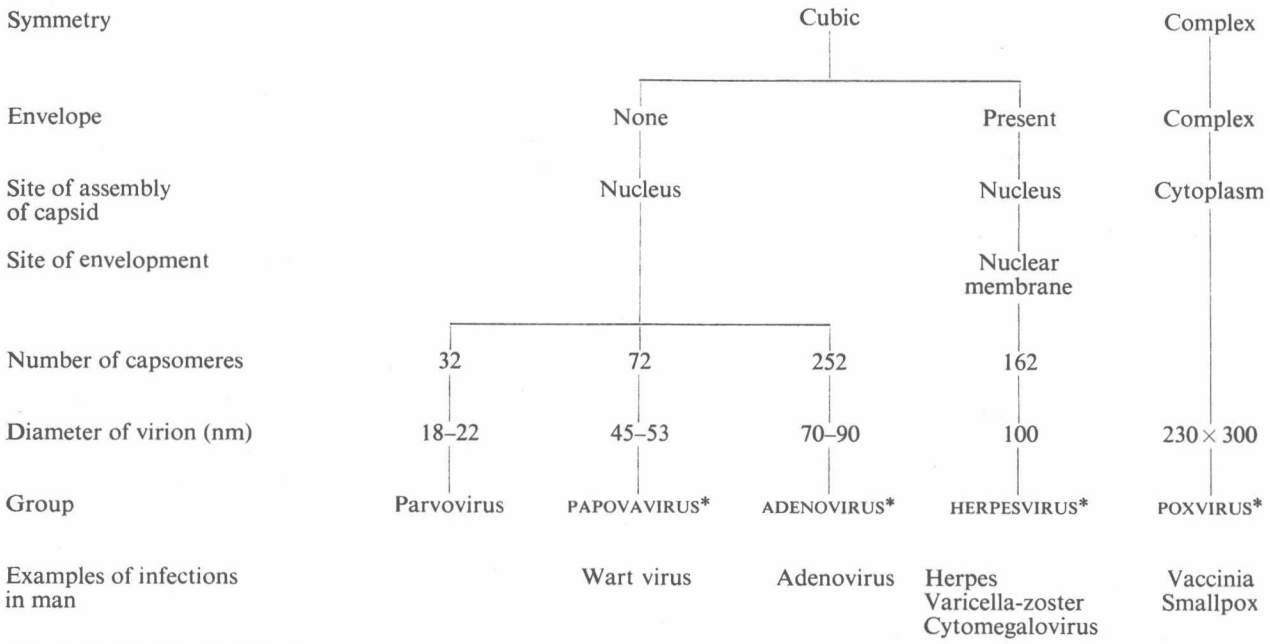
A similar classification for the RNA viruses is given in Table 1.2. All groups contain viruses of clinical importance except the oncorna and coronavirus groups. Respiratory syncytial virus, an important cause of upper respiratory infection, occupies an intermediate position between the orthomyxovirus and paramyxovirus groups, and together with a virus causing pneumonia in mice may constitute an independent group of metamyxoviruses.

VIRUS MULTIPLICATION

The first stage in the multiplication of a virus involves the entry of the virion into the host cell. It first becomes attached to the plasma membrane of the host cell by electrostatic forces. In some cases the membrane contains receptor sites which are specific for the virus. The virion then passes into the interior of the cell, either by fusion of its outer coat with the plasma membrane, or by engulfment in a phagocytic vacuole. If fusion occurs the nucleocapsid may pass into the cell, but if the virion is taken into the cell by phagocytosis the outer membrane is removed by cell enzymes, a process known as uncoating. When this has taken place infectious virus is no longer present in the cell. This stage of the growth cycle is known as the eclipse phase.

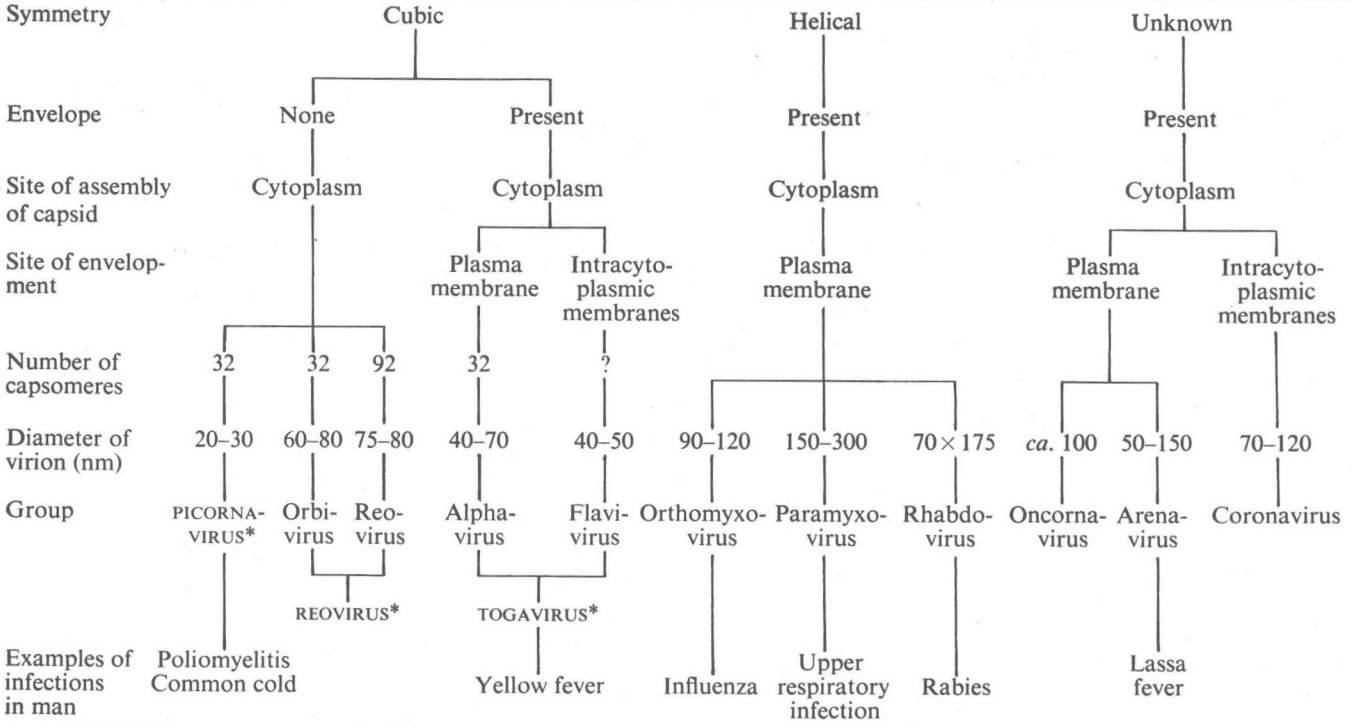
When the genetic material of the virion is exposed it undergoes two processes. It must be replicated in order to provide sufficient genetic material for assembly in the progeny virions, and the genetic message which it carries must be transcribed and translated so that the polypeptides of the virus may be synthesized on the host cell ribosomes. These processes are complicated and will be described here only in outline.

Table 1.1 Classification of DNA viruses of animals and man (after Melnick, 1974)



* Where the group name is in capitals it has been designated as a family

Table 1.2 Classification of RNA viruses of animals and man (after Melnick, 1974)



* Where the group name is in capitals it has been designated as a family

The DNA and RNA viruses differ in the methods employed for replicating their genetic material. In the former the DNA of the virion is replicated by a DNA polymerase, either present in the host cell or in some cases coded for by the virus and synthesized on the host cell ribosomes. RNA viruses can replicate RNA directly from their RNA genome by using it as a template. This process forms complementary strands, which act as templates for the synthesis of RNA in which the genetic code is restored for the purpose of incorporation into the progeny virions.

In the process of transcription RNA strands are generated which carry the equivalent of the genetic code and act as messenger RNA for the translation of virus proteins on the host cell ribosomes. The RNA genome of the virion may act directly as a messenger RNA and be translated into a very large polypeptide which is subsequently broken down by enzymes into shorter units. In some viruses, however, the RNA of the virion has an arrangement of bases which is complementary to the required code. It must therefore be transcribed by an RNA polymerase in order to generate messenger RNA with the required sequence of bases. The RNA of the genome is in some cases double-stranded, and one strand is transcribed into messenger RNA by a transcriptase carried in the virion.

Translation from messenger RNA follows a similar course in both DNA and RNA viruses, giving rise to enzymes required for virus multiplication and structural polypeptides required for making the new generation of virions.

When the components of the virus have been synthesized they are assembled into virions which are then released from the cell. The details of this process, together with the general features of the growth cycle, will be described separately for those groups of viruses which are of importance in chemotherapy.

Herpesviruses

The stages in the multiplication of the herpesviruses have been critically reviewed by Watson (1973). The virus particles enter the cell by fusion to the plasma membrane, or by engulfment in pinocytotic vesicles.

The early events in the multiplication cycle of the herpesviruses take place in the nucleus, with the formation of strands of RNA coded for by the virus. These pass into the cytoplasm and break down into shorter lengths which act as messenger RNA for the synthesis of capsid proteins, which then pass back into the nucleus, where the virus DNA has been replicating meanwhile. The various components then become assembled into capsids, which are spherical bodies bounded by a membrane and usually containing a dense core, which is presumably nucleoprotein. At this stage the particles are still inside the nucleus, but particles consisting of a core surrounded by two membranes now begin to appear in the cyto-

plasm. These are the mature virions, and it is considered that the intranuclear capsids enter the cytoplasm by passing through the nuclear membrane, acquiring a second coating in the process. The virions are liberated from the cell by budding through the plasma membrane, or by lysis of the cell. The length of the growth cycle is about 15 hours. The eclipse phase lasts for 8 hours, and infectious virions appear 2 hours later and reach peak titres 15 hours after the onset of infection.

Poxviruses

The member of the poxvirus group which has been most studied is vaccinia. The stages in its multiplication cycle have been reviewed by Fenner *et al.* (1974). The virion enters the cell by pinocytosis, and while it is in the pinocytotic vesicle the outer coats are removed by host cell enzymes leaving the central core which contains the virus DNA. The limiting membrane of the vacuole disappears and the core enters the cytoplasm. The virion contains a DNA-dependent RNA polymerase which synthesizes virus-specific messenger RNA, and it is thought that this codes for an enzyme which disrupts the core and liberates the virus DNA. The virus also codes for a DNA polymerase, by means of which the progeny DNA is synthesized. This takes place in localized areas in the cytoplasm. The progeny DNA is now transcribed, and the messenger RNA thus formed is translated on the host cell ribosomes to form structural polypeptides. These aggregate to form capsules, beginning at the margins of the areas in the cytoplasm where virus DNA is being synthesized, and gradually extending to surround a mass of DNA and finally enclose it. The material inside differentiates into a core and two lateral bodies and assembly of the progeny virions is then complete. Infective virus appears after 4 hours and by 20 hours or so the cycle of growth is complete. Liberation from the cell takes place as the result of lysis.

Orthomyxoviruses

The member of the orthomyxovirus group which is of importance in chemotherapy is influenza A. The virion contains seven strands of ribonucleoprotein and is bounded by a capsule which contains a haemagglutinin and the enzyme neuraminidase, which occur in the form of spikes projecting from the surface.

The existing state of knowledge of the replication cycle of influenza virus has been reviewed by Fenner *et al.* (1974). Although it is an RNA virus its replication involves the nucleus at some stage, since influenza virus will not multiply in cells which have been enucleated with cytochalasin B, or in which the nuclear DNA has been damaged by exposure to ultraviolet light. The virion attaches to the cell by means of the haemagglutinin, which

reacts with a specific receptor in the plasma membrane. The mechanism of penetration has not been established beyond doubt. There is evidence that the envelope of the virion fuses with the plasma membrane and also that the virions are taken up into pinocytotic vesicles. The strands of ribonucleoprotein contain a RNA polymerase which transcribes a messenger RNA which is complementary in its base sequence to the virus RNA. The proteins of the virus are synthesized by translation of the messenger RNA on the cytoplasmic ribosomes, but after synthesis they migrate to other parts of the cell. The protein forming the nucleocapsid migrates to the nucleus, and the haemagglutinin and protein of the virion membrane accumulate on the smooth endoplasmic reticulum. A non-structural protein is also formed, which may have a function in regulating transcription of RNA. It passes into the nucleolus. During the later stages of the growth cycle the RNA of the virus begins to replicate, so that virion RNA is formed at the expense of the complementary RNA synthesized during the early stages of infection. Assembly of the virions and their release from the cell take place as parts of a single process. Areas of plasma membrane develop visible projections, representing units of neuraminidase and haemagglutinin, and beneath them accumulations of ribonucleoprotein and a layer of membrane protein appear. The whole complex buds from the cell surface and becomes nipped off to form a complete virion. It is thought that the neuraminidase plays some part in enabling the virion to be released from the cell.

THE COURSE OF VIRUS INFECTIONS

After liberation from the cell at the end of the first cycle of infection the progeny virions may infect the cells in immediate contact, or may gain entry into the blood stream and give rise to infection in other parts of the body. The type of illness which ensues depends upon which process predominates. It may be acute, recurrent or chronic.

Acute infections

The acute virus infections can be divided into two groups: those in which the infection affects a single organ or tissue, and those in which an initial period of multiplication in some site, which may be entirely asymptomatic, is followed by a period of dissemination in which the virus multiplies in another tissue to give the characteristic features of the disease.

Typical examples of the first group are the common cold and influenza. The initial infection takes place in the nasal or bronchial mucosa, and the infection spreads laterally to involve a considerable part of the cell layer.

Chickenpox and smallpox are characteristic examples of the second group. The initial site of multiplication is uncertain, possibly the reticulo-

endothelial system or the respiratory tract. The virus undergoes several cycles of multiplication, until so much virus is produced that it spills over into the blood stream to produce a viraemia. The virus then undergoes further cycles of multiplication in the skin, producing the lesions characteristic of the disease. The extent of the skin involvement depends upon the number of virus particles liberated into the blood stream during the phase of viraemia. In both chickenpox and smallpox less than a dozen lesions may be present and the disease may escape detection. In other cases the eruption may be very extensive and associated with severe general illness.

Acute virus infections bring about an immune response which usually terminates the infection, with subsequent recovery. In cases of smallpox with a confluent eruption the immune mechanisms may be insufficient to arrest the infection and the death of the patient ensues.

The immune response resulting from an acute infection may often persist for many years. As a result a second attack may never occur, or only very rarely. However, second and further attacks occur quite commonly in a number of virus infections. There are two reasons for this. Firstly, the virus may not be completely eliminated from the body, but may persist in some site where it is not exposed to antibody. As a result of some stimulus it may become reactivated and give rise to a further attack of the disease. This behaviour is characteristic of viruses of the herpes group, and will be described in more detail in the chapters on the specific treatment of herpes, varicella-zoster and cytomegalic inclusion disease.

Secondly, further attacks may be due to infection with a virus of a serotype different from that of the initial infection. This situation is typical of the respiratory infections. There are at least 120 serotypes of rhinovirus which have no cross-immunity. Infection with one serotype leads to lasting immunity, but there are so many serotypes that fresh attacks of the common cold are a constant occurrence. Influenza virus undergoes a radical change of antigenicity every 10 years or so, a process known as antigenic shift. The general population has little or no immunity to the new type and an epidemic thus occurs. Immunity to the new type persists, but it undergoes further minor changes known as antigenic drift, which reduce the effectiveness of the existing antibody, thus allowing further attacks to occur.

Chronic virus infections

Certain virus infections are characterized by a chronic course which may last for weeks or months. Typical examples are warts and molluscum contagiosum. Here the infection does not cause lysis of the cell, so that the virus is not exposed to antibody. The cells are stimulated to proliferate and give rise to benign tumours, which increase in diameter by direct infection of cells lying on either side.

In patients in whom the ability to form antibody is congenitally absent

or reduced, or suppressed by treatment with immunosuppressant drugs, an infection which is normally acute may pursue a chronic course. A typical but rare example which is invariably fatal in the absence of treatment is vaccinia gangrenosa. In this condition the lesion of primary smallpox vaccination fails to heal, and continues to enlarge and give rise to metastatic lesions over a period of several months until the extent of tissue destruction is incompatible with survival. In transplant patients under immunosuppression the skin lesions of herpes may pursue a very prolonged course.

The virus infections considered so far have all been of the productive type, in which the growth cycle terminates in the formation of infective virions which proceed to spread the infection. In some conditions, however, infective virions are formed less frequently or not at all. Cell damage then progresses much less rapidly and a chronic infection is set up. Examples of this situation are seen in subacute sclerosing panencephalitis and progressive multifocal leukoencephalopathy. In the former condition electron microsocpy reveals microtubules resembling those of the myxoviruses in the nuclei of neurones, and strains of virus resembling measles have been isolated from brain biopsy material in tissue culture. In progressive multifocal leukoencephalopathy particles resembling those of a papovavirus can be found.

Transforming infections

The main feature of warts and molluscum contagiosum is a benign cell proliferation with production of infective virions. With some viruses this situation is carried a stage further; the infection induces a malignant transformation in the cells, which then proliferate in an uncontrolled manner, and the production of infective virions is reduced or completely abolished. A number of RNA viruses of animals behave in this manner, such as Rous sarcoma virus and various murine leukaemia viruses, and it is a theoretical possibility that a number of human malignant conditions result from infection with a virus of this type. Similar examples occur among the DNA viruses as well. Certain serotypes of human adenovirus will transform cells *in vitro* and induce malignant tumours in hamsters. Marek's disease of chickens is a malignant lymphomatosis due to infection with a virus of the herpes group.

Cell transformation by DNA viruses is of importance in human medicine also. There is some evidence that infection with type 2 herpes virus is linked with the development of cervical carcinoma. Epstein–Barr virus is a virus belonging to the herpes group which is present in a latent form in the cells of Burkitt's lymphoma. The part it plays in the causation of this tumour has not been established beyond doubt, but the extensive laboratory work carried out with the virus led to the chance finding that it is the causative agent of infectious mononucleosis. Lymphocytes infected with