

# Chapter 6

## Viruses and viral infections



Rubella, otherwise known as German measles

### KEY FACTS

- Viruses do not have a cellular structure and the simplest of them consist merely of nucleic acid surrounded by protein.
- All cellular organisms including animals, plants, bacteria, fungi and other microorganisms are vulnerable to virus infections.
- Viruses cannot be grown in simple media on Petri dishes. They can only be grown in suitable host cells.
- They can survive but cannot reproduce in medicines, so they are not potential spoilage organisms.
- Some viruses possess a lipid envelope; these are normally more susceptible to drying, solvents and disinfectants than nonenveloped ones.
- Viruses are not susceptible to common antibacterial antibiotics.
- Most viruses have a fairly limited range of hosts and they are transmitted from one individual to another by a wide variety of mechanisms.
- Some viruses may be latent (dormant) and survive in their host cells without killing them. They may be activated at a later date to reproduce and kill the host cell.
- Some viruses cause cancer.
- Most human viruses survive outside the body only for short periods and most of them are at least as vulnerable to heat, extremes of pH, drying and disinfectants as bacteria.

## 6.1 The pharmaceutical importance of viruses

Viruses are the most abundant organisms on the planet and their numbers exceed the global human population by a factor of  $10^{21}$ , or, putting it another way, for every human being on the planet, there are 1 000 000 000 000 000 000 viruses. They are parasites that can only reproduce inside a host cell; they have no metabolism of their own and possess few, if any, enzymes. All cellular organisms are vulnerable to virus infection; not just animals and plants, but bacteria and all other kinds of microorganisms too. Viruses do not, themselves, have a cellular structure and the simplest of them consist merely of nucleic acid surrounded by protein, so it is even debateable whether or not viruses should be regarded as living organisms. Just as the entire human genome has been mapped in recent years, so too have the genomes of several viruses, and in 2002 the polio virus was the first to be totally synthesized in the laboratory. Because they do not have a cellular structure, viruses do not possess mitochondria, ribosomes or the other organelles that are required for normal cell function and metabolism, and this factor further supports the view that they should be regarded as self-assembling clusters of complex chemicals rather than living organisms.

Viruses cannot be cultured on simple media in Petri dishes and they certainly cannot grow in medicines so, unlike bacteria, they have no potential as spoilage organisms. They can only be grown in an appropriate host cell; this means that a laboratory equipped for the cultivation of fertilized chickens' eggs or mammalian cells must be available for viruses to be studied. Such laboratories are expensive to operate and require skilled personnel so, despite the fact that viruses might survive in a medicine without actually growing in it, tests to detect or count contaminating viruses are rarely undertaken. Fortunately, most of them only survive for a short time outside the host organism (although there are important exceptions, such as hepatitis viruses), so medicines do not normally represent a major source of viral infection.

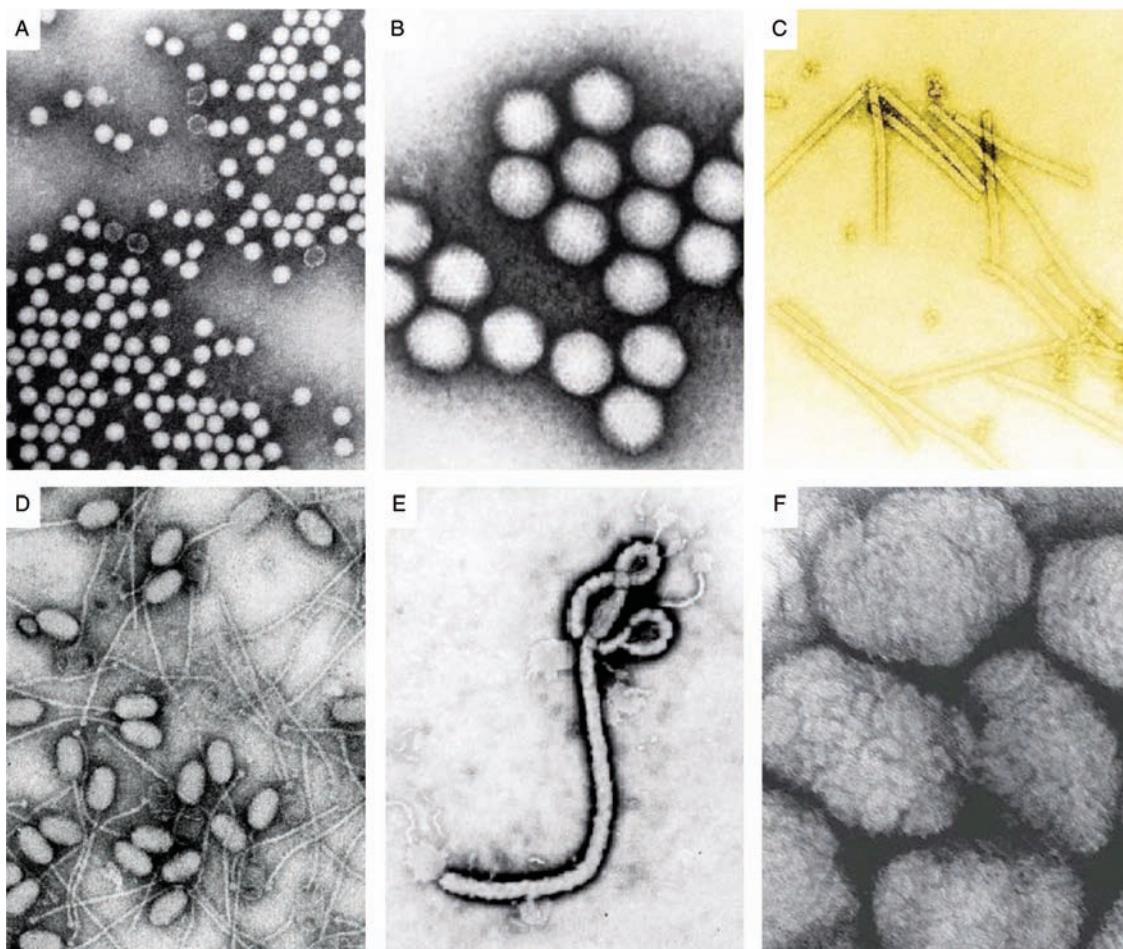
Viruses are totally resistant to the commonly used antibiotics, so they are of pharmaceutical interest not only because of the severity of the infections they may cause but also because of the difficulties of treating such infections. There are, however, two areas where viruses have applications in medicine, although it must be stressed that these are still areas of research and development rather than of widespread established use. They have potential as drug delivery systems, particularly for

diseases like cystic fibrosis that are a consequence of genetic disorders and for which 'gene therapy' might be a realistic option, because viruses may act as a carrier by which 'normal' genes may be introduced into affected human cells and tissues. The second application is for viruses that infect bacteria (bacteriophages, or 'phages' for short) to be used as alternatives to antibiotics for the treatment of human infections. Phage therapy, as it is called, was of more widespread interest in the first half of the twentieth century, but this interest lapsed in Western Europe and North America with the advent of the antibiotic era. The current problem of increasing antibiotic resistance has reawakened interest in phage therapy, but a phage-based medicine is still some way from receiving licensing approval for Western markets.

## 6.2 Virus structure and replication

Although viruses are not the smallest or simplest infectious agent known (viroids, consisting merely of RNA without surrounding protein, and prions, which are self-replicating proteins without nucleic acid, are both smaller and simpler) viruses are the smallest that can readily be seen with an electron microscope. Figure 6.1 shows electron micrographs of six different viruses selected to illustrate their variety of shapes and sizes. The individual virus particles (called virions) typically range from about 20 nm in diameter (Figure 6.1A), which is about one-fiftieth of the size of a typical bacterium like *Staphylococcus aureus*, up to about 350 nm which is the size of the so-called pox viruses (for example, the herpes group and the viruses responsible for chicken pox, shingles and smallpox (Figure 6.1F).

Many, particularly the smaller ones, appear to be approximately spherical (Figures 6.1A and 6.1B), but higher resolution electron microscope images show that the structure is frequently not a true sphere but an icosahedron (comprising 20 triangular sides; Figure 6.2). The protein coat that surrounds the nucleic acid and protects it from mechanical and chemical damage is termed the capsid and is made up of individual units called capsomeres; the nucleic acid and surrounding capsid are together termed the nucleocapsid. The common alternative to an icosahedron is for the capsid to have a helical structure, which, on an electron microscope, appears simply as a straight rod (Figure 6.1C); more complex structures also exist including those with tails, which are common amongst



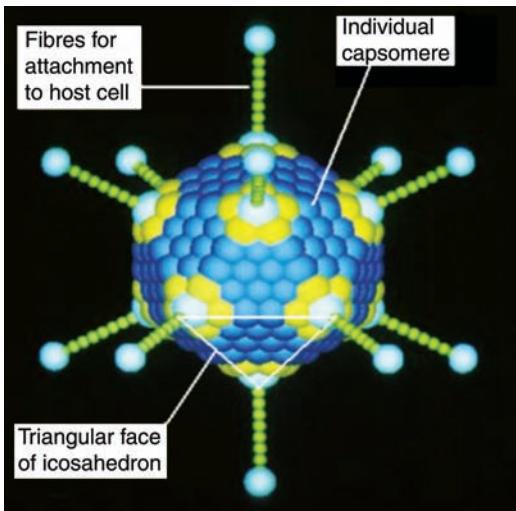
**Figure 6.1** Electron microscope images of viruses illustrating differences in shape and size. As displayed, the photographs reflect their true relative size (in brackets below). A. Polio virus (25–30 nm). B. Adenovirus (causing human respiratory infections, 75–100 nm). C. Tobacco mosaic virus (300 x 20 nm). D. Bacteriophage 3A of *Staphylococcus aureus* (300 nm including the tail). E. Ebola virus (800–1000 nm long). F. Smallpox virus (350 x 250 nm). Sources: A. PHIL ID #235; Photo Credit: Dr. Joseph J. Esposito and F.A. Murphy, Centers for Disease Control and Prevention. B. PHIL ID #237; Photo Credit: Dr. G. William Gary, Jr., Centers for Disease Control and Prevention. C. <http://commons.wikimedia.org/wiki/File:TobaccoMosaicVirus.jpg>. D. [http://commons.wikimedia.org/wiki/File:Phage\\_de\\_S\\_aureus\\_3A.jpg](http://commons.wikimedia.org/wiki/File:Phage_de_S_aureus_3A.jpg). E. PHIL ID #1181; Photo Credit: Frederick A. Murphy, Centers for Disease Control and Prevention. F. PHIL ID #2292; Photo Credit: Frederick A. Murphy, Centers for Disease Control and Prevention.

bacterial viruses (Figure 6.1D), and long curved or coiled filaments (Figure 6.1E).

When a virus infects a human cell it may cause immediate death and lysis of that cell, or it may cause a persistent infection whereby the cell is not immediately killed but releases (sheds) new virus particles steadily over a period of time. In order to persistently shed new virions however, the virus must avoid damaging the host cell membrane and it does this by allowing the newly created virions to 'bud' from the host cell (Figure 6.3).

Budding involves the new virus particle becoming wrapped in host cell membrane, which, on its outer

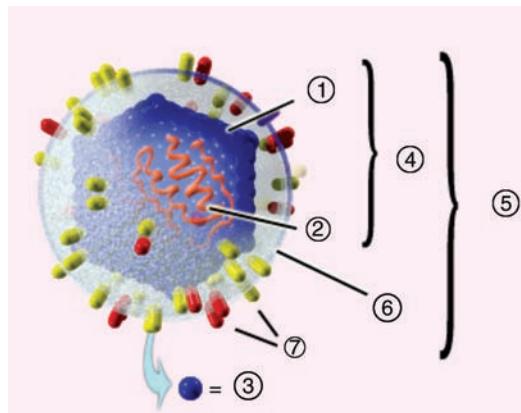
surface, has viral receptor glycoproteins that enable the new virion to attach to a new host cell; an envelope without such receptors would much reduce its infective capacity. Viruses that have adopted this shredding strategy therefore, are said to be 'enveloped' because they have a phospholipid bilayer membrane around them. The envelope does not afford any significant degree of protection against physical or chemical damage and is, itself, easily removed or damaged by surfactants and lipid solvents. So, although it might be expected that an additional layer outside the capsid would make the virus particle more robust, in fact the opposite is the case and



**Figure 6.2** Computer-generated diagram of adenovirus showing its icosahedral structure. *Source:* Adapted from an image by Dr. Richard Feldmann, National Cancer Institute; <http://commons.wikimedia.org/wiki/File:Adenovirus.jpg>.

enveloped viruses, such as herpes, measles, mumps, rubella, chicken pox, influenza and HIV, tend to be more sensitive to disinfectants and lipid solvents than nonenveloped ones, such as polio, adenovirus, rhino virus (common cold) and hepatitis A (see below).

Viruses normally contain only one type of nucleic acid, either DNA or RNA, which may be single or double stranded and is tightly coiled to fit into the available space within the capsid; it typically codes for between 10 and 200 genes depending on the size of the virus. Retroviruses, including HIV, are exceptional in that they contain both nucleic acids. The type of nucleic acid, capsid symmetry (icosahedral, helical or complex), the



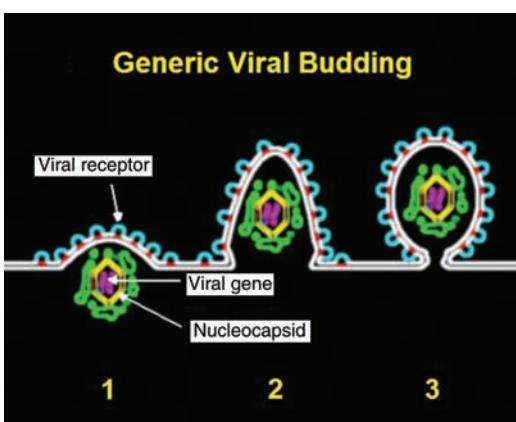
**Figure 6.4** Structural components of a generalized virus particle: 1. capsid; 2. nucleic acid; 3. individual capsomeres; 4. nucleocapsid, 5. virion; 6. envelope; 7. spike glycoproteins. *Source:* <http://commons.wikimedia.org/wiki/File:Virion.png>.

possession of an envelope or not, and the nucleic acid structure (single or double stranded) are the criteria by which viruses are classified. In addition to the structural (capsid) protein, viruses possess protein in the form of enzymes (exceptionally up to ten different types, but usually fewer, or none at all), which are commonly but not exclusively associated with reproduction of the viral genome. Thus the most complex virus is likely to exhibit a structure similar to that shown in Figure 6.4 and contain nucleic acid, protein, phospholipid and glycoproteins (sometimes referred to spike proteins).

## 6.3 Viral infections

Viruses usually have quite a limited host range. Rabies virus is rather unusual in that it can infect a variety of mammals; smallpox on the other hand has just one host – humans. Most mammalian viruses are somewhere between these two extremes and are able to infect a small number of related species – for example HIV, which infects humans and some other primates. Many bacteriophages are even more specific, attacking only a few strains within a species.

A virus must have an effective mode of transmission from one person to another. If it regularly killed its human host before being transmitted to another individual it would have a reduced chance of survival and would risk extinction. Transmission is often described as being either vertical (meaning through generations – from pregnant mother to embryo or from mother to baby via breast milk), or horizontal, where the virus is transmitted from one individual to another of the same species and they are not in a parent-child relationship.



**Figure 6.3** Viral budding. *Source:* [http://en.wikipedia.org/wiki/File:Budding\\_of\\_generic\\_virus,\\_pictorial\\_representation.jpg](http://en.wikipedia.org/wiki/File:Budding_of_generic_virus,_pictorial_representation.jpg).

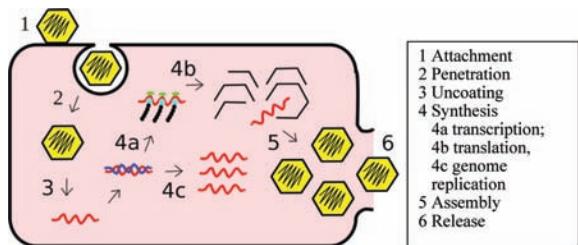
Several modes of transmission occur and these, not surprisingly, are related to the principal site of the virus infection in the body.

- Via droplet nuclei (particles expelled into the atmosphere during sneezing, coughing or talking), for example influenza, common cold, measles and other viruses infecting the respiratory system.
- The faecal-oral route is the common means of transmission for viruses whose *primary* infection site is the gastro-intestinal tract or associated organs, for example hepatitis A and polio.
- Via sexual intercourse, for instance HIV/AIDS, hepatitis B, genital herpes and cervical cancer.
- Insect vectors transmit dengue fever virus, West Nile disease and tickborne encephalitis.
- Direct contact with infected patients or contaminated objects by which the virus is introduced onto the skin, for example warts and verrucae and, in some cases, then into the blood stream by skin damage following scratching, for instance pox viruses.
- By direct introduction into the blood stream, for example hepatitis B, contaminating addicts' syringes and needles, and rabies following animal bites.

The ease by which an infection is transmitted via an aerosol depends upon humidity, because the virus is initially expelled from the infected lung as a mucus-coated droplet, but in low humidity (e.g. centrally heated houses in winter) the mucus rapidly dries so the aerosolized particle becomes lighter and remains suspended in the air for longer; it is partly for this reason that the common cold and influenza are more widespread in winter. Because viral envelopes are susceptible to damage by heat and drying the viruses that possess them normally survive outside the body only for short periods, typically a few hours, so they are more reliant on rapid person-to-person transmission via aerosols or direct contact.

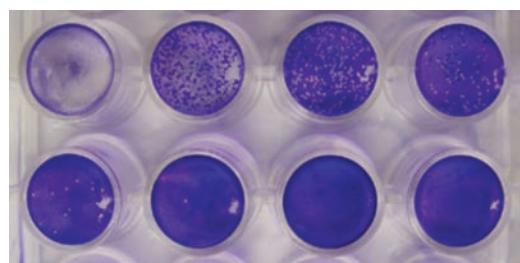
In order to replicate, a virus needs to be able to recognize and attach to a host cell, then to get inside; merely being adsorbed onto the outer surface of the cell is not enough. Recognition is achieved via the glycoproteins, which are the spikes of enveloped viruses or components of the capsid of nonenveloped viruses (Figure 6.2); these attach to receptor molecules on the host. Penetration is achieved via a process which may be considered the opposite of budding, whereby the attached virus is engulfed by the host plasma membrane (Figure 6.5).

After entering the cell, the infecting virus is transported to the area where the new virions will be assembled: RNA viruses remain in the cytoplasm, whereas DNA viruses go to the nucleus. The capsid is



**Figure 6.5** Virus replication cycle. Source: Wikimedia Commons: [http://commons.wikimedia.org/wiki/File:Virus\\_Replication\\_Cycle.svg](http://commons.wikimedia.org/wiki/File:Virus_Replication_Cycle.svg).

removed (termed 'uncoating') to expose the nucleic acids to the enzymes responsible for transcribing the viral genes. This is followed by assembly of the new virus particles and their release by budding or cell lysis. The time required for the complete replication cycle depends upon the environmental conditions and the generation time of the host cell. In rapidly growing bacterial cultures infection with a phage particle can lead to the creation and release by cell lysis of approximately 200 new virions (this is known as the 'burst size') within 20 minutes, but the process is much slower in mammalian cells and the burst size more variable; in some cases thousands or tens of thousands of new viruses are released. The liberated particles infect adjacent cells, and if those cells are themselves attached to, and covering, a surface (either a biological membrane as in a fertilized chicken's egg or the plastic surface of a laboratory culture vessel for



**Figure 6.6** Virus plaque assay. Kidney cells were grown to completely cover the bottom of wells in a plastic tray. The wells were infected with successive fourfold dilutions of herpes simplex virus and the cells cultured overnight to allow the formation of plaques (so the well at the top left received 4 times the virus concentration of the well immediately to its right, which, in turn, received 4 times the concentration of the third one in the row, and so on). The living cells were stained blue, but the plaques containing no living cells (seen most clearly in the two wells on the top right) show up as small colourless circular zones in the otherwise uniform blue cell monolayer. Source: [http://commons.wikimedia.org/wiki/File:Plaque\\_assay\\_dilution\\_series.jpg](http://commons.wikimedia.org/wiki/File:Plaque_assay_dilution_series.jpg).

example), the successive replication cycles produce a circular zone of cell lysis of several mm diameter called a plaque. If it is assumed that each plaque is formed from a single virus particle or an aggregate of several particles (either being described as a plaque forming unit – abbreviated to PFU) their formation and numbers form the basis for a method of counting viruses. This is called a 'plaque assay' and it would be required, for example, during the manufacture of a viral vaccine (Figure 6.6).

### 6.3.1 Latent viral infections

When a virus infects a human cell it usually reproduces and causes the cell to die and lyse in order to release the new virus particles, but that does not always happen. Some viruses may enter a latent (dormant) state in a small fraction of the infected cells; they do not cause immediate damage but give rise to a persistent infection. The dormant virus may exist free in the cytoplasm or become incorporated into the host cell's DNA and remain there for long periods – possibly throughout the life of the person concerned. In either case, suitable stimuli can reactivate the latent virus and so cause it to reproduce and kill the cell in the 'normal' manner. In humans, this reactivation may occur days, months or many years after the initial infection.

This situation is particularly common amongst herpes viruses, so cold sores due to herpes simplex (Figure 6.7) may recur following the reactivating stimulus of exposure to the ultraviolet component of sunlight or another viral infection (typically a common cold – hence the name), and the varicella zoster virus, which causes chicken pox in childhood, may be reactivated to cause shingles in adult life. Retroviruses, including HIV, become integrated into the host DNA and are almost

impossible to remove without killing the cell. The phenomenon of viral latency also occurs amongst bacteriophages and is a mechanism by which antibiotic resistance genes are transferred from one bacterial cell to another by means of a 'phage vector' (see the passage on transduction in Chapter 13).

### 6.3.2 Viral cancers

In addition to causing some of the most severe infections with the highest mortality rates and being implicated in the spread of antibiotic resistance, viruses have another major impact upon human health in that they can initiate several forms of cancer. It has been estimated that in 2002 approximately 1 in 6 human cancers worldwide were of viral origin, with the human papilloma viruses being responsible for approximately 5% of the total (mostly cases of cervical cancer) and the hepatitis B and C viruses together being responsible for a further 5% (causing liver cancer). Some herpes viruses (Figure 6.8), HIV and the human T-cell leukaemia viruses are also associated with cancer, though significantly less frequently than papilloma and hepatitis viruses. The term 'oncogenic' is sometimes used to describe viruses with cancer-causing ability and these have been the subject of intensive research in recent years because of the possibility of creating vaccines that would protect susceptible individuals against the forms of cancer in question. The first fruits of that research have been the two forms of cervical cancer vaccine first marketed worldwide in 2006.



**Figure 6.7** A 'cold'sore' resulting from a persistent herpes simplex infection. *Source:* PHIL ID #1573; Photo Credit: Dr. Herrmann, Centers for Disease Control and Prevention.



**Figure 6.8** Kaposi's sarcoma before (left) and after treatment with interferon. This cancer is caused by human herpes virus 8. *Source:* National Cancer Institute; [http://commons.wikimedia.org/wiki/File:Kaposi%27s\\_sarcoma\\_before.jpg](http://commons.wikimedia.org/wiki/File:Kaposi%27s_sarcoma_before.jpg) (left). National Cancer Institute; [http://commons.wikimedia.org/wiki/File:Kaposi%27s\\_sarcoma\\_after.jpg](http://commons.wikimedia.org/wiki/File:Kaposi%27s_sarcoma_after.jpg) (right).

**Table 6.1** Activity and required contact time for disinfectants acting on enveloped and nonenveloped viruses.<sup>a</sup>

Active ingredient	Enveloped viruses <sup>b</sup>	Nonenveloped viruses <sup>c</sup>	Contact time required (min)
Ethanol-based 60–95%	Good	Poor	Rapid 0.5–2.0
Hypochlorite (4–6% chlorine)	Good	Good	Rapid 0.5–2.0
Iodine-based 0.5–5%	Good	Fair	Medium 5–10
Phenols 0.2–3%	Fair	Poor	Medium 5–10
Quaternary ammonium compounds 2%	Good	Poor	Medium 5–10
Hydrogen peroxide 3% or less	Fair	Poor	Slow 10–20

<sup>a</sup>Based on New York State's categorization of disinfectants for hospital use.

<sup>b</sup>Including herpes, simplex, HIV, hepatitis C, cytomegalovirus, measles, mumps, rubella, influenza, respiratory syncytial virus, varicella zoster, coronavirus and hepatitis B, which, although not strictly enveloped, has similar sensitivity.

<sup>c</sup>Including hepatitis A, coxsackie, polio, rhinovirus, human papilloma virus, adenovirus, rotavirus and parvovirus.

## 6.4 Virus survival outside the body and susceptibility to disinfection

Apart from their possible roles as a drug-delivery system and in phage therapy (mentioned above) the main pharmaceutical interest in viruses is killing them, or at least preventing or treating the infections they cause. An understanding of their potential to survive outside the body and their susceptibility to physical and chemical methods of inactivation is clearly relevant in pharmaceutical science.

The environmental factors that influence a virus's survival outside the body include temperature, pH and moisture availability, whilst its possession of a lipid envelope (or not) will strongly influence its susceptibility to detergents and solvents like ethanol or isopropanol, which are commonly used as the basis for disinfectants. Viruses are not particularly heat resistant so pasteurization is a means by which they can be removed. Just as bacteria vary in their sensitivity to heat, so too do viruses, so it is difficult to quote a single temperature/time combination that is certain to destroy all viruses, nevertheless a temperature of 60 °C for periods between 1–10 hours has been found satisfactory for removing different viruses from liquid blood products, but removal of hepatitis from dried blood products requires temperatures of at least 80 °C and significantly longer exposures.

Viruses similarly vary in their sensitivity to extremes of pH. Most human pathogenic viruses are inactivated by acid, and exposure to pH 4 for 6 hours is sufficient to kill some sensitive viruses, whereas several days'

exposure may be required for more resistant species. Enteroviruses – those causing infection in the gastrointestinal tract – are transmitted by the faecal-oral route so they survive transient exposure to stomach acid at pH 1–3.

Ultraviolet (UV) light damages nucleic acids and so has the potential to kill all kinds of microorganisms. Viruses are at least as susceptible to UV light as bacteria, and both groups of organisms are readily killed by the germicidal lamps that are used to decontaminate both water (used for medicines' manufacture) and air (as in biological containment cabinets and operating theatres).

Enveloped viruses are generally much more sensitive to the effects of drying, detergents, organic solvents and disinfectants than nonenveloped ones because any process or chemical that damages or removes the lipid envelope is likely to render the virus particle noninfective, which, for practical purposes, is the same as dead. Despite their relatively greater sensitivity however, some enveloped viruses may survive on dry solid surfaces for a significant period of time; influenza virus, for example, despite its normal mode of transmission as an aerosol, has been shown to survive and remain an infection hazard for 24–48 hours on stainless steel or plastic, and hepatitis A (nonenveloped) can survive in dried faeces for 30 days or more. Table 6.1 shows the relative efficacies of some frequently used disinfectants against many of the common enveloped and nonenveloped viruses.

## Acknowledgement

Chapter title image: PHIL ID #10145; Centers for Disease Control and Prevention.

