CHAPTER 3

Virus Disease in Populations and Individual Animals

Since viruses must replicate to survive, actively infected populations are the usual source of infection. Some viruses, such as poxviruses, have a high resistance to desiccation. Smallpox virus can remain actively infectious in soiled clothing, in contaminated households, and in soil for several years. The last documented cases of smallpox in Somalia were apparently acquired from contaminated soil. The persistence of some viruses in fecal material is also a potential long lasting, essentially passive, reservoir of infection. Aerosols of infectious hantavirus and canine **parvovirus** can be infectious for many months after secretion. Also, some viruses, especially hepatitis A virus, can be isolated from contaminated water sources for several days or even weeks after inoculation.

Even though infectious virus can be maintained for a time in a passive state, in nature the ultimate source of a viral pathogen is an active infection in another host. The two most usual reservoirs for human disease are other humans or other animals. Modes of spread of some human viruses are illustrated in Fig. 3.1, and pathogenic viruses and their reservoirs discussed in this section are listed in Table 3.1.

Some viruses with human reservoirs

The majority of human viruses leading to either mild or life-threatening disease are maintained in human populations. The list runs the gamut from colds caused mainly by rhinoviruses, warts caused by papillomaviruses, to HIV. The mode of passage of viruses between humans (i.e., the vector) is intimately involved with human behavior. This behavior can be modified by the disease

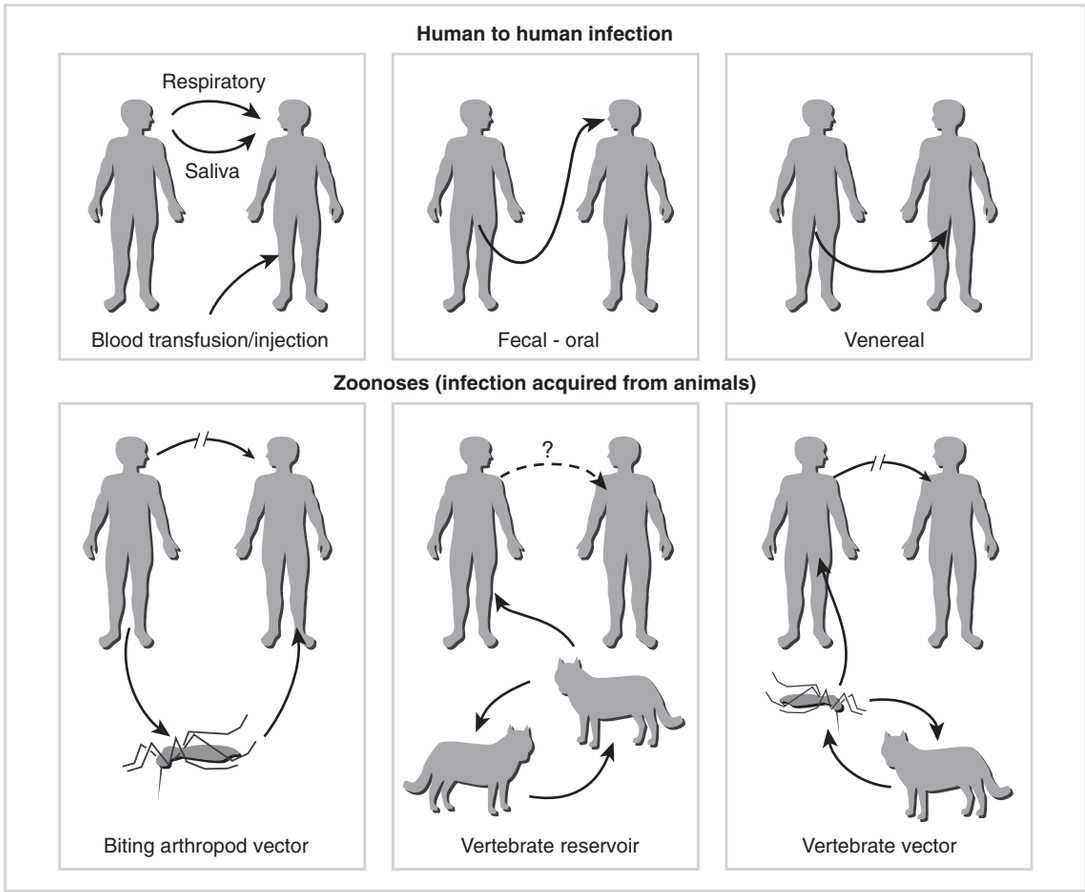

Fig. 3.1 Some transmission routes of specific viruses from their source (reservoir) to humans. The mode of transmission (vector) is also shown. (Based on Mims C. A., and White D. O. *Viral pathogenesis and immunology*. Boston: Blackwell Science, 1984.)

Table 3.1 Some pathogenic viruses and their vectors.

Virus	Vector	Host	Disease
Poliovirus	Human – fecal contamination of water or food	Human	Enteric infection, in rare cases CNS infection (poliomyelitis)
Western equine encephalitis	Mosquito	Horse	Viral encephalitis in the horse–occasional infection of human
La Crosse encephalitis	Mosquito	Squirrel, fox, human	No obvious disease in squirrel or fox; viral encephalitis in human
Sin nombre (*Hantavirus*)	Deer mouse	Deer mouse, other rodents	Hantavirus hemorrhagic respiratory distress syndrome
HIV	Direct injection of virus-infected body fluids into blood	Human	AIDS
Measles	Aerosol	Human	Skin rash, neurological involvement
Yellow fever	Mosquito	Tropical monkeys	Malaise, jaundice
Dengue fever	Mosquito	Human, mosquito, primates	Mild to severe hemorrhagic disease

Table 3.1 *Continued*

Virus	Vector	Host	Disease
Ebola	Unknown	Unknown	Often fatal hemorrhagic fever
Hepatitis A	Fecal contamination of water or food	Human	Acute hepatitis
Hepatitis B	Direct injection of blood	Human	Chronic hepatitis, liver carcinoma
Hepatitis C	Direct injection of blood	Human	Acute and chronic hepatitis
Hepatitis delta	Blood, requires coinfection with hepatitis B	Human	Acute hepatitis
Hepatitis E	Fecal contamination of water or food	Human	Mild acute hepatitis except often fatal to pregnant women
Rabies	Bite of infected animal	Vertebrates	Fatal encephalitis
Herpes simplex (HSV)	Saliva, other secretions	Human	Surface lesions followed by latency, rare encephalitis
Varicella-zoster (VZV, chicken pox)	Aerosol	Human	Rash, shingles, latency
Epstein-Barr (EBV)	Saliva	Human	Infectious mononucleosis, latency
Influenza	Aerosol	Human, many vertebrates	Flu
Smallpox	Aerosol	Human	Variola
Myxoma	Insect bite	Rabbits	Variable mortality, skin lesions
Rhinovirus	Aerosol	Human	Colds
Coronavirus	Aerosol	Human	Colds
Rubella (German measles)	Aerosol	Human	Mild rash, severe neurological involvement in first-trimester fetus
Adenovirus	Aerosol, saliva	Human	Mild respiratory disease
Papillomavirus	Contact	Human	Benign warts, some venereally transmitted, some correlate with genital carcinomas
HTLV (human T-cell leukemia virus)	Injection of blood	Human	Leukemia
Tomato spotted wilt (bunyavirus)	Thrip	Broad range of plant species	Necrosis of plant tissue, destruction of crops
Cadang-cadang (viroid)	Physical transmission via pruning	Coconut palm	Coconut palm pathology
Prion (protein pathogen?)	Ingestion or inoculation of prion protein	Human, other mammals have specific types, cross species spread possible	Noninflammatory encephalopathy
Plant rhabdoviruses	Leaf hoppers, aphids, plant hoppers	Broad range of plant species	Necrosis of plant tissue, destruction of crops

symptoms themselves. Thus, a respiratory infection leads to coughing and sneezing, which spreads an aerosol of droplets containing virus. HSV is spread in saliva, but is not spread by an aerosol; rather, it requires direct transfer of an aqueous suspension. In contrast, the closely related varicella zoster virus (VZV), the agent of chicken pox, is spread by aerosols. With HIV, body fluids, including blood, serum, vaginal secretions, and seminal fluid, are sources of infection. The virus can be spread by passive inoculation of, for example, a contaminated hypodermic syringe, by transfusion, or by sexual activity.

Some viruses with vertebrate animal reservoirs

While the majority of human viral diseases are maintained in the human population itself, some important pathogens are maintained primarily in other vertebrates. A disease that is transmissible from other vertebrates to humans is termed a **zoonosis**. Rabies is a classic example of a zoonosis that affects humans only sporadically. Because humans rarely transmit the virus to other animals or other humans, infection of a human is essentially a dead end for the virus. The rabies virus, which is transmitted in saliva via a bite, is maintained in populations of wild animals, most generally carnivores. The long incubation period and other characteristics of the pathogenesis of rabies mean that an infected animal can move great distances and carry out many normal behavioral patterns prior to the onset of disease symptoms. These symptoms may include hypersensitivity to sound and light, and finally, hyperexcitability and frenzy. Except in rare instances of inhalation of aerosols, humans only acquire the disease upon being bitten by a rabid animal; however, the fact that the disease can be carried in domestic dogs and cats means that when unvaccinated pets interact with wild animal sources, the pets can then transmit the disease to humans. Vaccination of pets provides a generally reliable barrier.

Viral zoonoses often require the mediation of an arthropod vector for spread to humans. The role of the arthropod in the spread can be mechanical and passive in that it inoculates virus from a previous host into the current one without virus replication having occurred (a favored route with animal poxviruses), but the arthropod's role as a vector can be dynamic. For viruses with RNA genomes that are transmitted between hosts via arthropods (such as those responsible for yellow fever, a number of kinds of encephalitis, and dengue fever), virus replication in the vector provides a secondary reservoir and a means of virus amplification. This makes spread to a human host highly efficient since even a small inoculation of the virus into the arthropod vector can result in a large increase in virus for transmission to the next host.

VIRUSES IN POPULATIONS

Most (but certainly not all) virus infections induce an effective and lasting immune response. Some of the basic features of this response are described in Chapters 7 and 8. An effective immune response means that local outbreaks of infection result in the formation of a population of resistant hosts. This means that any virus that induces protective immunity must maintain itself either in another reservoir or by dynamically spreading in "waves" through the population at large. If enough members of the susceptible population become immune, virus cannot spread effectively and it becomes extinct. This herd immunity is a major factor in both gradual and abrupt changes in the virulence of many viruses resulting from the random acquisition of genetic alterations.

The occurrence of mild respiratory infections (such as a common cold) in isolated communities provides graphic evidence of this phenomenon. For example, when scientists visit the Antarctic research stations at the beginning of the Antarctic summer, they bring in colds to infect the resident population. When scientists stop arriving with the onset of winter, the prevailing respiratory diseases run their course and disappear. Figure 3.2 charts a classic epidemiological study of

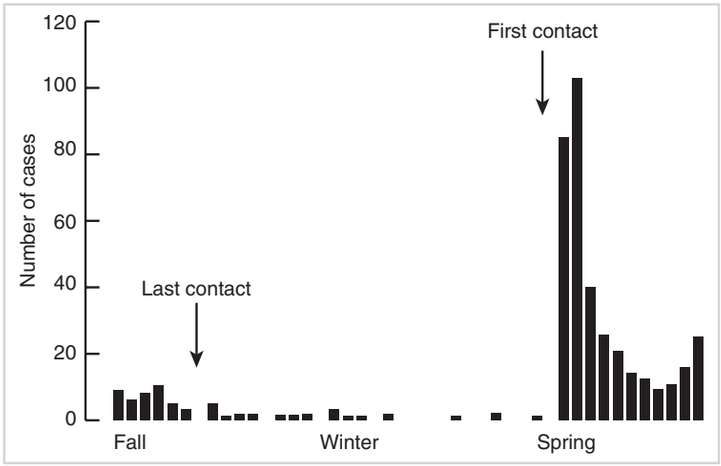

Fig. 3.2 Occurrence of respiratory illness in an arctic community (Spitzbergen Island off Norway) that is isolated during the winter months. Following the last boat communication with the European mainland, the number of respiratory illnesses declines from a low number to almost nil. With the first boat arriving in the Spring, new serotypes of respiratory viruses are communicated from the crew and passengers and a miniepidemic occurs. As the virus passes through the population, resistance builds and infections decline to a low level. (Based on data originally published by Paul, J. H., and Freese, H. L. An epidemiological and bacteriological study of the "common cold" in an isolated Arctic community (Spitsbergen [sic]). *American Journal of Hygiene* 1933;7:517.)

respiratory illness in an isolated fishing and mining population on Spitzbergen Island in the Arctic Ocean. Note, that after the last contact with the "outside world," the incidence of such viral borne respiratory infections rapidly declines to an undetectable level.

Generation of lasting immunity provides an effective means of controlling and even eradicating certain viral diseases. The antigenic stability of the smallpox virus and effective immunity against it allowed effective vaccination programs to eradicate the disease from the population. Polio and measles are current candidates for partial or total elimination from the population due to availability of effective vaccines. In addition, currently a program is underway to try to vaccinate wild populations of raccoons and other small carnivores against rabies with use of vaccine-laced bait. It is hoped that such an approach will reduce or eliminate the growing incidence of rabies in US wild animal populations. Of course, the reason for this solicitude has little to do with the animals involved; rather, it is to afford protection to domestic animals, and ultimately to humans.

Despite our considerable abilities, not all virus diseases can be readily controlled even under the most favorable economic and social conditions. Flu virus variants arise by genetic mixing of human and animal strains, and it is not practical to attempt a widespread vaccination campaign with so many variables. HIV remains associated with lymphatic tissue in infected individuals even when antiviral drugs effectively eliminate virus replication. The intimate association of HIV with the immune system may make vaccination campaigns only partially effective. The ability of herpesviruses to establish latent infections and to reactivate suggests that a completely effective vaccine may be difficult if not impossible to generate.

A major obstacle to the control of viral and other infectious diseases in the human population as a whole is economic. It costs a lot of money to develop, produce, and use a vaccine. Many of the nations most at risk of deadly infectious disease outbreaks are financially unable to afford effective control measures, and pharmaceutical corporations involved with vaccine research and production are primarily interested in bottom-line profit. Perhaps more tragically, some nations at risk also lack the political will and insight to mount effective efforts to counter the spread of viral disease. Such problems constantly change character but are never ending.

ANIMAL MODELS TO STUDY VIRAL PATHOGENESIS

The great German clinical microbiologist Robert Koch formulated a set of rules for demonstrating that a specific microorganism is the causative agent of a specific disease. These rules are very much in force today. In essence, Koch's rules are as follows:

1 The same pathogen must be able to be cultured from every individual displaying the symptoms of the disease in question.
2 The pathogen must be cultivated in pure form.
3 The pathogen must be able to cause the disease in question when inoculated into a suitable host.
While these rules can be applied (with *caution*) to virus-mediated human diseases, it is clearly not ethical to inoculate a human host with an agent suspected to cause a serious or life-threatening disease (criterion 3). Regrettably, this ethical point has been missed more than once in the history of medicine. Examples of the excesses of uncontrolled human experimentation stand as a striking indictment of Nazi Germany, but excesses are not confined to totalitarian forms of government. The infamous Tuskegee syphilis studies are an example of a medical experiment gone wrong. These studies, ostensibly to evaluate new methods to treat syphilis, were carried out on a large group of infected black men in the rural southern United States by physicians of the US Public Health Service in the 1930s and 1940s. Even though effective treatments were known, a number of men were treated with **placebos** (essentially sugar pills) to serve as "controls" and to allow the physicians to accumulate data on progression of the disease in untreated individuals. Other examples of potentially life-threatening experiments with little effort to explain the dangers or potential benefits (the criteria for **informed consent**) using volunteer prisoners as test subjects are also well documented.

This discussion should not lead to the conclusion that it is never appropriate to use human subjects to study a disease or its therapy. Human experimentation is critical to ensuring treatment safety and effectiveness, but to do such studies in an ethical manner, the risks and benefits must be fully understood by all those involved.

One extremely effective way to obtain reliable data on the dynamics of disease and its course in an individual is to develop an accurate animal model. A researcher's need to experimentally manipulate variable factors during infection in order to build a detailed molecular and physiological picture of the disease in question can only be accomplished with a well-chosen model. The lack of a suitable animal model for a viral disease is almost always a great impediment to understanding its control and treatment.

Another important reason for using an animal model to study virus infection is that useful information can often be obtained with very simple experimental processes. The ability of a virus to cause specific symptoms can be determined by careful control of the viral genotype and site of inoculation in the animal, followed by observation of the symptoms as they develop. The passage of a virus throughout the body during infection can be studied by dissection of specific organs, careful gross and microscopic observation, and simple measurement (**assay**) of virus levels in those organs. The host response to infection can be determined (in part, at least) by measuring the animal's production of antibodies and other immune factors directed against viruses.

More detailed and specific information concerning the interaction between a virus and its animal host can be obtained by using more sophisticated techniques, many of which are outlined in Part III. For example, transcription of a portion of the HSV genome in latently infected neurons can be observed by use of sophisticated methods to detect viral RNA in tissue *in situ*. Viral genomes integrated into the genomes of specific cells in an animal can be detected and characterized using restriction enzyme analysis and hybridization techniques. Specific immune responses can be assayed and localized by use of involved immunohistochemical methods. All these techniques add detail and richness to the "picture" of the virus–host interaction, and all are required for a full understanding of the interaction between virus and cell and virus and host. However, none of these techniques is required for basic knowledge. The basics can be obtained by using the most simple and readily applied experimental tools: observation, dissection, and measurement of virus.

There are also ethical problems with the use of animals, and the suffering caused must be thoroughly considered in the design of appropriate experimental protocols. For example, an

experimental study that establishes important aspects of a disease may be too devastating to repeat as a casual laboratory exercise. Further, and very importantly, animal models often only approximate the disease of interest, as it would occur in humans.

Despite very real problems with the use of animals to study virus-caused disease, it often is the only way to proceed. Careful and accurate clinical observations of infected individuals, animals, or plants provide many details concerning the course of viral infection. But only in a complete plant or animal model can the full course of disease and recovery as a function of controlled variations of infection and physiological state of the host be studied. This is true even when many aspects of virus infection can be studied in cultured cells and with cloned fragments of the viral genome.

Working with plants may be slow owing to their generation times, but working with experimental animals poses more serious problems. Animals are expensive to obtain and keep. They require significant care in handling and studying, for both humane and "hard" scientific reasons. It would be pointless to invest time, effort, and expense in the study of a disease in starving, improperly caged, or unclean animals.

Still, any experimental animal model for a human disease is a compromise with the real world. For example, the amounts of virus inoculated into the animal and the site of inoculation (i.e., mouth, eye, **subcutaneous** tissue, intracerebral tissue) must be the same for all test subjects, a situation very different from the "real" world. Also, the model disease in the animal may well be different, in whole or in part, from the actual disease seen in a human population. Genetic makeup of the animal (inbred, outbred, specific genetic markers present or absent), age, and sex of the infected host must be controlled to generate interpretable and reproducible results. Obviously, while certain diseases favor certain age groups, an infected population will evidence a wide range of variation in genetic and physical details.

Another complication is that the viral pathogen usually must be specifically adapted to the test animal. Virus directly isolated from an infected population often will not provide a dependable set of experimental **parameters** of infection. In addition, safety considerations must be taken into account. Working with virus characterized by a very high mortality rate, such as that caused by Ebola virus, would require heroic and expensive precautions and containment facilities for study.

The animal models for virus disease described in this chapter demonstrate some of the methods, successes, and limitations involved in the use of animals. Despite the problems associated with working with experimental animals, some basic data could not have been and cannot be obtained any other way.

A mouse model for studying poxvirus infection and spread

Many of the models developed for the study of virus pathogenesis involve the use of mice. These animals have an excellent immune system, can be infected with many viruses adapted from human diseases, and are relatively inexpensive to use. Frank Fenner's studies on the pathogenesis of mouse pox carried out in the 1950s provided a classic model for experimental study of viral pathogenesis.

Although smallpox virus is extinct in the wild, the recent realization that smallpox has been extensively studied as a weapon, possibly in the possession of terrorists, brings these classic studies into sharp focus. Further, other animal poxviruses such as monkey pox can infect humans, and human encroachment of tropical habitats has led to significant occurrence of this disease in tropical Africa. Another poxvirus, myxoma virus, is endemic in rabbit populations in South America, and was used in a temporarily successful attempt to control the ecological threat posed by the high rate of rabbit multiplication in Australia. While touted at the time as an example of successful biological control, numerous complications occurred with its use. Thus, this "experiment" is a valuable example of the benefits and problems involved with biological control.

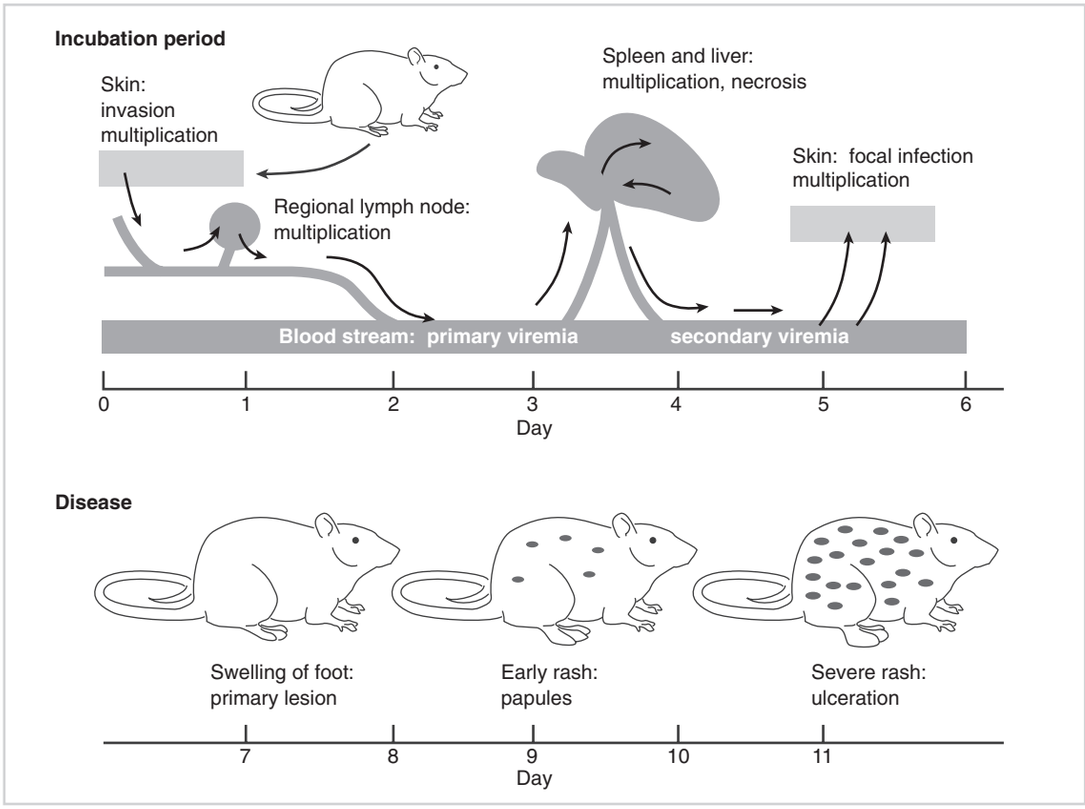

Fig. 3.3 The course of experimental poxvirus infection in laboratory mice. Virus is inoculated at day 0 in the footpad of each member of a large group of genetically equivalent mice. Mice are observed daily, and antibody titers in their serum are measured. Selected individuals are then killed, and various organ systems assayed for appearance and presence of virus. Note that symptoms of the disease (rash and swollen foot) become noticeable only after a week.

In a classic study of mouse pox pathogenesis, virus is introduced by subcutaneous injection of the footpad, and virus yields in various organs, antibody titer, and rash are scored. As noted, the basic experiment thus requires only careful dissection of the infected animal, measurement of virus titers, and careful observation. The patterns of virus spread and the occurrence of disease symptoms are illustrated in Fig. 3.3.

Of course, the model is just that; it does not completely describe virus infection in the wild. An example of a significant deviation from one "natural" mode of infection is when poxvirus is transmitted as an aerosol, leading to primary infection in the lungs. This is a difficult infection route to standardize and is only rarely utilized. Also, examining single animals in the laboratory ignores the dynamics of infection and the interactions between virus and the population. As a consequence, genetic changes in virus and the host, both of which are the result of the disease progressing in the wild, are ignored.

Reovirus infection of mice – the convenience of a virus with a fragmented genome for identifying genes involved in pathogenesis

Reoviruses contain a genome made up of 10 specific fragments of double-stranded RNA (**dsRNA**). Their structure and replication are described in Chapter 16. For the present, it is sufficient to be aware that each of the 10 segments encodes essentially one protein. Most of these pro-

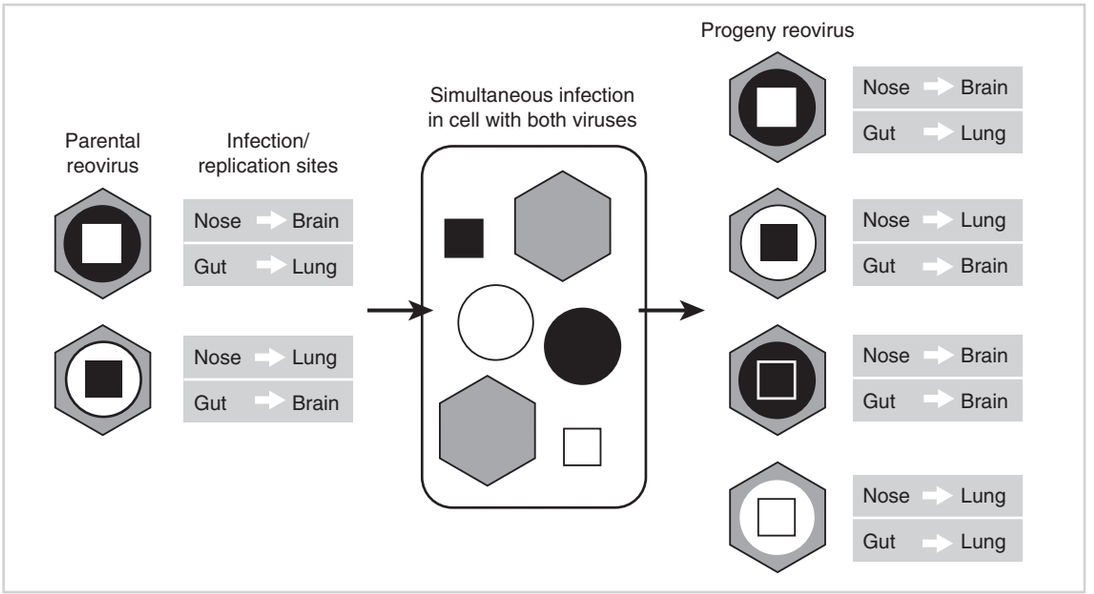

Fig. 3.4 Schematic representation of the relationship between reovirus genotypes and the phenotypes of pathogenesis in newborn mice. The two capsids indicate two different genotypes of the virus. The first contains a solid circle and an open square to represent 2 of the 10 segments of the viral genome, while the second genotype has an open circle and a solid square to represent the same segments with different genetic characteristics. The first genotype is able to cause an infection of the brain of newborn mice when introduced in the nose, and an infection of the lung when introduced into the gut. The second genotype causes the converse: A nasal infection leads to virus replication in the lung and a gut infection leads to virus infection of the brain. Infection of a cell with both viruses leads to mixing of all the viral genomic segments and the new virus can either be parental genotypes or two new genotypes, depending on which genomic segments are packaged. Each genotype can be propagated by itself using careful virological techniques that are described in Part III of this text. The two new genotypes demonstrate the different pathogenic phenotypes shown. This result suggests that the ability of the virus to enter the brain or lung from a gut infection is determined, in part, by the genomic segment characteristics represented with the square. The ability of the virus to enter the brain or lung from a nasal infection is determined, in part, by the genomic segment characteristic represented with the circle. In practice, the results are more complex and interpretation not so simple!

teins make up the virus's outer structure. If two different genotypes of reovirus infect the same cell, a **mixed infection** ensues in which the genomic fragments undergo **random reassortment** so that progeny virus can contain segments from either parent. Thus, if genomic segments of the two parents can be distinguished, perhaps by slight differences in their relative sizes, and if the parents differ in the way they replicate in an animal model (i.e., in their **phenotypes**), it is possible to assign the differences to a specific gene.

A simplified method to analyze results of a (hypothetical) mixed infection with two different strains of reovirus is shown in Fig. 3.4. In this example, the mixed infection results in four different viruses (two are parental), each of which has a different pathogenic character in the test animal. Purification of each genotype, followed by analysis of the size of the proteins expressed during a single infection with each, allows one to determine which gene segment is in each progeny virus. Then experimental study of the course of disease allows an assignment of the gene segment to a phenotype.

Human reovirus does not cause any major disease or syndrome in humans. Despite this, the mouse reovirus model gives us the ability to follow the influence of virus genes on infections in newborn and immune-impaired mice. This approach allows a thorough study of aspects of pathogenesis controlled by specific viral genes. This model system, developed in large part by Bernard Fields and his colleagues, has served as an excellent prototype of all studies on the genetics of viral patho-

genesis. In the newborn mouse, infection of the lung or the digestive tract with reovirus leads to a number of readily observable systemic and neurological symptoms. These symptoms result from the virus destroying specific tissues of the CNS following viremia and invasion of neural tissue. Distinct genotypes of the virus can be readily differentiated by immunological methods into specific **serotypes**, and these serotypes have different courses of infection (i.e., they infect different tissues and spread by different routes). Phenotypes can be ascribed to the function of individual virus genes, since each serotype has genomic segments that are slightly different in size from the corresponding segment of a different serotype.

The study of reovirus pathogenesis establishes the general methodology for such studies, but it does not address how the virus causes disease and spreads in humans. Still, many interesting things can be learned from such studies. For example, alteration in the amino acid composition of a specific virus protein makes the virus very sensitive to acidic pH. Thus, a virus that has the genomic segment responsible for this protein will not be able to cause a disease in a mouse that is infected in the stomach. If the virus is introduced directly into the small intestine, however, a disease can ensue. This is a good example of the importance in knowing the actual route of infection in describing pathogenesis.

Another example of the theoretical use of such studies is found in the fact that infection of a newborn mouse with a certain virus genotype may result in a different course of infection than when the same virus is infected in the same way in an adult mouse that has been genetically altered to have a severely impaired immune system (an **SCID,** or severely combined immunodeficient, **mouse**). Such a result demonstrates that even though a newborn mouse has a limited capacity for immune responses, this is not equivalent to a complete loss of immune capability (i.e., even a newborn mouse has the ability to mount some immune defenses).

Rabies: where is the virus during its long incubation period?

Rabies and its transmission by the bite of infected animals to other animals and humans are well known in almost all human cultures. The disease and its transmission were carefully described in Arabic medical books dating to the Middle Ages, and there is evidence of the disease in classical times. One of the puzzles of rabies virus infection is the very long incubation period of the disease. This long period plays an important role in the mechanism of spread, and it is clear that animals (or humans) infected with the virus can be vaccinated *after* infection and still mount an effective immune response.

The pathogenesis of rabies has been studied for over a century, and our current understanding is well founded in numerous careful studies made at varying levels of sensitivity using a number of approaches. An example of the use of immunological methods is shown in Fig. 3.5. The basic course of infection starts with inoculation of virus at a wound caused by an infected animal followed by limited virus replication at the site of primary infection. For the disease to develop, the virus must enter a neuron at a sensory nerve ending. These sensory nerve endings exist in all sites where the virus is known to enter an animal. Following this, the virus spreads passively to the nerve cell body in a dorsal root ganglion where it replicates to a high level. Either this replicated virus, or other virus moving directly, passes into neurons of the cerebellum and cerebral cortex where it replicates to high levels. Such replication leads to distinct behavioral changes associated with virus transmission. The virus also moves away from the CNS to sensory neurons and salivary glands of the oral mucosa where it replicates and is available for injection into another animal.

As early as 1887, CNS involvement was shown to result from direct spread of the virus from the site of infection into the CNS, as experimental animals that had their sciatic nerve severed prior to injection of the footpad with rabies virus did not develop the disease. The following experiment showed that the virus can remain localized at the site of infection for long periods of time: The footpad of several experimental animals was injected with virus at day 0 and then the inoculated foot

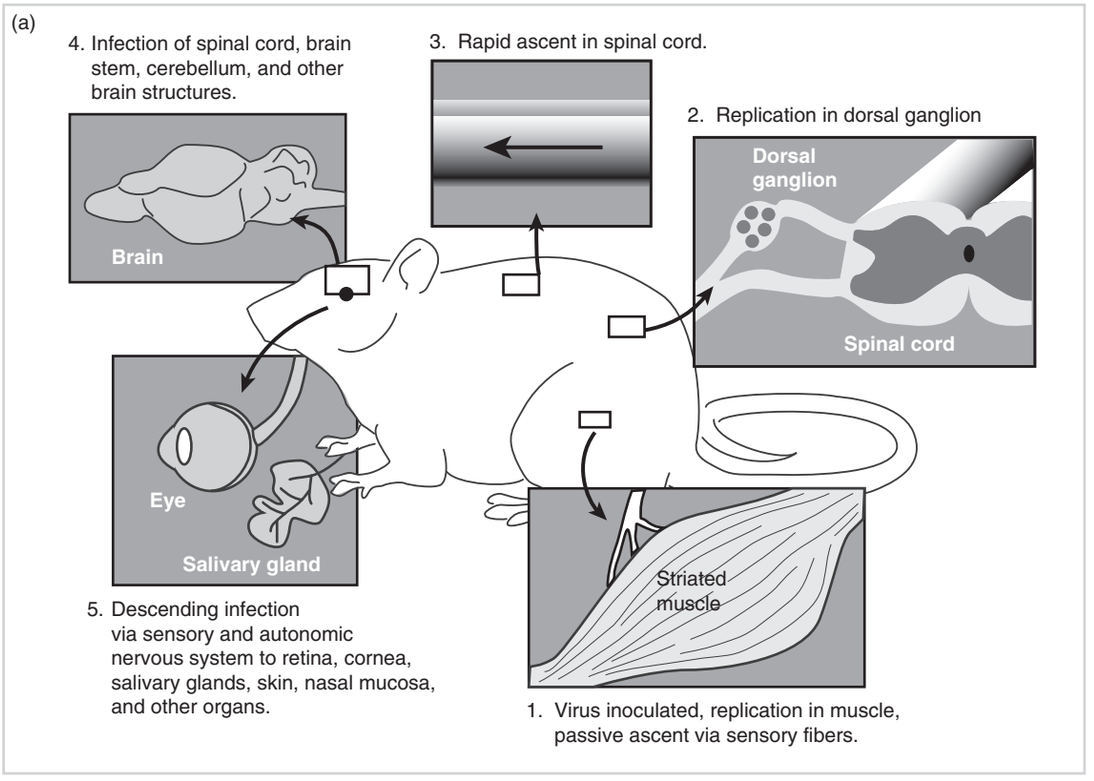

Fig. 3.5 Visualization of rabies virus–infected neurons in experimentally infected animals. (*a*) A schematic representation of the pathogenesis of rabies in an experimentally infected laboratory animal (*b*) Immunofluorescent detection of rabies virus proteins in neurons of infected animals. As described in Chapters 7 and 12, the ability of an antibody molecule to specifically combine with an antigenic protein can be visualized in the cell using the technique of immunofluorescence. The cell and the antibody bound to it are then visualized in the microscope under ultraviolet light, which causes the dye to fluoresce (a yellow-green color). The top left panel shows replication of rabies virus in a sensory nerve body in a dorsal root ganglion along the spine of an animal infected in the footpad. The bottom left panel shows the virus replicating in a neuron of the cerebellum, while the top right panel shows infected neurons in the cerebral medulla. Infection of the brain leads to the behavior changes so characteristic of rabies infections. Finally, the sensory nerve endings in the soft palate of a hamster infected with rabies virus at a peripheral site contains virus, as shown by the fluorescence in the bottom right panel. This virus can move to the saliva where it can be spread to another animal. The arrows point to selected cells showing the variation in signal intensity that is typical of infections in tissues. See Plate 1 for color image.

was surgically removed from different groups at days 1, 2, 3, and so on, after infection. Mice whose foot was removed as long as 3 weeks after infection survived without rabies, but once neurological symptoms appeared, the mice invariably died. Since removal of the foot saved the mice, it is clear that the virus remained localized there until it invaded the nervous system.

Finally, a similar experiment showed that rabies virus virulence for a specific host could be increased by multiple **virus passages** (rounds of virus replication) in that host. Virus isolated from a rabid wild animal takes as long as a week to 10 days to spread to the CNS of an experimentally infected laboratory animal. In contrast, isolation of virus from animals developing disease and reinoculation into the footpad of new animals several times result in a virus stock that can invade the test animal's CNS in as little as 12 to 24 hours. Further, the virus stock that has been adapted to the laboratory animal is no longer able to efficiently cause disease in the original host. As described in Chapter 8, this is one way of isolating strains of virus that are avirulent for their natural host and have potential value as vaccines.

(b)

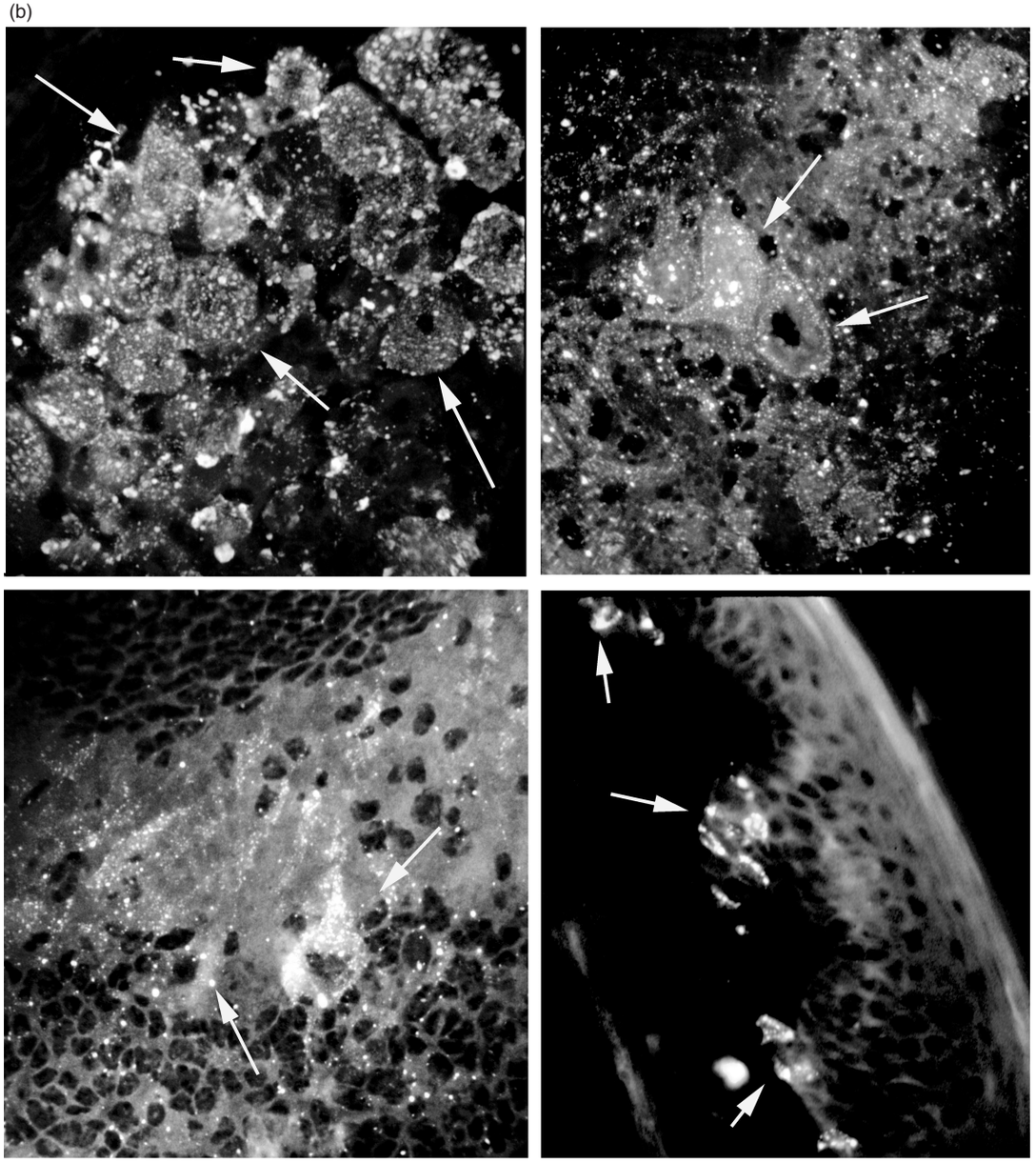

Fig. 3.5 *Continued*

Herpes simplex virus latency

There are two closely related types of herpes simplex virus: type 1 (facial, HSV-1) and type 2 (genital, HSV-2). Both establish latent infections in humans, and reactivation from such infections is important to virus spread. Some details concerning latent infection by herpes simplex virus are discussed in Chapter 18. Different animal models demonstrate both general similarities and specific differences. These differences illuminate a major limitation of many animal models for human disease: A model often only partially reflects the actual course of disease in the natural host—in this case, in humans.

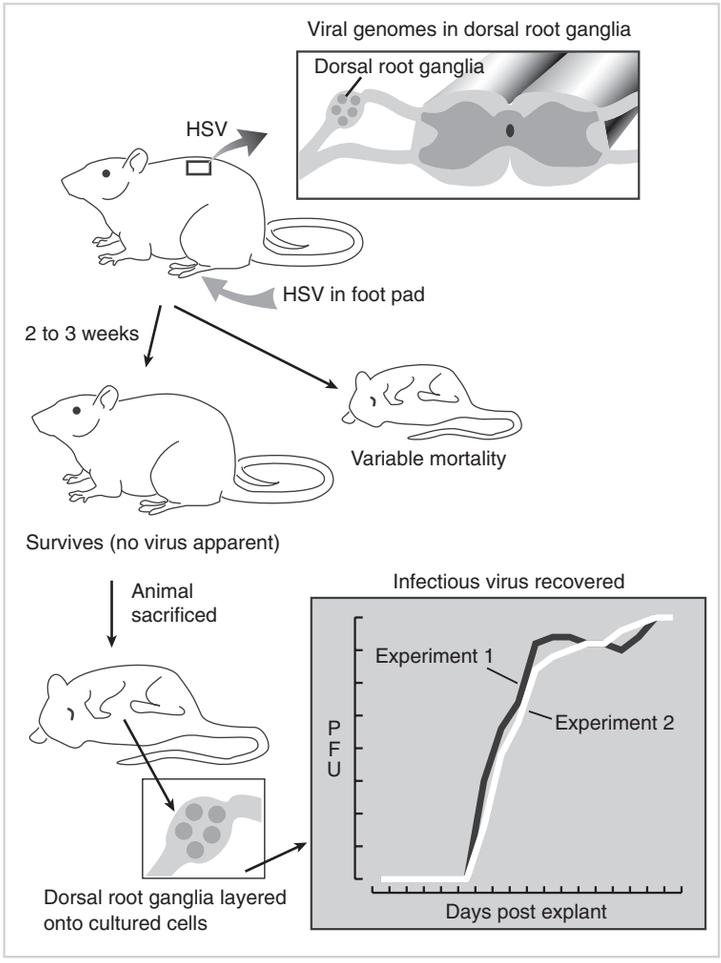

Fig. 3.6 Analysis of the establishment and maintenance of latent HSV infections in mice. A number of mice are inoculated in the footpad, and following the symptoms of primary disease, which includes foot swelling and minor hind-quarter paralysis, many mice recover. Those that do not recover have infectious virus in their CNS. The mice that recover are latently infected and no infectious virus can be detected, even with high-sensitivity measurements of nervous and other tissue. HSV genomes, but not infectious virus, can be detected in nuclei of sensory nerve dorsal root ganglia. When these ganglia are cultured with other cells that serve both as an indicator of virus replication and as a feeder layer for the neurons (i.e., explanted), a significant number demonstrate evidence of virus infection and infectious virus can be recovered, as shown on the inset graph (two separate experiments are shown).

Murine models

HSV infection in the eye or the footpad of mice can lead to a localized infection with spread of virus to the CNS and then to the brain. Although some animals die, as shown in Fig. 3.6, survivors maintain a latent infection in sensory nerve ganglia. During this latent infection, no infectious virus can be recovered from nerve tissue, but if the nerves are **explanted** (dissected) and maintained on a "**feeder layer**" of cultured cells, virus will eventually appear and begin to replicate. This observation demonstrates both that the viral genome is intact in the latently infected neuron, and that virus is not present in infectious form until something else occurs.

This model is quite useful for the study of genetic and other parameters during *establishment* and *maintenance* of a latent infection. For example, the sensory neurons can be isolated and viral DNA can be recovered. But since mice do not spontaneously reactivate HSV, the physiological process of reactivation, where virus can be recovered at the site of initial infection, cannot be effectively studied in mice.

Rabbit models

Infection of rabbit eyes with HSV leads to localized infection and recovery. The rabbits maintain virus in their trigeminal ganglia, and viral DNA or virus or both can be recovered using methods

described for the murine model. Unlike mice, rabbits spontaneously reactivate HSV and virus occasionally can be recovered from the rabbit's tear film. Further, this reactivation can be *induced* by **iontophoresis** of epinephrine with high frequency. Rabbits, because HSV can reactivate in them, are vital to the design of experiments to investigate induced reactivation, although they are more expensive to purchase and keep than mice.

Guinea pig models

Guinea pigs are favored experimental animals for the study of infection and disease because they are readily infected with many human pathogens. They are an important model for the study of HSV-2, which cannot be studied effectively in the murine and rabbit models just described.

Guinea pigs can be infected vaginally with inoculation of virus, and following a localized infection, latency can be established. As occurs in the murine and rabbit models, virus or viral DNA can be recovered from latently infected neurons (those enervating the vaginal area in this case). As in rabbits, latent infection in guinea pigs will spontaneously reactivate, and periodic examination can be used to measure reactivation rates. Unlike rabbits, however, guinea pig reactivation cannot be induced. Also, HSV-2 reactivates much more frequently than does HSV-1 in the guinea pig model; therefore, this model may be of some value in establishing the subtle genetic differences between these two types of viruses that manifest as a differential tropism for **mucosa**.

Can virus be spread across "kingdoms"?

The same principles concerning the source and transmission of viruses outlined for human diseases apply to viruses infecting plants and bacteria. The question naturally arises of whether viruses infecting a host in one of the three biological kingdoms can establish infections in another kingdom. The classifying of viruses by shape, type of genome, and general properties of replication reveals quite clearly that certain viruses that infect bacteria are related to those infecting plants and animals. These close relationships are especially striking among some viruses that utilize RNA as their genomes. In spite of the evidence for close relationships, there are few reliably documented instances of a specific virus type being able to replicate in host cells of different biological kingdoms. The strongest evidence exists for certain plant rhabdoviruses and bunyaviruses that are spread by arthropods, and that can replicate in cells of the arthropod's gut.

Despite a dearth of evidence for infection between biological kingdoms, the ecosystem's increasing stress engendered by human economic and agricultural activities causes situations where a rare cross-kingdom replication event *could* happen. If such an event occurs, it could conceivably have a role in the emergence of a new agricultural or animal disease. Clearly, this topic bears continuing scrutiny.

QUESTIONS FOR CHAPTER 3

1 In the case of rabies virus, how would you classify humans with respect to their role as a host?

2 What characteristics are shared by *all* hepatitis viruses?

3 Using the data presented in Table 3.1, answer the following questions:

a Which of the viruses in the table are vectored by mosquitoes?

b Which of the viruses in the table are transmitted in an aerosol?

c Which of the viruses in the table are transmitted by injection of blood?

d Which of the viruses in the table are neurotropic?

4 You are a viral epidemiologist studying the population of Spitzbergen Island off the coast of Norway (see Fig. 3.2). Suppose that a team of scientists plans to visit this island by special boat during the Christmas holiday season. How might this visit change the pattern of respiratory infections you have been observing? What criteria must exist for this visit to have an effect on the pattern of viral respiratory illness on the island?

5 You have isolated two mutant strains of virus Z – mutant 1 and mutant 2. Neither strain can replicate when infected into cells, but either can be propagated in cell culture when coinfected with mutant virus 3. When you coinfect cultured cells with mutants 1 and 2 together, infection proceeds, but only mutant 1 and mutant 2 can be recovered from the infected cells. What is the best explanation of these results?