Veterinary Virology

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Preface

Diseases caused by viruses contribute significantly to animal morbidity and mortality and produce substantial economic losses. When the disease involves companion animals there is an additional sense of individual human loss. Much of this loss, even in developed countries, is due to acute diseases most commonly caused by viruses that are constantly present in the animal environment. Viruses that occur intermittently or are exotic in the animal environment of developed countries are the basis of additional costs to society because of the complexity of public programs that are necessary to prevent their introduction. Viruses that are zoonotic, i.e., viruses that are transmitted between animals and humans, add further to society's burden, both in terms of human suffering and in terms of demands on resources.

Losses due to acute viral diseases have been appreciated for many years, but through recent spectacular advances in our basic understanding of viral infections we now recognize additional, more subtle causes of morbidity and mortality. We have begun to appreciate the importance of losses due to virus-induced cancers, virus-induced immunosuppression and opportunistic infections, virus-induced slow central nervous system diseases, and virus-induced teratogenesis and reproductive failure. The rapidity with which advances are occurring in virology serves to emphasize the need for better understanding on the part of everyone involved in veterinary medicine, from the student to the teacher, and from the research worker in basic science to the veterinary practitioner. It is an exciting time in virology, especially veterinary virology, but it is also a demanding time.

Many books have appeared in recent years providing comprehensive information on basic, molecular biologic virology. In addition, many books have appeared for the clinical specialist, especially the single species clinical specialist. There is a need for each of these types of books,

but we believe that there has been a growing need to bring the two poles of virology together—represented by basic biomedical virology and the clinical disciplines of infectious diseases, respectively. We believe that the underlying principles of virology hold the key to future improvements in the prevention and management of infectious diseases at the level of the individual animal, the herd or flock, and the animal population as a whole. This book has been written with such a purpose and perspective in mind, primarily for veterinary students and other graduate students interested in animal diseases, but we hope it will also prove useful to practicing veterinarians, animal scientists, biologists, microbiologists, and other allied professionals. We hope, for all readers, that our enthusiasm for the subject, our sense of excitement, is as infectious as some of the viral pathogens we describe.

The overall pattern follows that of "Medical Virology," written by two of the present authors and now in its third edition, to which this book is a companion volume. However, the detailed pattern differs in both structure and content. Part I, Principles of Animal Virology, consists of 16 chapters and uses examples drawn from studies of the viral diseases of domestic animals. Part II, Viruses of Domestic Animals, describes in 19 chapters the more important viral diseases of domestic animals according to viral family. In a series of tables in the last chapter, we have attempted to "cut the cake" in another way by listing disease syndromes found in various species of domestic animals, the viruses that cause them, their geographic distribution, and the availability of vaccines. The glossary comprises short definitions of words or terms that may not have been encountered before by students. Each entry is printed in italics when first used in the text.

We are grateful to Mrs. Marj Lee and Mr. Kevin Cowan, both of the Australian National University, for their devotion and skill in preparing the manuscript and line drawings, and to the staff of Academic Press for their assistance in the production of this book.

Frank Fenner
E. Paul J. Gibbs
Frederick A. Murphy
Michael J. Studdert
David O. White

Peter A. Bachmann

The progress of this book from idea to reality involved six people, each representing a different perspective, each influencing the others on the basis of a different professional experience, each contributing to the accomplishment of the overall goal. As the book proceeded there was a sense of having come to the right balance, but this sense of balance was shattered on May 26, 1985, when Peter Bachmann died. We have done our best to make up for this great loss. The book does reflect the continuing influence of our friend and colleague.

We dedicate the book to Peter Bachmann. He was a fine person, a fine veterinary scientist, a fine virologist. He leaves to his colleagues and students a legacy of commitment to the science of veterinary virology and an equal commitment to the *joie de vivre.*

CHAPTER 1

Structure and Composition of Viruses

The unicellular microorganisms can be arranged in order of decreasing size and complexity: protozoa, fungi, bacteria, mycoplasmas, rickettsiae, and chlamydiae. These microorganisms, however small and simple, are cells. They always contain DNA as the repository of their genetic information, they contain RNA, and they have their own machinery for producing energy and macromolecules. Microorganisms grow by synthesizing their own macromolecular constituents (nucleic acid, protein, carbohydrate, and lipid), and they multiply by binary fission.

Viruses, on the other hand, are smaller and simpler in construction than unicellular microorganisms, and they contain only one type of nucleic acid—either DNA or RNA, never both. Furthermore, since viruses have no ribosomes, mitochondria, or other organelles, they are completely dependent on their cellular hosts for energy production and protein synthesis. They replicate only within cells of the host that they infect. Indeed, unlike any microorganism, many viruses can, in suitable cells, reproduce themselves from their genome, a single nucleic acid molecule; i.e., their nucleic acid alone is infectious. Are viruses alive? The question is rhetorical. Outside a susceptible cell, the virus particle,

TABLE 1-1
Contrasting Properties of Unicellular Microorganisms and Viruses

Property	Bacteria	Mycoplasmas	Rickettsiae	Chlamydiae	Viruses
<300 nm diameter	−	±	−	±	+
Growth on nonliving media	+	+	−	−	−
Binary fission	+	+	+	+	−
DNA and RNA	+	+	+	+	−
Nucleic acid infectious	−	−	−	−	+[a]
Ribosomes	+	+	+	+	−
Metabolism	+	+	+	±	−
Sensitivity to antibiotics	+	+	+	+	−
Sensitivity to interferon	−	−	−	+	+

[a]Some, among both DNA and RNA viruses.

like a bacterial spore, is metabolically inert; on the other hand, when replicating in a cell it exhibits all the characteristics of life. The key differences between viruses and microorganisms are listed in Table 1-1.

Several important practical consequences flow from these differences. For example, some viruses (but no microorganisms) may persist in cells by the integration of their DNA (or a DNA copy of their RNA) into the genome of the host cell, and they are not susceptible to antibiotics that act against specific steps in the metabolic pathways of bacteria.

MORPHOLOGY

For many years it has been known that viruses are smaller than unicellular microorganisms. Independently, in 1898–1899, the Dutch plant pathologist Beijerinck, working on tobacco mosaic disease, and the German veterinarians Loeffler and Frosch, working on foot-and-mouth disease of cattle, showed that these diseases could be transmitted by material which could pass through a filter with pores too small to allow passage of bacteria. The new group of "microorganisms" became known as the "filterable viruses." Filtration studies showed that virus particles (*virions*) range from about the size of the smallest unicellular microorganisms (300 nm) down to objects little bigger than the largest protein molecules (20 nm). For a time they were also called "ultra-

microscopic," since they are too small to be seen with the ordinary light microscope. Only with the advent of the electron microscope did it become possible to study their morphology properly. In 1959, our knowledge of viral ultrastructure was transformed when Brenner and Horne applied *negative staining* to the electron microscopy of viruses. Potassium phosphotungstate, which is electron-dense, fills the interstices of the viral surface, giving the resulting electron micrograph a degree of detail not previously possible. Electron micrographs of negatively stained preparations of the virions of all families of viruses of vertebrates are shown in the relevant chapters of Part II of this book.

Viral Structure

In the simpler viruses the virion consists of a single molecule of nucleic acid surrounded by a protein coat, the *capsid;* the capsid and its enclosed nucleic acid together constitute the *nucleocapsid.* In some of the more complex viruses the capsid surrounds a protein core (Fig. 1-1A), and in other viruses the capsid is surrounded by a lipoprotein *envelope* (Fig. 1-1D). The capsid is composed of morphological units called *capsomers,* which are held together by noncovalent bonds. Individual capsomers, which consist of one or more polypeptide molecules, are usually visible by electron microscopy. In helical nucleocapsids, the viral nucleic acid is folded throughout its length in a specific relationship with the capsomers (Fig. 1-1C), but there is no such specific relationship between RNA and protein in the small icosahedral picornaviruses.

Within an infected cell, the capsomers of the simpler viruses self-assemble to form the capsid. The manner of this assembly is strictly defined by the nature of the bonds formed between individual capsomers, which imparts symmetry to the capsid. Only two kinds of symmetry have been recognized: icosahedral and helical (Fig. 1-1).

Icosahedral Symmetry. The icosahedron is one of the five classical "Platonic solids" of geometry; it has 12 vertices (corners) and 20 faces, each an equilateral triangle. It has axes of two-, three-, and fivefold rotational symmetry, passing through its edges, faces, and vertices, respectively (see Fig. 1-2). The icosahedron is the optimum solution to the problem of constructing, from repeating subunits, a strong structure to enclose a maximum volume. The same principles were applied by the architect Buckminster Fuller to the construction of icosahedral buildings ("geodesic domes").

Only certain arrangements of the repeating morphological units, the capsomers, can fit into the faces, edges, and vertices. In adenovirus particles, for example, capsomers on the faces and edges bond to six

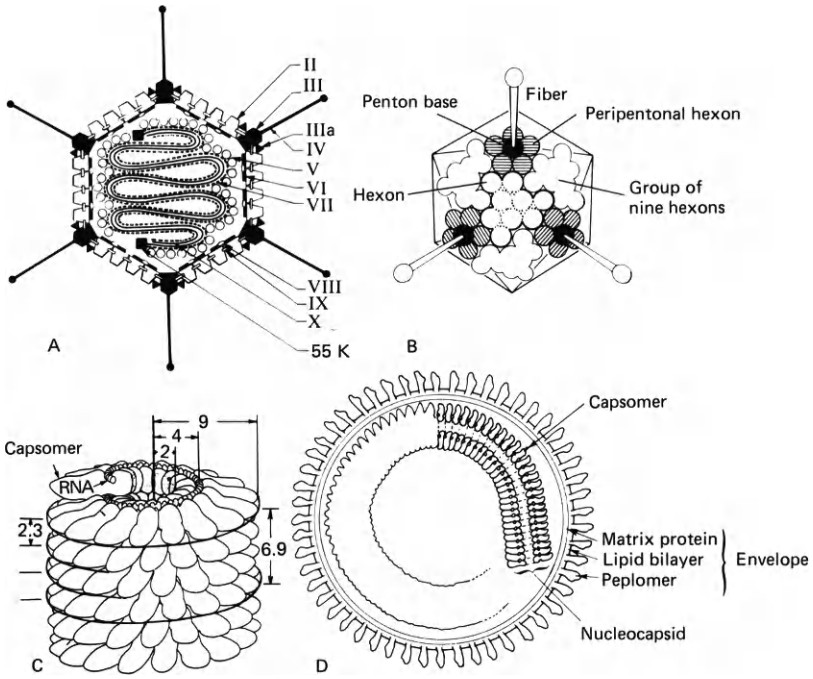

FIG. 1-1. *Features of virion structure, exemplified by adenovirus (A,B), tobacco mosaic virus (C), and paramyxovirus (D). Not to scale. (A,B) Icosahedral structure of adenovirion. All hexon capsomers are trimers of the same polypeptide (II), distinguished as "group of nine" or "peripentonal," respectively, only by their location in the capsid. The penton base is a pentamer of polypeptide III; the fiber is a trimer of polypeptide IV. Several other viral polypeptides occur just beneath the capsid (VI, VIII, IX) and others again in the core (V, VII, 55K), where they are intimately associated with the viral DNA. (C) The structure of helical nucleocapsids has been elucidated by studies of a nonenveloped plant virus, tobacco mosaic virus, but the principles apply to animal viruses with helical nucleocapsids, all of which are enveloped. In tobacco mosaic virus a single polypeptide is folded to form a capsomer. A total of 2130 capsomers assemble in a helix with a pitch of 2.3 nm and an axial repeat of 6.9 nm (49 subunits in each three turns). The 6-kb RNA genome sits in a groove on the inner part of the capsomer, and is wound to form an RNA helix of the same pitch, 8 nm in diameter, which extends the length of the virion. The virion is 300 nm long and 18 nm in diameter, with a hollow cylindrical core 4 nm in diameter. (D) All animal viruses with a helical nucleocapsid and some of those with an icosahedral capsid are enveloped. The envelope consists of a virus-specified matrix protein (M; absent in* Arenaviridae, Bunyaviridae, *and* Coronaviridae, *as well as in the enveloped viruses with icosahedral capsids), beneath a lipid bilayer in which are inserted numerous glycoprotein peplomers. [A, from H. S. Ginsberg,* In *"Comprehensive Virology" (H. C. Fraenkel-Conrat and R. R. Wagner, eds.), Vol. 13, p. 409. Plenum Press, New York, 1979. B, by John Mack, from R. M. Burnett,* In *"Biological Macromolecules and Assemblies: Virus Structures" (F. Jurnak and A. McPherson, eds.), Vol. 1, p. 337. Wiley, New York, 1984; C, from C. F. T. Mattern,* In *"Molecular Biology of Animal Viruses" (D. P. Nayak, ed.), Vol. 1, p. 5. Dekker, New York, 1977; and D, modified from D. L. D. Caspar* et al., Cold Spring Harbor Symp. Quant. Biol. **27,** *49 (1962).]*

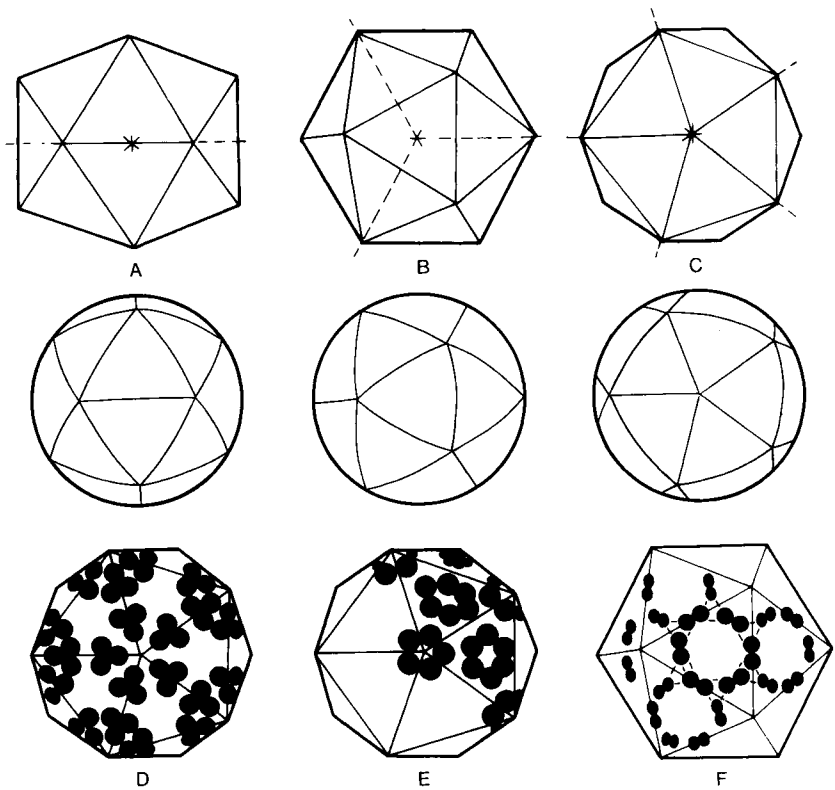

FIG. 1-2. *Features of icosahedral structure. A regular icosahedron viewed along two-fold (A), threefold (B), and fivefold (C) axes of symmetry. In negatively stained electron micrographs, virions may appear hexagonal in outline (upper row) or apparently spherical (middle row). Various clusterings of capsid polypeptides give characteristic appearances of the capsomers in electron micrographs (lower row). For example, they may be arranged as 60 trimers (D), capsomers being then difficult to define, as in poliovirus; or they may be grouped as 12 pentamers and 20 hexamers (E), which form bulky capsomers as in parvoviruses; or as dimers on the faces and edges of the triangular facets (F), producing an appearance of a bulky capsomer on each face, as in caliciviruses.*

neighboring capsomers and are called *hexamers;* those at the vertices bond to five neighbors and are called *pentamers* (Fig. 1-1A,B; Plate 1-1A). In some viruses both hexamers and pentamers consist of the same polypeptide; in others they are different. The varied arrangements of hexamers between pentamers have been systematically codified by Caspar using terms such as "P-number" and "T-number" (see references). Some possible arrangements of capsomers are shown in Fig. 1-2D, E, and F.

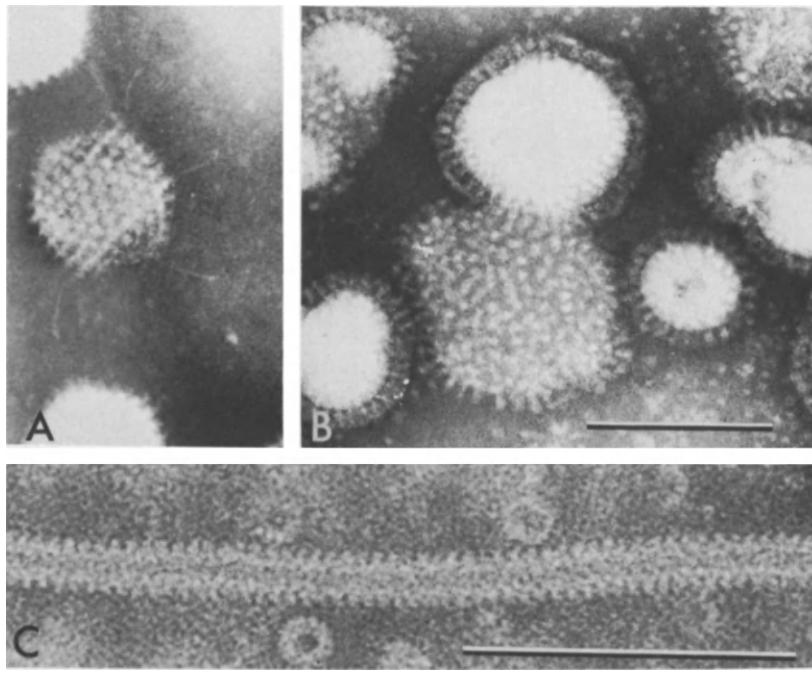

PLATE 1-1. *Morphological features of viral structure revealed by negative staining and electron microscopy (bars = 100 nm). (A) Icosahedral structure of adenovirus capsid. At each of the 12 vertices there is a penton base capsomer from which projects a fiber with a small terminal knob; each of the 20 triangular facets contains 18 identical hexon capsomers, of which 6 are unshared and 12 shared with adjacent facets. The capsid encloses a protein core with which the DNA is associated. (B) Envelope of influenza virus (family:* Orthomyxoviridae*). The peplomers are of two morphological types: the hemagglutinin is a rod-shaped trimer and the neuraminidase is a stud-shaped tetramer. Both are embedded in lipid, beneath which there is a matrix protein; this lipoprotein envelope encloses a helical nucleocapsid. (C) Nucleocapsid of parainfluenza virus (family:* Paramyxoviridae*). The RNA is wound within and protected by a helix composed of identical capsomers. The complete nucleocapsid is 1000 nm (1 μm) long, but in the intact particle is folded within a roughly spherical envelope about 180 nm in diameter. (A and B, courtesy Dr. N. G. Wrigley; C, courtesy Dr. A. J. Gibbs.)*

In a practical sense, the examination of negatively stained icosahedral virions in the electron microscope, and analysis of their capsomer arrangement, can often provide immediate and unambiguous information for the identification of a virus as a member of a known family—or, in very rare instances, as a candidate prototype for a new family. For example, the visualization of a nonenveloped virion with a row of four

hexamers in line between vertex pentamers would identify a virus as an adenovirus (Plate 1-1A).

The recent demonstration by X-ray crystallography of the atomic resolution structure of two picornaviruses (poliovirus and rhinovirus) has provided a remarkable insight into the organization and assembly of their virions, the location of the antigenic sites involved in neutralization, and aspects of their penetration into cells. Similar detail can be expected as these new technical capabilities are applied to other viruses and to problems of replication, assembly, and pathogenesis.

Helical Symmetry. The nucleocapsids of several RNA viruses have a different type of symmetry: the capsomers and nucleic acid molecule(s) self-assemble as a helix (Fig. 1-1C,D; Plate 1-1C). In all such viruses each capsomer consists of a single polypeptide molecule. The plant viruses with helical nucleocapsids are rod shaped and naked (nonenveloped). However, in all animal viruses helical nucleocapsids are wound into a coil and enclosed within a lipoprotein envelope (see Plate 27-1), possibly to give the very long nucleocapsids stability.

Viral Envelopes. Viral envelopes are acquired at host cell membranes—some at the plasma membrane, others at internal cell membranes such as the nuclear membrane, endoplasmic reticulum, and Golgi complex—during the maturation of the virus by the process known as "budding." The lipids of the viral envelope are derived directly from the cell, but the proteins in the envelope are virus coded. One kind is the glycoprotein *peplomer* (*peplos* = envelope) or spike. These peplomers can often be seen clearly in electron micrographs as projections from the outer surface of the envelope (Plate 1-1B). The other kind of envelope protein, *matrix protein*, is nonglycosylated and is found on the inside of the envelope of virions of several families; it provides added rigidity. The envelope of rhabdoviruses is closely applied to a bullet-shaped matrix protein that encloses a helical nucleocapsid. Arenaviruses, bunyaviruses, and coronaviruses have no matrix protein and consequently are rather more pleomorphic than other enveloped viruses.

Envelopes are not restricted to viruses of helical symmetry; some icosahedral viruses (ranaviruses, African swine fever virus, herpesviruses, togaviruses, flaviviruses, and retroviruses) have envelopes. The infectivity of most enveloped viruses depends on the integrity of the envelope, but some poxviruses have an envelope which is not necessary for infectivity.

CHEMICAL COMPOSITION

The essential components of infectious viral particles are nucleic acid (the genome) and protein. In addition, all enveloped viruses contain

lipid in the envelope and carbohydrate in their glycoprotein peplomers (as well as that in the nucleic acid). The largest and most complex viruses (poxviruses, ranaviruses, and African swine fever virus) also have lipids associated with other parts of the virion.

Nucleic Acid

Any particular virus contains only a single kind of nucleic acid. However, this may be DNA or RNA; indeed, the RNA viruses provide the only instance in nature in which RNA is the exclusive repository of genetic information. All viral genomes are *haploid*, i.e., they contain only one copy of each gene, except for retrovirus genomes, which are *diploid*. Viral DNA or RNA can be *double-stranded* (*ds*) or *single-stranded* (*ss*). Since 1978 the genomes of many of the smaller animal viruses have been sequenced, and there are now no insuperable technical impediments to the sequencing of any viral genome. By 1985, the largest genome to be completely sequenced was that of a herpesvirus (EB virus), which consist of 172,000 base pairs (172 kilobase pairs, kbp).

When carefully extracted from the virion, the nucleic acid of viruses of certain families of both DNA and RNA viruses is infectious; i.e., when introduced into a cell it can initiate a complete cycle of viral replication, with the production of a normal yield of progeny virions. In these cases, messenger RNA (mRNA) is transcribed from the viral DNA in the nucleus, by a cellular transcriptase, or in the case of RNA viruses the genomic RNA itself functions as mRNA. In other cases, the isolated nucleic acid is not infectious even though it contains all the necessary genetic information. Among DNA viruses, failure to infect occurs if transcription requires a viral rather than a cellular transcriptase; among RNA viruses failure occurs when the viral RNA is of minus (−) sense or is double-stranded; its transcription to produce plus (+) sense mRNA then requires a virion-associated transcriptase. The (+) sense RNA of retroviruses is not infectious, because replication of the RNA occurs only after the production of a DNA provirus by a virion-associated reverse transcriptase (see Table 1-3).

DNA. The genome of all DNA viruses consists of a single molecule, which is double-stranded except in the case of the parvoviruses, and may be linear or circular.

The DNA of papovaviruses and hepadnaviruses is circular. Within the virion, the circular DNA of the papovaviruses, like that of mitochondria and bacterial plasmids, is a supercoiled circle, known as a superhelix (Plate 1-2A). When an enzyme relieves the tension by introducing a nick into one strand, the molecule becomes a relaxed circle (Plate 1-2B). One

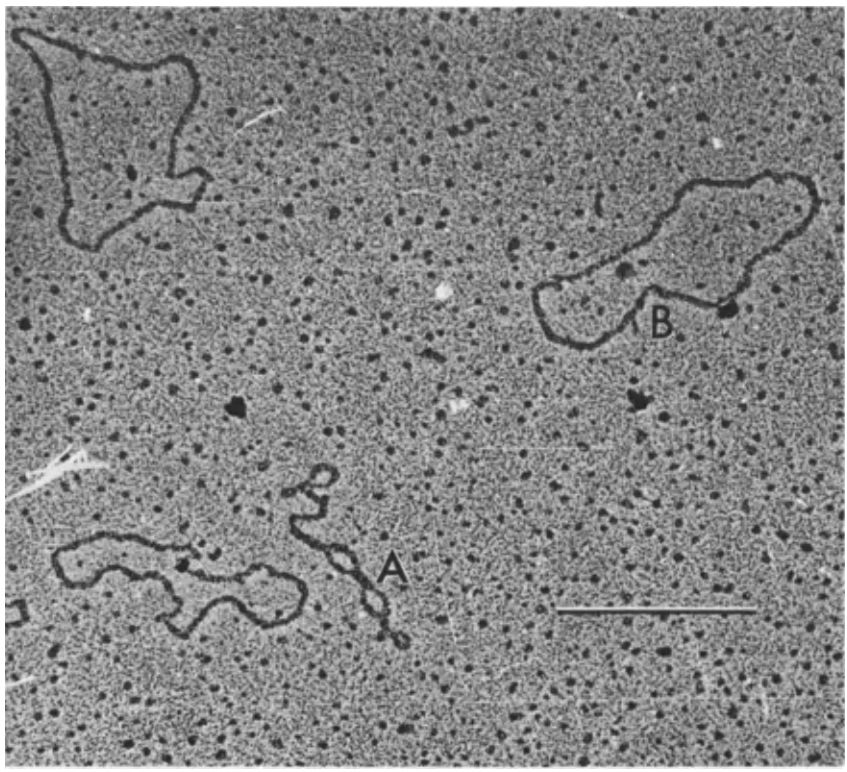

PLATE 1-2. *DNA molecules extracted form the papovavirus SV40 (bar = 500 nm). Molecules of SV40 DNA exist in two major forms. (A) When it is isolated from the virions, most of the DNA occurs as a double-stranded, supercoiled, circular molecule (superhelix). (B) If one of the DNA strands is nicked, the superhelix becomes a circle. (Courtesy Dr. P. Sharp.)*

strand of the circular DNA of hepadnaviruses is shorter than the other; the genome is thus only partially double-stranded.

Most of the linear DNAs from viruses of other families have characteristics which enable them to adopt a circular configuration temporarily, presumably during replication. The two strands of poxvirus DNA are covalently cross-linked at each end, so that on denaturation, the molecule becomes a large single-stranded circle (Fig. 1-3C). The linear dsDNA of some herpesviruses (and the linear ssRNA of retroviruses) contains *repeat sequences* at the ends of the molecule. Following partial digestion of both DNA strands from their 5′ ends by an exo-

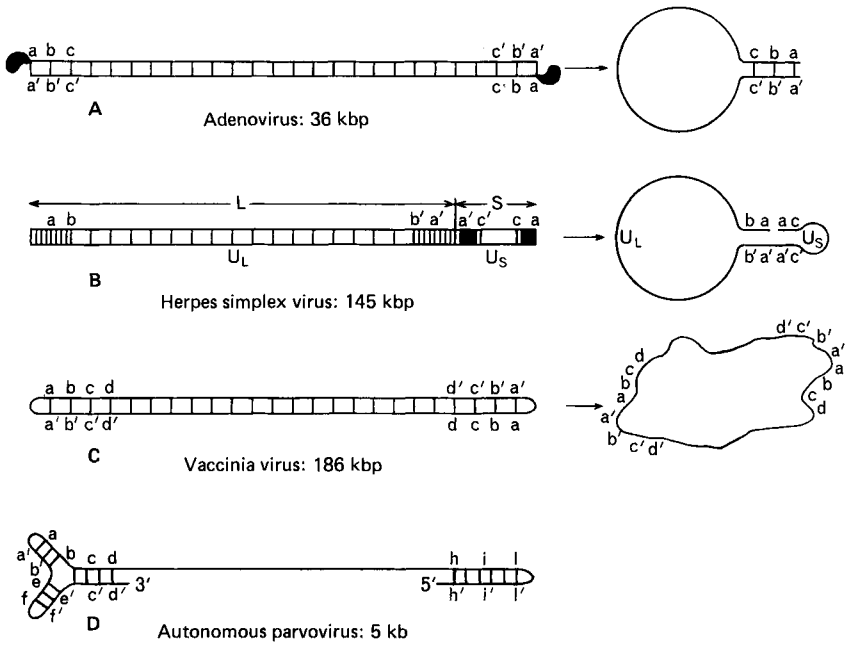

FIG. 1-3. *Specialized arrangements at the termini of linear DNA viral genomes. Not to scale. (A) Adenovirus DNA has inverted terminal repeats, with a covalently linked protein located at each end of the molecule. Termini of single strands anneal to form "saucepan" structures, as shown on the right. (B) Herpes simplex virus DNA consists of two covalently linked components, long (L) and short (S), each of which consists of a large unique sequence (U_L and U_S, respectively) flanked by inverted repeats. In a viral population, four isomeric forms differing in the orientation of the unique regions relative to each other occur in equimolar amounts. Intact single strands anneal as shown on the right. (C) Vaccinia virus DNA has inverted terminal repeats and each end is covalently closed, so that on denaturation it forms a large, single-stranded circular molecule. (D) Parvovirus DNA is single-stranded, with a palindromic sequence at the 3′ end that folds back to form a Y-shaped hairpin structure stabilized by hydrogen bonding. The 5′-terminal sequence is also palindromic, but the sequence is unrelated to that at the 3′ end.*

nuclease, the exposed single-stranded ends are complementary in their nucleotide sequences, thus providing "cohesive" or "sticky" ends, so that, if the molecule is melted, it will reanneal as a circular dsDNA. In the case of the adenoviruses, these terminal repeats are inverted; hence, even without enzymatic digestion, denatured molecules self-anneal to form single-stranded circles (Fig. 1-3A). Inverted terminal repeat sequences, which give rise to "hairpin" structures, are also a feature of the ssDNA parvoviruses (Fig. 1-3D).

Another type of terminal structure occurs in adenoviruses, hepadnaviruses, parvoviruses, and the ssRNA picornaviruses and caliciviruses, in all of which a protein is covalently linked to the 5′ terminus. This has an essential function in replication of the genome.

The DNA of certain iridoviruses (genus *Ranavirus*) contains a high proportion of 5-methylcytosine instead of cytosine.

The size of viral DNA genomes ranges from 4.5 kilobases (kb) (molecular mass, $M_r = 1.5 \times 10^6$) for the small ssDNA parvoviruses to over 200 kbp ($M_r = 185 \times 10^6$) for the large dsDNA poxviruses. As 1 kb or 1 kbp contains enough genetic information to code for about one average-sized protein, we recognize as an approximation that viral DNAs contain from about 4 to 200 genes and code for 4 to 200 proteins. However, the relationship between any particular nucleotide sequence and its protein product is not as straightforward as this.

First, the DNA of most of the larger viruses—like that of cells—contains what appears to be redundant information, in the form of (1) *repeat (reiterated) sequences* and (2) *introns*, i.e., regions which are *spliced* out and discarded from the RNA transcript. On the other hand, a single such RNA transcript may be spliced and/or cleaved in several different ways to yield several distinct mRNAs, which may be translated into different proteins. Furthermore, a given mRNA sequence may be read in two different reading frames (theoretically, up to three, because each *codon* is a triplet), giving rise to two (or three) proteins with different amino acid sequences. These fascinating examples of genetic economy are well illustrated by the papovaviruses (see Fig. 4-4) and will be discussed in Chapter 4. Suffice it to say at this point that nowadays we cannot always talk in terms of a direct one-to-one relationship between a "gene" and its "gene-product," although such a relationship does sometimes occur.

Viral DNAs contain several kinds of noncoding sequences, in addition to introns and various types of terminal repeat sequences, described above. Consensus sequences, which tend to be conserved through evolution because they serve vital functions, include those of RNA splice sites, polyadenylation sites, RNA polymerase recognition sites and promoters, initiation codons for translation, and termination codons.

RNA. The genome of RNA viruses may also be single-stranded or double-stranded. Furthermore, while some occur as a single molecule, others are segmented. Arenavirus and birnavirus RNAs consist of 2 segments, bunyavirus RNA of 3, orthomyxovirus RNA of 7 or 8 (in different genera), and reovirus 10, 11, or 12 (in different genera). Each of these molecules is unique (often a single "gene"). All viral RNAs are linear; none is a covalently closed circle. However, the ssRNAs of arenaviruses and bunyaviruses have sticky ends, hence these molecules

occur as circles. The genomes of ssRNA viruses have considerable secondary structure, regions of base pairing probably serving as signals controlling transcription, translation, and/or packaging into the capsid.

Single-stranded viral nucleic acid, which is generally RNA, can also be defined according to its *sense* (also known as *polarity*). If it is of the same sense as mRNA, it is said to have *positive* (+) *sense*). This is the case with picornaviruses, caliciviruses, togaviruses, flaviviruses, coronaviruses, and retroviruses. If, on the other hand, its nucleotide sequence is complementary to that of mRNA, it is said to have *negative* (−) *sense*). Such is the case with the paramyxoviruses, orthomyxoviruses, rhabdoviruses, arenaviruses, and bunyaviruses, all of which have an RNA-dependent RNA polymerase (*transcriptase*) in the virion, in order that mRNA can be transcribed. With the arenaviruses and at least one genus of bunyaviruses one of the RNA segments is *ambisense*, i.e., part (+) sense, part (−) sense.

Where the viral RNA is of (+) sense, it is usually polyadenylated at its 3′ end (in picornaviruses, caliciviruses, togaviruses, and coronaviruses, but not in flaviviruses) and capped at its 5′ end (togaviruses, flaviviruses, coronaviruses) (see Chapter 4). The picornaviruses and caliciviruses have a protein attached to the 5′ end of the viral RNA.

The size of ssRNA viral genomes varies from 7.5 to 18 kb (M_r = 2.5 to 7 × 10^6) and that of the dsRNA viruses from 7 to 22 kbp (M_r = 4.8 to 15 × 10^6)—a much smaller range than seen with the DNA viruses. Accordingly they code, in general, for fewer than a dozen proteins. In the case of the segmented RNA genomes of orthomyxoviruses and reoviruses, one can consider most of the segments to be individual genes, each coding for one unique protein. No such simple relationship applies to the other RNA viruses. For example, the picornavirus genome [(+) sense ssRNA] is directly translated into a single "polyprotein," which is subsequently cleaved to give the several viral polypeptides.

The essential features of the genomes of viruses of vertebrates are summarized in Table 1-2. Their remarkable variety is reflected in the diverse ways in which the information encoded in the viral genome is transcribed to RNA, then translated into proteins, and the ways in which the viral nucleic acid is replicated (see Chapter 4).

Viral preparations often contain some particles with an atypical content of nucleic acid (see Chapter 5). Host cell DNA is found in some papovavirus particles, and cellular ribosomes are incorporated in arenaviruses. Several copies of the complete viral genome may be enclosed within a single particle, or viral particles may be formed that contain no nucleic acid (empty particles) or that have an incomplete genome (*defective interfering particles*).

TABLE 1-2
Structure of the Genome in Viruses of Different Families[a]

Family	Structure of nucleic acid
Papovaviridae	Circular superhelical dsDNA (see Plate 1-2)
Adenoviridae	Linear dsDNA with inverted terminal repeats and a covalently bound protein (see Fig. 1-3A)
Herpesviridae	Linear dsDNA; two unique sequences flanked by reiterated sequences; isomeric configurations occur (see Fig. 1-3B)
Poxviridae African swine fever virus	Linear dsDNA; both ends covalently closed, with inverted terminal repeats (see Fig. 1-3C)
Parvoviridae	Linear ssDNA, (−) sense; with repeated sequences and a hairpin structure at one end (see Fig. 1-3D)
Hepadnaviridae	Circular dsDNA with ss region
Picornaviridae *Caliciviridae* *Togaviridae* *Flaviviridae* *Coronaviridae*	Linear ssRNA, (+) sense; serves as mRNA; 3′ end polyadenylated (except *Flaviviridae*); 5′ end capped, or protein covalently bound (*Picornaviridae, Caliciviridae*)
Paramyxoviridae *Rhabdoviridae*	Linear ssRNA, (−) sense
Orthomyxoviridae	Segmented genome; 7 or 8 molecules of linear ssRNA, (−) sense
Arenaviridae	Segmented genome; 2 molecules of ssRNA, (−) sense or ambisense[b]; "sticky ends" allow circularization
Bunyaviridae	Segmented genome; 3 molecules of ssRNA, (−) sense or ambisense; "sticky ends" allow circularization
Retroviridae	Diploid genome, dimer of linear ssRNA, (+) sense; hydrogen bonded at 5′ ends; terminal redundancy; both 3′ termini polyadenylated, both 5′ ends capped; may carry oncogene
Reoviridae	Segmented genome; 10, 11, or 12 molecules of linear dsRNA
Birnaviridae	Segmented genome; 2 molecules of linear dsRNA

[a]There is considerable variation within some families, e.g., *Herpesviridae, Reoviridae.*
[b]Ambisense indicates that part of molecule is (+) and part (−) sense.

Protein

Some virus-coded proteins are *structural,* i.e., they are part of the virion; some are *nonstructural* and are concerned with various aspects of the replication cycle. A major role of structural proteins is to provide the viral nucleic acid with a protective coat. The virions of all viruses of vertebrates contain several different proteins, the number ranging from 3 in the case of the simplest viruses to over 100 in the case of the complex poxviruses. In *isometric* viruses, the structural proteins form an icosahedral capsid which sometimes encloses a polypeptide core that is inti-

mately associated with the nucleic acid. Some virions, e.g., those of reoviruses, appear to have two concentric capsids.

The capsid proteins are assembled in the virion in groups, to form the capsomers visible in electron micrographs. Each capsomer is composed of one to six molecules of polypeptide, usually of the same kind (homopolymers) but sometimes different (heteropolymers). Capsomers from the vertices and the faces are usually composed of different polypeptides. A few viruses have a double capsid, each being composed of a different set of polypeptides. Other proteins, invariably glycoproteins, make up the peplomers projecting from the envelope; a second type of envelope protein is the nonglycosylated matrix protein that occurs as a layer at the inner surface of the lipid envelope of orthomyxoviruses, paramyxoviruses, and rhabdoviruses. One or more of the proteins on the surface of the virion has a specific affinity for complementary receptors present on the surface of susceptible cells; the same viral protein contains the antigenic determinants against which neutralizing antibodies are made. Virions of several families carry a limited number of enzymes, transcriptases being the most important (Table 1-3).

Lipid

Lipid constitutes about 30–35% of the dry weight of enveloped viruses, the viral envelope being derived from cellular lipids. As a consequence, the composition of lipids of particular viruses differs according to the composition of the membrane lipids of the cells in which they have replicated. About 50–60% of the envelope lipid is phospholipid, and most of the remainder is cholesterol.

The poxviruses, ranaviruses, and African swine fever virus contain cellular lipid in their envelopes, and other lipids in the inner part of the virion. Lipid occurs in the outer membrane of poxviruses, and has a different composition from that of host cell lipids. In ranaviruses and African swine fever virus the additional viral lipid occurs within the icosahedral capsid.

Carbohydrate

Apart from that associated with viral nucleic acid, carbohydrate occurs as a component of viral glycoproteins, which usually occur as peplomers, with their hydrophobic ends buried in the lipid bilayer of the envelope, while their glycosylated hydrophilic ends project into the medium. Poxviruses also contain internal glycoproteins, in the membrane of the core, and one of the outer capsid proteins of rotaviruses is glycosylated.

TABLE 1-3

Virion-Associated Enzymes and Their Functions

Family	Virus	Function
Enzymes affecting interaction of virions with the host cell surface		
Neuraminidase	Orthomyxovirus, paramyxovirus	Cleaves *N*-acetylneuraminic acid from surface polysaccharides
Enzymes transcribing the viral genome into mRNA[a]		
DNA-dependent RNA polymerase	Poxvirus, African swine fever virus	Transcribes RNA
dsRNA-dependent RNA polymerase	Viruses with dsRNA	Transcribes RNA
ssRNA-dependent RNA polymerase	Viruses with (−) sense ssRNA	Transcribes RNA
Enzymes adding specific terminal groups to viral mRNA		
Nucleotide phosphohydrolase	Viruses synthesizing mRNA in virions (e.g., poxvirus, reovirus)	Converts terminal 5′ triphosphate to diphosphate as prelude to guanylylation
Guanylyltransferase	Viruses synthesizing mRNA in virions (e.g., poxvirus, reovirus)	Adds guanylyl residue to 5′ end diphoshpate in mRNA
RNA methylases	Viruses synthesizing mRNA in virions (e.g., poxvirus, reovirus)	Methylate guanylyl residue at 5′ end of mRNA and some riboses in 2′ position
Poly(A) polymerase	Viruses synthesizing mRNA in virions (e.g., poxvirus, reovirus)	Synthesizes poly(A) tail at 3′ end of mRNA
Enzymes involved in copying virion RNA into DNA		
RNA-dependent DNA polymerase (reverse transcriptase)	Retrovirus	Makes DNA–RNA hybrids
RNase H (an activity of the reverse transcriptase)	Retrovirus	Breaks down RNA strand in RNA–DNA hybrid
Polynucleotide ligase	Retrovirus	Closes ss breaks in dsDNA
Enzymes for nucleic acid replication or processing		
DNA-dependent DNA polymerase	Hepadnavirus	Synthesizes dsDNA
Deoxyribonuclease (exo- and endo-)	Poxvirus, retrovirus, adenovirus	Break DNA chains and cross-links
Endoribonuclease	Poxvirus	Processing of mRNA
Other enzymes		
Protein kinases	Retrovirus, orthomyxovirus, paramyxovirus, herpesvirus, adenovirus	Phosphorylate proteins
tRNA aminoacylases	Retrovirus	Aminoacylate tRNA

[a]Also called transcriptases.

PRESERVATION OF VIRAL INFECTIVITY

In general, viruses are more sensitive than bacteria or fungi to inactivation by physical and chemical agents. A knowledge of their sensitivity to environmental conditions is therefore important for ensuring the preservation of the infectivity of viruses as reference reagents, and in clinical specimens collected for diagnosis, as well as for their deliberate inactivation for such practical ends as sterilization, disinfection, and the production of inactivated vaccines (see Chapters 14 and 16).

The principal environmental condition that may adversely affect the infectivity of viruses in clinical specimens is too high a temperature; other important conditions are pH and lipid solvents.

Temperature

Viruses vary considerably in heat stability. Surface proteins are denatured within a few minutes at temperatures of 55° to 60°C, with the result that the virion is no longer infectious, because it is no longer capable of normal cellular attachment and/or uncoating. At ambient temperature the rate of decay of infectivity is slower but significant, expecially in hot summer weather or in the tropics in any season. Viral preparations must therefore be stored at low temperature; 4°C (ice or a refrigerator) is usually satisfactory for a day or so, but longer term preservation requires temperatures well below zero. Two convenient temperatures are −70°C, the temperature of frozen CO_2 ("dry ice") and of some freezers, or −196°C, the temperature of liquid nitrogen. As a rule of thumb, the half-life of most viruses can be measured in seconds at 60°C, minutes at 37°C, hours at 20°C, days at 4°C, and years at −70°C or lower. The enveloped viruses are more heat labile than nonenveloped viruses. Some enveloped viruses, notably respiratory syncytial virus, tend to be inactivated by the process of freezing and subsequent thawing, probably as a result of disruption of the virion by ice crystals. This poses problems in the collection and transportation of clinical specimens. The most practical way of avoiding such problems is to deliver specimens to the laboratory as rapidly as practicable, packed without freezing, on ice (see Chapter 13).

In the laboratory, it is often necessary to preserve stocks of viable virus for years. This is achieved in one of two ways: (1) rapid freezing of small aliquots of virus suspended in medium containing protective protein and/or dimethyl sulfoxide, followed by storage at −70°C or −196°C; (2) freeze-drying (lyophilization), i.e., dehydration of a frozen viral suspension under vacuum, followed by storage of the resultant powder at

4°C or −20°C. Freeze-drying prolongs viability significantly even at ambient temperatures, and is important in enabling live viral vaccines to be used in tropical countries.

Ionic Environment and pH

On the whole, viruses prefer an isotonic environment at physiological pH, but some virions tolerate a wide ionic and pH range. For example, whereas most enveloped viruses are inactivated at pH 5–6, adenoviruses and many picornaviruses survive the acidic pH of the stomach.

Lipid Solvents

The infectivity of enveloped viruses is readily destroyed by lipid solvents such as ether or chloroform, or detergents like sodium deoxycholate, so that these agents must be avoided in laboratory procedures concerned with maintaining the viability of viruses. On the other hand, detergents are commonly used by virologists to solubilize viral envelopes and liberate proteins for use as vaccines or for chemical analysis. Sensitivity to lipid solvents is also employed as a preliminary screening test in the identification of new viral isolates, especially by arbovirologists.

FURTHER READING

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CHAPTER 2

Classification and Nomenclature of Viruses

It is reasonable to assume that all organisms are infected with viruses: vertebrate and invertebrate animals—including mammals, birds, reptiles, amphibia, helminths, and arthropods—as well as plants, algae, fungi, protozoa, bacteria, and mycoplasmas. Since every species that has been intensively searched has yielded several different viruses belonging to several viral families (including some that are host species-specific), it is not unreasonable to believe that the number of distinct species of viruses on earth may be greater than the number of species of all other living things. There are even some "satellite" viruses the replication of which depends on other viruses.

All viruses, whatever their cellular hosts, share the features summarized in Table 1-1; hence, viral taxonomists have developed a system of classification and nomenclature that embraces all viruses. From the operational point of view, however, we can divide viruses into those that affect vertebrate animals, insects, plants, and bacteria, respectively. We are concerned with the viruses of vertebrate animals, although some of

these viruses, the arthropod-borne viruses (arboviruses), also replicate in insects or other arthropods.

Several hundred species of viruses have been recovered from humans, who are the best-studied vertebrate hosts, and new ones are still occasionally discovered. Somewhat fewer have been recovered from each of the common species of farm and companion animals, and from the commonly used laboratory animals. In order to simplify the study of this vast number of infectious agents we need to sort them into groups that share certain common properties. The most important criteria for classification are the physical and chemical characteristics of the virion and the mode of replication of the virus. Before discussing formal viral taxonomy, which is based on these criteria, a simpler, clinically useful classification based on modes of transmission from one host to another will be described.

CLASSIFICATION BASED ON EPIDEMIOLOGICAL CRITERIA

Viruses of animals can be transmitted by ingestion, inhalation, injection (including arthropod bites), contact (including coitus), or congenitally. In discussing the pathogenesis and epidemiology of viral infections we will frequently make use of the following terminology as a useful way of classifying viruses.

Enteric viruses are usually acquired by ingestion and replicate primarily in the intestinal tract. The term is restricted to viruses that tend to remain localized in the intestinal tract, rather than becoming generalized. Enteric viruses important in veterinary practice include the rotaviruses, coronaviruses, enteroviruses, and some adenoviruses.

Respiratory viruses are usually acquired by inhalation and replicate in the respiratory tract. The term is usually restricted to those viruses that remain localized in the respiratory tract, and includes the orthomyxoviruses and rhinoviruses, and some of the paramyxoviruses, coronaviruses, and adenoviruses.

Arboviruses (*ar*thropod-*bo*rne viruses) infect arthropods that ingest vertebrate blood; they replicate in the arthropod's tissues and can then be transmitted by bite to susceptible vertebrates. Viruses that belong to six families are included: all orbiviruses (a genus of the reoviruses), most bunyaviruses, flaviviruses, togaviruses and rhabdoviruses, and African swine fever virus.

CLASSIFICATION BASED ON PHYSICOCHEMICAL CRITERIA

Although classification on epidemiological criteria is convenient for certain purposes, each group contains viruses with very disparate phys-

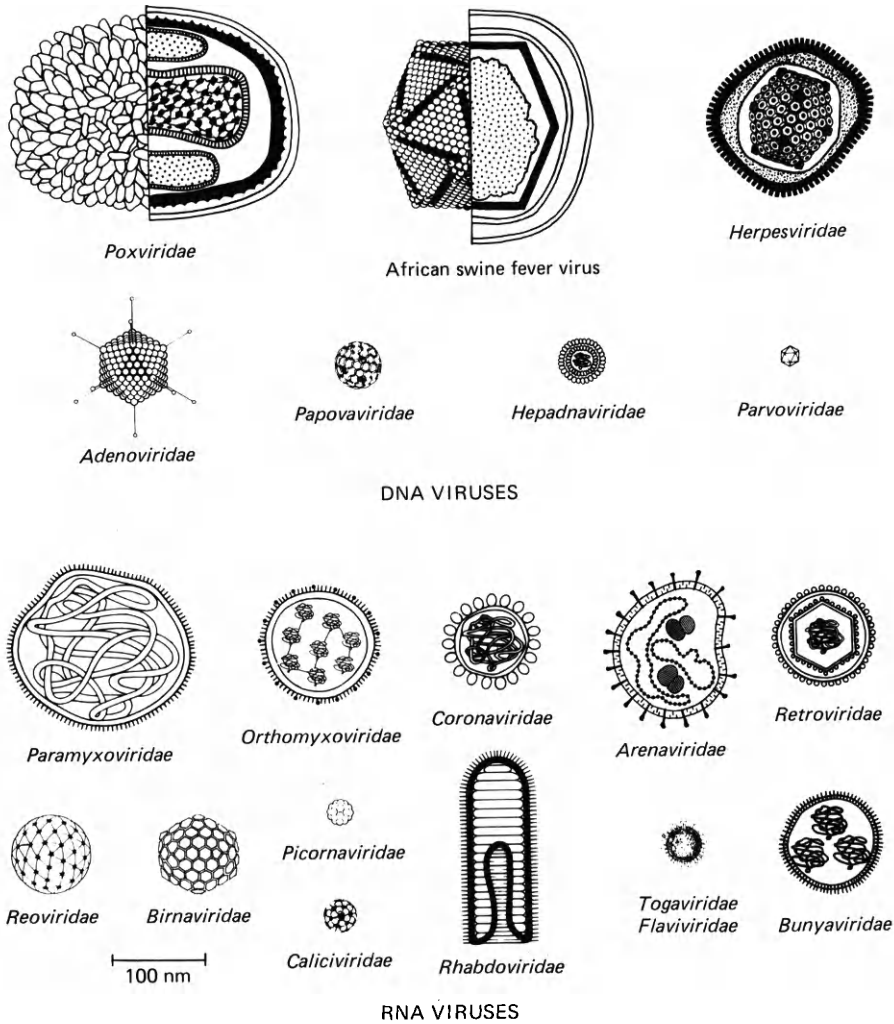

FIG. 2-1. *Diagram illustrating the shapes and sizes of animal viruses. The virions are drawn to scale, but artistic license has been used in representing their structure. In some, the cross-sectional structure of capsid and envelope are shown, with a representation of the genome; with the very small virions, only their size and symmetry are depicted.*

icochemical and biological properties. As knowledge of the properties of viruses increased it became clear that the physicochemical properties of the virion and the strategy of replication provided a more fundamental basis for viral classification. Classification into major groups called families, and the subdivision of families into genera, has now reached a position of substantial international agreement.

The primary criteria for delineation of families are (1) the kind of nucleic acid and the strategy of viral replication, and (2) the morphology of the virion, including its size, shape (see Fig. 2-1), symmetry of the nucleocapsid, and the presence or absence of an envelope, all of which are readily determined by electron microscopy.

Subdivision of families into genera depends on criteria that vary for different families. Genera contain from one to over a hundred species, usually defined by antigenic differences. The rationale for subdivision of genera into species is controversial. Most virologists agree that to be designated as distinct species, two viruses should differ substantially in nucleic acid sequence. However, there is as yet no consensus on how such differences should be quantified, and perhaps weighted. Antigenic differences are widely agreed to be of considerable importance. There is less unanimity about whether viruses distinguishable only by neutralization tests should be regarded as separate species, or merely as serotypes within a species. The effects of this ongoing debate on the designation of viral species should not be a major concern for the student, since we will use the common vernacular terms to distinguish viruses that cause different diseases.

Monoclonal antibodies are proving of great value in the differentiation of viruses at the species level and below: types, subtypes, strains, and variants—terms that have no generally accepted taxonomic status. At the research rather than the routine diagnostic level, the composition of the nucleic acid of the viral genome—as revealed by molecular hybridization, oligonucleotide fingerprinting or restriction endonuclease mapping, electrophoresis in gels (especially useful for RNA viruses with segmented genomes), and nucleotide sequence analysis—is used to identify species and to distinguish minor differences between viral strains and mutants.

NOMENCLATURE

Since 1966 the classification and nomenclature of viruses at the higher taxonomic levels (families and genera) has been systematically organized by the International Committee on Taxonomy of Viruses. The highest taxonomic group among viruses is the family; families are named with a suffix *-viridae.* Subfamilies have the suffix *-virinae;* genera the suffix *-virus.* The prefix may be another latin word or a *sigla,* i.e., an abbreviation derived from some initial letters. Latinized family, subfamily, and generic names are written in italics; vernacular terms derived from them are written in roman letters. For example, the term

poxviruses is used to designate members of the family *Poxviridae*. It is still customary to use vernacular terms rather than latinized binomials for viral species, e.g., foot-and-mouth disease virus.

THE FAMILIES OF DNA VIRUSES

A brief description of each family of viruses of vertebrates is given below and summarized in Tables 2-1 and 2-2, in order to orient the reader before embarking upon Part I of this book. Most families are allocated separate chapters in Part II, which deals with the role of the viruses in diseases of veterinary importance. We begin with the DNA viruses.

Papovaviridae

The papovaviruses (sigla: *pa* = papilloma; *po* = polyoma; *va* = vacuolating agent) are small nonenveloped icosahedral viruses which replicate in the nucleus and may transform infected cells. In the virion their nucleic acid occurs as a cyclic double-stranded molecule, which is infectious. There are two genera: *Papillomavirus* (wart viruses) (55 nm diameter) have a larger genome (8 kbp) which may persist in an episomal form in transformed cells; *Polyomavirus* (45 nm in diameter) has a smaller genome (5 kbp) which may persist in cells integrated into the host cell DNA.

Papillomaviruses occur in many species of animals, but each is usually of narrow host specificity. Bovine papillomaviruses, which cause cutaneous papillomas of cattle and sarcoids of horses, and the virus of canine oral papillomatosis, are among the most important. The genus *Polyomavirus* includes SV40 (from rhesus monkeys) and mouse polyoma virus, both of which have been very useful models for the study of viral tumorigenesis.

Adenoviridae

The adenoviruses (*adeno* = gland) are nonenveloped icosahedral viruses 70 nm in diameter, whose genome consists of a single linear molecule of dsDNA, 30–37 kbp. They replicate in the nucleus. Adenoviruses are usually associated with infection of the respiratory tract, the intestinal tract, and occasionally the eye. Many are characterized by prolonged subclinical infection.

There are two genera, *Mastadenovirus* and *Aviadenovirus*. Mastadenoviruses that are animal pathogens include equine adenovirus, bovine

TABLE 2-1

Properties of the Virions of the Families of DNA Viruses

Family	Virion					Genome[a]	
	Diameter (nm)	Envelope	Nucleocapsid		Transcriptase	Nature[b]	Size (kb or kbp)
			Symmetry	Capsomers			
Papovaviridae	45, 55[c]	−	Icosahedral	72	−	ds, circular	5, 8[c]
Adenoviridae	70	−	Icosahedral	252	−	ds, linear	30–37
Herpesviridae	150	+	Icosahedral	162	−	ds, linear	120–220
Poxviridae	300–450 × 170–260	+[d]	Complex	—	+	ds, linear	130–280
African swine fever virus	220	+[d]	Icosahedral	1892	+	ds, linear	150
Parvoviridae	20	−	Icosahedral	32	−	ss, (−), linear	5
Hepadnaviridae	45	−	Icosahedral	?	+[e]	ds, circular[f]	3.2

[a]Genome of DNA viruses is invariably a single molecule.

[b]ds, Double-stranded; ss, single-stranded; sense of single-stranded nucleic acid (+) or (−).

[c]Lower figures, *Polyomavirus;* higher figures, *Papillomavirus.*

[d]Not essential for infectivity.

[e]Reverse transcriptase.

[f]Circular molecule is double-stranded for most of its length but contains a single-stranded region.

TABLE 2-2

Properties of the Virions of the Families of RNA Viruses

Family	Virion					Genome	
	Diameter[a] (nm)	Envelope	Nucleocapsid		Transcriptase	Nature[b]	Size (kb or kbp)
			Symmetry	Capsomers			
Picornaviridae	25	−	Icosahedral	60	−	ss, 1, (+)	7.5–8.5
Caliciviridae	35	−	Icosahedral	32	−	ss, 1, (+)	7.9
Togaviridae	65	+[c]	Icosahedral	?	−	ss, 1, (+)	12
Flaviviridae	45	+[c]	Icosahedral	?	−	ss, 1, (+)	12
Orthomyxoviridae	100	+	Helical	—	+	ss, 8, (−)	13.6
Paramyxoviridae	180	+	Helical	—	+	ss, 1, (−)	18–20
Coronaviridae	100	+[c]	Helical	—	−	ss, 1, (+)	17–24
Arenaviridae	120	+[c]	Helical	—	+	ss, 2, (−)	10–14
Bunyaviridae	110	+[c]	Helical	—	+	ss, 3, (−)	13.5–21
Retroviridae	100	+[c]	Icosahedral	?	+[d]	ss, 1, (+)	2 × (3.5–9)[e]
Rhabdoviridae	180 × 75	+	Helical	—	+	ss, 1, (−)	13–16
Filoviridae	790–970 × 80	+	Helical	—	+	ss, 1, (−)	12.7
Reoviridae	60–80	−	Icosahedral	32, 92[f]	+	ds, 10 to 12[g]	17–22
Birnaviridae	60	−	Icosahedral	92	+	ds, 2	7

[a]Some enveloped viruses are very pleomorphic and sometimes filamentous.

[b]All molecules linear; ss, single-stranded; ds, double-stranded; 1 to 12, number of molecules in genome; (+) or (−), sense of single-stranded nucleic acid.

[c]No matrix protein.

[d]Reverse transcriptase.

[e]Genome is diploid, two identical molecules being held together by hydrogen bonds at their 5′ ends.

[f]Inner capsid of *Orbivirus*, *Rotavirus*, and *Reovirus*, 32; outer capsid of *Reovirus*, 92.

[g]*Reovirus* and *Orbivirus*, 10; *Rotavirus*, 11; Colorado tick fever virus, 12.

adenoviruses, infectious canine hepatitis virus, and canine adenovirus type 2. Pathogenic aviadenoviruses include those which cause egg drop syndrome and marble spleen disease. Some adenoviruses of other species of mammals and birds are also pathogenic. Some of the adenoviruses of humans, cattle, and chickens cause malignant tumors when inoculated into newborn hamsters and have been used in experimental studies on tumorigenesis, but none causes tumors in nature.

Herpesviridae

The herpesviruses (*herpes* = creeping) have enveloped virions 120–200 nm in diameter, with icosahedral nucleocapsids about 100 nm in diameter. Their genome is a single linear molecule of dsDNA, 120–220 kbp. They replicate in the nucleus and mature by budding through the nuclear membrane, thus acquiring an envelope. This large family includes many important veterinary and human pathogens, and has been subdivided into three subfamilies. *Alphaherpesvirinae* includes infectious bovine rhinotracheitis virus, bovine mammillitis virus, B-virus, pseudorabies virus, equine rhinopneumonitis and coital exanthema viruses, herpesviruses of cats, dogs, and chickens, and herpes simplex types 1 and 2 and varicella virus of humans. *Betaherpesvirinae* comprises the cytomegaloviruses, which are highly host-specific viruses of humans, cattle, pigs, horses, mice, guinea pigs, and other animals. *Gammaherpesvirinae* are often associated with tumors, and include baboon and chimpanzee herpesviruses, Marek's disease virus of chickens, turkey herpesvirus, *Herpesvirus ateles, Herpesvirus saimiri* of monkeys, and Epstein–Barr (EB) virus in humans.

A feature of all herpesvirus infections is lifelong persistence of the virus in the body, usually in latent form. Excretion may occur continuously or intermittently without disease, or episodes of recurrent clinical disease and recurrent excretion may occur years after the initial infection.

Iridoviridae and African Swine Fever Virus

Originally a number of large icosahedral DNA viruses that affect insects, nematodes, fish, and amphibians were grouped together with African swine fever virus into the family *Iridoviridae*. In 1984 African swine fever virus was removed from the family *Iridoviridae*, but it has not yet been allocated to any other family. The family *Iridoviridae* (*irido* = iridescent) was defined on the characteristics of certain insect viruses, but it includes two genera that affect vertebrates. They are the largest icosahedral viruses (capsid, 125 nm in diameter), and those that affect

vertebrates have enveloped virions 200–250 nm in diameter. The genome is a single linear molecule of dsDNA, 150 kbp. Replication occurs in the cytoplasm, using a virion-associated transcriptase, but nuclear involvement is required for viral DNA synthesis.

The genus *Ranavirus* includes several members that affect amphibians; and a member of the genus *Lymphocystivirus* produces lymphocystis disease of fish. African swine fever virus is an important pathogen of swine, which can be transmitted by contact and also by ticks.

Poxviridae

The poxviruses (*pock* = pustule) are the largest and most complex viruses of vertebrates. The virions are brick shaped, measuring 300–450 × 170–260 nm in all genera except *Parapoxvirus*, in which virions are ovoid, 220–300 × 140–170 nm. All have an inner core which contains a single linear molecule of dsDNA, 130–280 kbp. Unlike all other DNA viruses of vertebrates except the iridoviruses and African swine fever virus, poxviruses replicate in the cytoplasm; mRNA is transcribed by a virion-associated transcriptase.

The family is divided into two subfamilies, one of which, *Chordopoxvirinae*, comprises the poxviruses of vertebrates. This subfamily is divided into six genera, all of which include animal pathogens. The genus *Orthopoxvirus* includes cowpox, camelpox, ectromelia (mousepox), rabbitpox (a variant of vaccinia virus), and monkeypox viruses. Variola virus, which caused human smallpox, and vaccinia virus, used to control that disease, also belong to this genus. *Avipoxvirus* includes many specific bird poxviruses; *Capripoxvirus* includes the viruses of sheeppox, goatpox, and lumpyskin disease of cattle. *Leporipoxvirus* includes myxoma and rabbit fibroma viruses, while the genus *Suipoxvirus* has only one member, swinepox virus. *Parapoxvirus* includes contagious pustular dermatitis, pseudocowpox (milker's nodules), and bovine papular stomatitis viruses.

Parvoviridae

Parvoviruses (*parvus* = small) are only about 20 nm in diameter, have icosahedral symmetry and a genome of ssDNA, 5 kb. The virions are relatively heat stable, and most species have a narrow host range. Members of two genera affect vertebrates. Animal pathogens of the genus *Parvovirus* include feline panleukopenia, mink enteritis, and Aleutian mink disease viruses, and canine, bovine, goose, porcine, and murine parvoviruses. Members of the genus *Dependovirus* are defective viruses,

which depend on adenovirus for replication. They occur in birds, cattle, horses, and dogs, as well as humans, but are not known to cause disease.

Hepadnaviridae

Human hepatitis B virus and related viruses of other animals form a family for which the name *Hepadnaviridae* (*hepa* = liver; *dna*) has been proposed. The virion is a spherical particle 42 nm in diameter, consisting of a 27-nm icosahedral core within a closely adherent outer capsid that contains cellular lipids, glycoproteins, and a virus-specific surface antigen (HBsAg). The genome is a small, circular, partially double-stranded DNA molecule, which consists of a long (3.2 kb) and a short (1.7–2.8 kb) strand.

The hepadnaviruses replicate in the nucleus of hepatocytes and cause hepatitis, which may progress to cirrhosis and primary hepatocellular carcinoma. The most important species is human hepatitis B virus, but hepadnaviruses also occur in Pekin ducks, woodchucks, and squirrels.

THE FAMILIES OF RNA VIRUSES

Picornaviridae

The *Picornaviridae* (name originally derived from *p*oliovirus, *i*nsensitivity to ether, *c*oxsackievirus, *o*rphan virus, *r*hinovirus, and *r*ibonucleic *a*cid (omitting one "r"), but conveniently also consistent with the sigla, *pico* = small; *rna* = ribonucleic acid) comprise small nonenveloped icosahedral viruses 25–30 nm in diameter, which contain a single molecule of (+) sense ssRNA (7.5–8.5 kb), and replicate in the cytoplasm.

There are four genera. Two genera, *Enterovirus* and *Rhinovirus*, include large numbers of species that affect domestic animals, often without causing significant disease. An enterovirus causes swine vesicular disease, which may be confused with foot-and-mouth disease. Porcine enterovirus 1 causes polioencephalomyelitis. These two genera also include several important human pathogens. The only species in the genus *Aphthovirus* is foot-and-mouth disease virus, which occurs as seven types and many antigenically distinct subtypes. It is among the most important of all viruses in veterinary medicine. The genus *Cardiovirus* comprises the viruses of encephalomyelocarditis of swine and rodents.

Caliciviridae

The caliciviruses (*calix* = cup) were first classified as a genus of the family *Picornaviridae,* but were later found to differ enough in morphology and mode of replication to warrant classification as a separate family. They are slightly larger than picornaviruses (diameter 35–40 nm) but have a genome of similar size. Most species have a narrow host range. Several are of veterinary importance: vesicular exanthema viruses of swine, San Miguel sea lion virus, and feline calicivirus. Caliciviruses have also been isolated from vesicular lesions of dogs, mink, dolphins, calves, and chimpanzees. Virions with caliciviruslike morphology have been demonstrated in the feces of humans, calves, pigs, and sheep with diarrhea.

Togaviridae

The togaviruses (*toga* = cloak) are small spherical enveloped viruses 60–70 nm in diameter, containing (+) sense ssRNA (12 kb) enclosed within an icosahedral core. They replicate in the cytoplasm and mature by budding from cell membranes. Three genera, *Alphavirus, Pestivirus,* and *Arterivirus,* contain species of veterinary importance. *Alphavirus* contains many species, all of which are arthropod-transmitted. Important alphaviruses that infect both livestock and humans include eastern, western, and Venezuelan equine encephalitis viruses. In nature the alphaviruses usually produce inapparent viremic infections of birds, mammals, or reptiles, but in domestic animals and humans generalized disease or encephalitis can sometimes result.

Non-arthropod-borne togaviruses include several important animal pathogens. The genus *Pestivirus* includes bovine virus diarrhea virus, hog cholera virus, and border disease virus of sheep. The genus *Arterivirus* includes only equine arteritis virus. The genus *Rubivirus* contains only one species, rubella virus of humans. The ungrouped non-arthropod-borne togaviruses include lactic dehydrogenase virus and simian hemorrhagic fever virus.

Flaviviridae

Formerly classified with the togaviruses, this group (*flavi* = yellow) has now been given family status, principally because of a distinctly different in strategy of replication. In most other respects, including their transmission by arthropods, the flaviviruses resemble the *Alphavirus* genus of the family *Togaviridae,* but they are somewhat smaller (40–50 nm in diameter). There is only one genus, *Flavivirus.*

Flavivirus diseases of veterinary importance include louping ill, turkey meningoencephalitis, and Wesselsbron disease. Central European tick-borne encephalitis virus produces an inapparent infection in ruminants but may be excreted in the milk and cause disease in humans who drink contaminated milk. Other important human pathogens include the viruses of yellow fever, dengue, and several kinds of arthropod-borne encephalitis.

Orthomyxoviridae

The orthomyxoviruses (*myxo* = mucus) are spherical or filamentous RNA viruses 80–120 nm in diameter, with a helical nucleocapsid enclosed within an envelope acquired by budding from the plasma membrane. Their genome consists of seven or eight segments of (−) sense ssRNA (total size, 13.6 kb), and is associated with a viral transcriptase. The envelope is studded with peplomers, which are of two kinds: a hemagglutinin and a neuraminidase.

The family includes two important species: influenzavirus A, which infects birds, horses, and swine, as well as humans; and influenzavirus B, which is a human pathogen only. Influenza C virus is a specifically human pathogen, which has seven segments of RNA in its genome, but rarely causes serious disease. Influenza A viruses of animals (swine, birds) and humans undergo genetic reassortment, to generate novel subtypes ("antigenic shift") which cause major pandemics of influenza in humans.

Paramyxoviridae

The paramyxoviruses have a large, roughly spherical enveloped virion 150–300 nm in diameter, with a helical nucleocapsid. Their genome consists of a single linear molecule of (−) sense ssRNA (18–20 kb), and the virion contains a transcriptase. The envelope contains two glycoproteins: a hemagglutinin (in some species with neuraminidase activity also) and fusion protein.

The family *Paramyxoviridae* includes the etiological agents of some of the most important veterinary diseases: canine distemper, rinderpest of cattle, and Newcastle disease of chickens. It is subdivided into three genera: *Paramyxovirus, Morbillivirus* (*morbilli* = measles), and *Pneumovirus* (*pneumo* = lung). Viruses of the genera *Paramyxovirus* and *Pneumovirus* cause respiratory infections and occasionally generalized infections [*Paramyxovirus:* Newcastle disease virus, parainfluenza 1 virus (Sendai virus of mice), parainfluenza 3 virus of cattle, and other parainfluenza viruses of cattle and birds; *Pneumovirus:* bovine and ovine

respiratory syncytial viruses, pneumonia virus of mice]. Viruses of the genus *Morbillivirus* usually cause generalized infections: rinderpest of cattle, peste-des-petits-ruminants of sheep and goats, and canine distemper, as well as measles.

Coronaviridae

The coronaviruses (*corona* = crown) are somewhat pleomorphic viruses 75–160 nm in diameter, with widely spaced, pear-shaped peplomers in their lipoprotein envelope. This lacks a matrix protein, and encloses a core of undetermined symmetry with a single linear molecule of (+) sense ssRNA, 17–24 kb. Animal pathogens include calf coronavirus (neonatal diarrhea), transmissible gastroenteritis virus of swine, hemagglutinating encephalomyelitis virus of swine, feline infectious peritonitis virus, infectious bronchitis virus of fowl, and turkey bluecomb virus.

Arenaviridae

Areanviruses (*arena* = sand) acquired their name because of the presence of ribosomes (resembling grains of sand in thin sections examined with the electron microscope) incorporated within pleomorphic enveloped virions 50–300 nm in diameter, which contain no matrix protein. Their genome consists of two pieces of (−) sense or ambisense ssRNA (total size, 10–14 kb), each held in a circular configuration by "sticky ends," with an associated transcriptase. All cause natural inapparent infections of rodents.

None of them are of veterinary importance, but humans may occasionally contract a serious generalized disease, e.g., Lassa fever or lymphocytic choriomeningitis. Lassa, Machupo, and Junin viruses are "biosafety level 4" pathogens, i.e., they may be worked with in the laboratory only under maximum biocontainment conditions.

Bunyaviridae

Over 100 bunyaviruses (Bunyamwera is a locality in Africa) comprise the largest single group of arboviruses. The enveloped virions are 90–120 nm in diameter, with a nucleocapsid of helical symmetry. Their genome consists of three pieces of (−) sense or ambisense ssRNA (total size, 13.5–21 kb), each held in a circular configuration, and there is an associated transcriptase. They replicate in the cytoplasm and bud from Golgi membranes. Because of their segmented genome, bunyaviruses readily undergo genetic reassortment, which may produce "antigenic shift." Some strains have been shown to undergo antigenic drift as well.

All have wild-animal reservoir hosts and some are transovarially transmitted in mosquitoes, with a high frequency. The genus *Phlebovirus* includes the important pathogen of sheep and humans, Rift Valley fever virus, and the genus *Nairovirus* includes Nairobi sheep disease virus. Recently it has been shown that the important human disease, hemorrhagic fever with renal syndrome, is caused by a bunyavirus which does not appear to be arthropod-transmitted but is enzootic in rodents.

Retroviridae

This name (sigla: *re* = reverse; *tr* = transcriptase) is used for a large family of enveloped viruses 80–100 nm in diameter, with a complex structure and an unusual enzyme, reverse transcriptase. Uniquely among viruses, the genome is diploid, consisting of an inverted dimer of (+) sense ssRNA (2 × 3.5–9 kb). The dsDNA copy of the viral genome transcribed by the viral reverse transcriptase is integrated into the cellular DNA as an essential part of the replication cycle. Proviral DNA is found in the DNA of all normal cells of many species of animals and may under certain circumstances be induced to produce virus. These are known as endogenous retroviruses. Others, called exogenous retroviruses, are transmitted horizontally.

The family *Retroviridae* is subdivided into three families, two of which contain pathogens of veterinary importance. The subfamily *Oncovirinae* (*onkos* = tumor) includes the oncogenic retroviruses: bovine leukemia virus, feline leukemia and sarcoma viruses, baboon, gibbon, and woolly monkey leukemia–sarcoma viruses, as well as avian reticuloendotheliosis, avian leukemia and sarcoma viruses, and the mouse mammary tumor virus. The subfamily *Lentivirinae* (*lenti* = slow) includes viruses responsible for several slowly developing, often fatal diseases: maedi–visna and progressive pneumonia of sheep, caprine arthritis–encephalitis, equine infectious anemia, and human acquired immune deficiency syndrome (AIDS). *Spumavirinae* (*spuma* = foam) includes the "foamy agents," which are a problem when they contaminate cultured cells, but are not recognized as pathogens.

Rhabdoviridae

The rhabdoviruses (*rhabdos* = rod) are bullet-shaped viruses, about 180 × 75 nm, containing a single molecule of (−) sense ssRNA (13–16 kb), which is associated with a transcriptase. Their helical capsid is enclosed within a shell to which is closely applied an envelope with peplomers. The virion matures by budding through the plasma membrane. Animal pathogens in the genus *Vesiculovirus* include vesicular

stomatitis, Chandipura, Piry, and Isfahan viruses. The genus *Lyssavirus* includes rabies virus and several serologically related agents from Africa. Ungrouped rhabdoviruses that cause diseases in animals include bovine ephemeral fever virus and several rhabdoviruses of fish.

Reoviridae

The family name is a sigla, *r*espiratory *e*nteric *o*rphan virus, reflecting the fact that members of the first discovered genus, *Reovirus*, were found in both the respiratory and intestinal tract of humans and most animals, but were not associated with any disease. The distinctive feature of the family is that the virions contain dsRNA, in 10, 11, or 12 segments (total size, 17–22 kbp). The virions are nonenveloped icosahedrons 60–80 nm in diameter. There are two other genera, each of which causes infections of veterinary importance: *Orbivirus* (*orbi* = ring) and *Rotavirus* (*rota* = wheel). Orbiviruses are arboviruses that include bluetongue viruses of sheep, epizootic hemorrhagic disease virus of deer, and African horsesickness virus. The genus *Rotavirus* includes viruses that are important causes of diarrhea in domestic animals and humans.

Birnaviridae

This recently named family (sigla: *bi* = two; *rna*) contains viruses with nonenveloped virions of icosahedral symmetry, 60 nm in diameter, which replicate in the cytoplasm. The genome consists of two segments of linear dsRNA (total size, 7 kbp). Animal pathogens include infectious bursal disease virus of chickens and infectious pancreatic necrosis virus of fish.

OTHER VIRUSES

There are a number of other viruses that belong to groups that are as yet unclassified. They are the proposed family *Filoviridae*, the toroviruses, the astroviruses, the unclassified virus of Borna disease, and the still mysterious agents that cause the subacute spongiform encephalopathies, which include scrapie.

Filoviridae

The "biosafety level 4" pathogens, Ebola and Marburg viruses, that cause African hemorrhagic fever of humans, have been tentatively allocated to a new family, *Filoviridae*. In many respects they resemble rhabdoviruses, but the virions are pleomorphic and sometimes very long (*filum* = thread), maximun infectivity being associated with a particle

790–970 nm long and 80 nm wide. Their genome is a single molecule of (−) sense ssRNA, 12.7 kb. They are known only by the sporadic infections and occasional nosocomial epidemics produced in humans in Africa.

Toroviruses

The name *Toroviridae* (*torus* = object shaped like a donut) has been suggested for hitherto undescribed viruses that are associated with diarrhea in horses (Berne virus) and calves (Breda virus). The virions are enveloped and disk-shaped (35 × 170 nm) and contain a nucleocapsid of presumed helical symmetry. The genome is a single molecule of (+) sense ssRNA, 20 kb.

Astroviruses

Astrovirus (*astro* = star) is a name accorded unofficially to viruses with small spherical virions with a characteristic star-shaped surface pattern, which have been visualized by electron microscopy in the feces of calves, lambs, and humans. Their genome consists of one molecule of ssRNA about the same size as that of the picornaviruses, but unlike picornaviruses, there are only two capsid proteins.

Borna Disease Virus

An encephalomyelitis of horses and sheep, called Borna disease (Borna is a town in Germany where the disease was first observed) is caused by an unclassified enveloped RNA virus.

Subacute Spongiform Encephalopathies

The causative agents of the subacute spongiform encephalopathies, which include the sheep disease scrapie and the human diseases kuru and Creutzfeldt–Jakob syndrome, remain enigmatic. Their infectivity is highly resistant to inactivation by physical and chemical agents, and they appear to be nonimmunogenic. They may be small and unusual viruses, or they may not be viruses at all, as the term is currently used. All produce slow infections with incubation periods measured in months or even years, followed by progressive disease, which leads inexorably to death from a degenerative condition of the brain characterized by a spongiform appearance.

PROVING A CAUSAL RELATIONSHIP BETWEEN VIRUS AND DISEASE

Before we embark upon the analysis of viral diseases of domestic animals, it will be useful to discuss briefly the problem of establishing causal relationships between a virus and a disease. One of the great landmarks in the scientific study of infectious diseases was the development of what have come to be called the Henle–Koch postulates of causation. They were originally drawn up for bacteria and protozoa, not viruses, and were revised in 1937 by Rivers, who developed a modified set of criteria to cover viruses. With the advent of cell culture for viral diagnosis in the early 1950s, a large number of new viruses were discovered. Many were not associated with disease and were referred to as

TABLE 2-3
Criteria for Causation: A Unified Concept Appropriate for Viruses as Causative Agents, Based on the Henle–Koch Postulates[a]

1. *Prevalence* of the disease should be significantly higher in those exposed to the putative cause than in controls not so exposed
2. *Exposure* to the putative cause should be present more commonly in those with the disease than in controls without the disease when all other risk factors are held constant
3. *Incidence* of the disease should be significantly higher in those exposed to the putative cause than in those not so exposed as shown in prospective studies
4. *Temporally,* the disease should *follow* exposure to the putative agent with a distribution of incubation periods on a bell-shaped curve
5. *A spectrum* of host responses should follow exposure to the putative agent along a logical biological gradient from mild to severe
6. *A measurable host response* following exposure to the putative cause should *regularly appear* in those lacking this before exposure (i.e., antibody, cancer cells) or should *increase* in magnitude if present before exposure; this pattern should not occur in those not so exposed
7. *Experimental reproduction* of the disease should occur in higher incidence in animals or man appropriately exposed to the putative cause than in those not so exposed; this exposure may be experimentally induced in the laboratory, or demonstrated in a controlled natural exposure (as with sentinel animals)
8. *Elimination or modification* of the putative cause or of the vector carrying it should decrease the incidence of the disease
9. *Prevention or modification* of the host's response on exposure to the putative cause should decrease or eliminate the disease (immunization, drugs)
10. The whole thing should make biological and epidemiological sense

[a]From A. S. Evans, *Yale J. Biol. Med.* **49,** 175 (1976).

"orphan" viruses or "viruses in search of disease." Later the problem arose of determining whether viruses were causally involved in various chronic diseases including cancer, a problem that is still of major interest. Since the relevant disease may not be reproducible by experimental inoculation of animals, virologists have had to evaluate the probability of "guilt by association," a difficult procedure that relies to a significant degree on epidemiological and serological methods.

Serological criteria for proving a causal relationship between virus and disease were first formulated by Evans (Table 2-3) in an assessment of the relationship of EB virus to infectious mononucleosis, a human disease, at a time when there was no method of isolation of the virus.

Evans' serological criteria are difficult to apply. With ubiquitous viruses that only occasionally cause diseases such as tumors or chronic neurological disorders, the presence of antibodies in a population is impossible to interpret; an antibody response might precede the development of the disease by so long a period that the relationship is blurred. Antibody may even play a role in the pathogenesis of the disease, or it might appear to develop after the onset of disease, e.g., in bovine leukemia (Chapter 31). Nevertheless, the criteria set out in Table 2-3 provide a useful guide to investigators, which if followed carefully can minimize the number of false attributions of causation.

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CHAPTER 3

Cultivation and Assay of Viruses

Viruses replicate only within living cells. Some viruses are restricted n the kinds of cells in which they replicate, and a few have not yet been :ultivated at all under laboratory conditions. Fortunately, however, nost viruses can be grown in cultured cells, embryonated hen's eggs, or aboratory animals.

In veterinary virology, the natural host animal can be used for the :ultivation of viruses; indeed the earliest viral assay was carried out by Loeffler and Frosch with foot-and-mouth disease virus in cattle. The natural host is still useful for studies of pathogenesis and immunology, vaccine trials, experiments in chemotherapy, and occasionally for diagnostic purposes. However, *in vitro* cultivation of viruses in cell cultures s essential for the study of their mode of replication (Chapter 4) and for diagnostic virology (Chapter 13).

CELL CULTURE

Although over 70 years have elapsed since mammalian cells were first grown *in vitro*, it is only since the advent of antibiotics that cell culture (also referred to as tissue culture) became a matter of simple routine.

Aseptic precautions are still essential, but the problems of contamination with bacteria, mycoplasmas, fungi, and yeasts are no longer insurmountable. Today, most kinds of animal cells can be cultivated *in vitro* for at least a few generations, and numerous immortal cell lines have been derived. Since 1949, when Enders, Weller, and Robbins reported that poliovirus could be grown in cultured nonneural cells with the production of recognizable cytopathic changes, hundreds of previously unknown viruses have been isolated and identified in cell cultures. The discovery of the adenoviruses, rhinoviruses, and many others during the 1950s and 1960s was directly attributable to their use, as were the consequent revolution in the diagnosis of viral diseases, the development of vaccines, and the dramatic advances in knowledge of the molecular biology of animal viruses.

Methods of Cell Culture

Cells may be grown *in vitro* as explants of tissue, such as respiratory or intestinal epithelium, or as cell cultures. Explant cultures are occasionally used for research purposes or for the cultivation of certain viruses, but almost all diagnostic and research work involving viral cultivation is carried out in cell cultures—usually in monolayers, occasionally as suspension cultures.

To produce cell monolayers, tissue is cut into small pieces and placed in a medium containing a proteolytic enzyme such as trypsin. After the cells have dispersed into a single-cell suspension, they are washed, counted, diluted in a growth medium, and permitted to settle on the flat surface of a glass or plastic container. Most types of cells adhere quickly, and under optimal conditions they divide about once a day until the surface is covered with a confluent monolayer.

Media

Cell culture has been greatly aided by the development of chemically defined media containing almost all the nutrients required for cell growth. The best known of these media, developed by Eagle, is an isotonic salt solution with added glucose, vitamins, and amino acids, buffered at pH 7.4, and containing antibiotics to inhibit the growth of bacteria and fungi. Serum must be added to Eagle's medium, and to most others, to supply additional growth factors, without which most cells will not multiply satisfactorily. In recent years several growth factors have been identified, and certain cell lines can now be grown in media that are totally defined chemically. For instance, the Madin–Darby canine kidney cell line has been grown on fibronectin or in poly-

lysine- or collagen-coated dishes, in serum-free medium supplemented with hormones including insulin, binding proteins (e.g., transferrin and albumin), and attachment factors. Such serum-free media are particularly useful for the cultivation of "hybridoma" cells used for the production of monoclonal antibodies, where there is a need to ensure that all the immunoglobulin in the medium is antibody of a single specificity, produced by the hybridoma cells. Defined media also present advantages for the isolation of viruses that are likely to be neutralized by antibody present in "normal" animal serum, but this is generally not a problem if fetal calf serum is employed. Fetal calf serum (5–10%) is therefore incorporated in the media used for the initial growth of cells in culture. Once the monolayer is established, the "growth medium" is removed, virus is inoculated, and "maintenance medium" containing little or no serum is added to the culture.

Types of Cultured Cells

Many types of cells undergo only a few divisions *in vitro* before dying out, whereas others will survive for up to a hundred cell generations and some can be propagated indefinitely. These differences, the nature of which are not fully understood, give us three main types of cultured cells (Plate 3-1).

Primary Cell Cultures. When cultures are established initially from tissue taken directly from animals (often from fetal organs or tissues), they contain several cell types, most of which are capable of only limited growth *in vitro*—perhaps 5 or 10 divisions at most. This restricts their value, whether for routine diagnostic work or vaccine production, because of the high cost and inconvenience of having to obtain fresh tissue each time, as well as lack of consistency from batch to batch. Furthermore, the donor animals often harbor latent viruses which can confuse diagnosis or contaminate vaccines. Nevertheless, the presence of a diverse range of differentiated cell types in such primary cultures means that they tend to be very sensitive to many animal viruses. In veterinary diagnostic virology, it is common practice to inoculate samples suspected to contain virus into primary cultures derived from the same species of animal as that providing the samples.

Diploid Cell Strains. These are cells that are capable of undergoing a number of divisions in culture that is roughly related to the life span of the species of animal—about 50 for fetal human cells and about 10 for fetal cells from horses and cows. They retain their original diploid chromosome number throughout. Diploid strains of fibroblasts established from human fetuses or embryos are widely used in human diagnostic

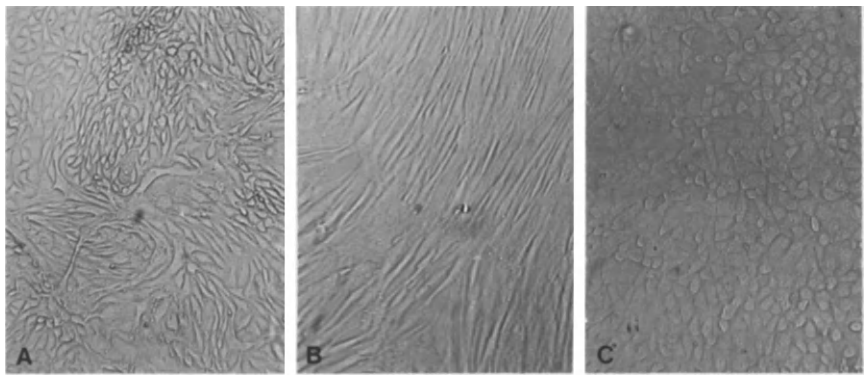

PLATE 3-1. *Unstained confluent monolayers of the three main types of cultured cells, as they appear by conventional low-power light microscopy, through the wall of the tissue culture vessel (×45). (A) Primary monkey kidney epithelium. (B) Diploid strain of fetal fibroblasts. (C) Continuous line of epithelial cells. (Courtesy I. Jack.)*

virology and vaccine production, but diploid strains have not been much used in veterinary vaccine production.

Continuous Cell Lines. These are cells of a single type that are capable of indefinite propagation *in vitro*. Such immortal cell lines originate from cancers, or by spontaneous transformation of a diploid cell strain. Often they no longer bear close resemblance to their cell of origin, as they undergo many mutations during their prolonged culture. The usual indication of these changes is that the cells have lost the specialized morphology and biochemical abilities that they possessed as differentiated cells *in vivo*. For example, it is no longer possible to distinguish microscopically between the epithelial cell lines arising from various cells of ectodermal or endodermal origin, or between the fibroblastic cell lines arising from cells of mesodermal origin. Cells of continuous cell lines are often aneuploid in chromosome number, especially if of malignant origin.

Continuous cell lines derived from monkey (e.g., the Vero cell line), dog (MDCK), cattle (MDBK), pig (PK15), cat (CFK), mouse (L929, 3T3), hamster (BHK-21), rabbit (RK-13), and others are widely used in experimental and diagnostic virology (see Table 13-3).

The great advantage of continuous cell lines over primary cell cultures is that they can be propagated indefinitely by subculturing the cells at regular intervals. Like other cells, they retain viability for many years when frozen in serum-containing medium with added dimethylsulfoxide and stored at very low temperature, e.g., in liquid nitrogen (−196°C) or

an electric deep-freezer at −70°C or lower. Good laboratories follow the general microbiological precept that the surest way of faithfully maintaining the characteristics of a cultured cell line is to replace it periodically from frozen stocks.

Some continuous cell lines have been adapted to grow as suspensions of single cells. Such suspension cultures are particularly useful for biochemical studies of viral replication, because large numbers of identical cells are continuously available for regular sampling and processing.

Various methods have been devised to maximize the surface area to which cells can attach, while keeping the overall size of the vessel and the volume of medium within reasonable bounds. Round bottles can be continuously rolled, or may be filled with glass tubes, glass beads, or spiral plastic film. Perhaps the most useful method for growing cells on a large scale for vaccine production is on plastic or Sephadex beads (microcarriers) maintained in suspension in large fermentation tanks.

Applications of Cell Culture

Of paramount inportance for virologists is the selection of cell lines that will allow optimal growth of the virus under study. Some viruses replicate in almost any cell line and some cell lines support the replication of many different types of viruses. On the other hand, many viruses are quite restricted in the kinds of cells in which they can be isolated from an infected animal. On adaptation by serial passage, however, mutants with somewhat greater growth potential for a given cell line can be selected. A useful general rule is to use a cell strain of low passage number from the same animal species for primary isolation of a virus. Once the virus has been isolated, alternative more convenient cell substrates may be sought.

Cultured cells serve three main purposes: (1) isolation of viruses from clinical specimens (see Chapter 13), for which purpose a type of cell culture should be selected which is known for its high sensitivity and in which cell abnormality is readily recognized, (2) production of vaccines and antigens for serological diagnosis, for which the principal requirement is for a cell line giving a high yield of virus and free from contaminating agents (see Chapter 14), and (3) biochemical studies of viral replication (see Chapter 4), for which continuous cell lines, preferably growing as suspension cultures, are usually chosen.

Recognition of Viral Growth in Cell Culture

The growth of viruses in cell culture can be monitored by a number of biochemical procedures indicative of the intracellular increase in viral macromolecules and virions (see Chapter 4). In addition, there are sim-

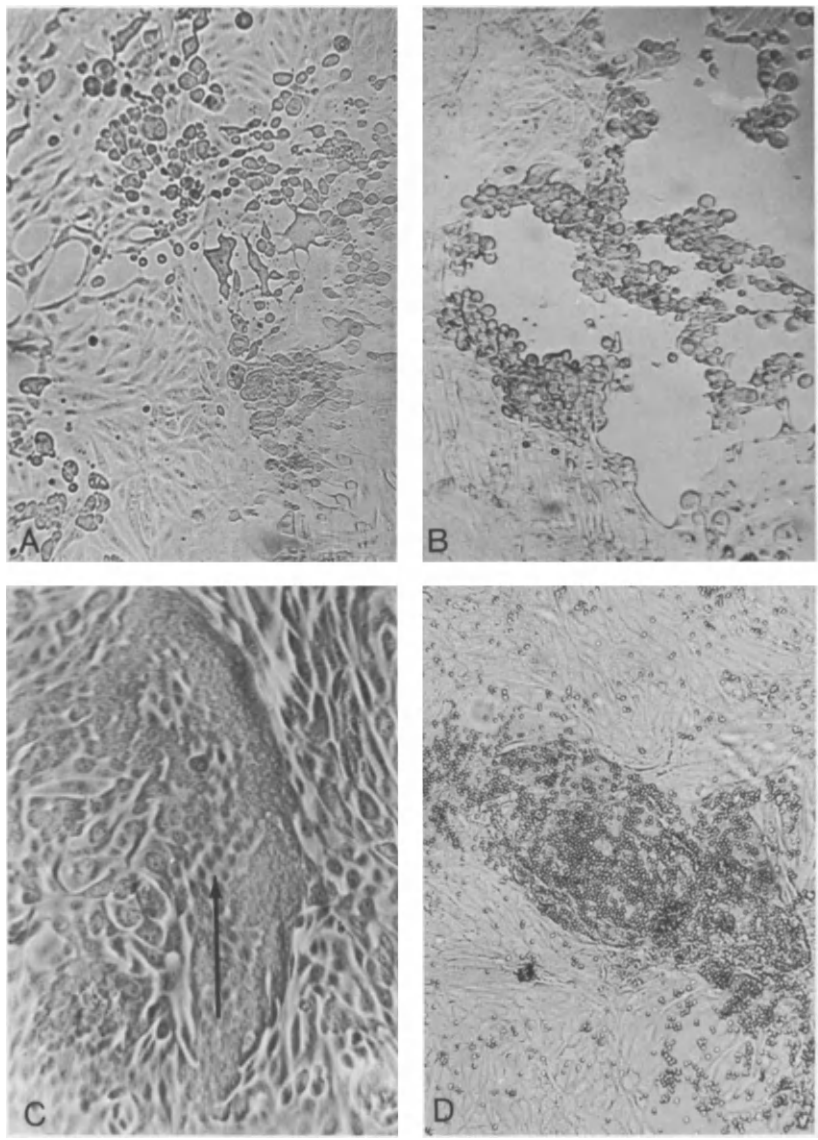

PLATE 3-2. *Cytopathic effects produced by different viruses. The cell monolayers are shown as they would normally be viewed in the laboratory, unfixed and unstained (×60). (A) Enterovirus—rapid rounding of cells, progressing to complete cell destruction. (B) Herpesvirus—focal areas of enlarged, rounded cells. (C) Paramyxovirus—focal areas of cells are fused to form syncytia or giant cells. (D) Hemadsorption. Erythrocytes adsorb to those cells in the monolayer that are infected. The technique is applicable to any virus that incorporated hemagglutinin into the plasma membrane. Most of the enveloped viruses that mature by budding from the cell membranes produce hemadsorption. (Courtesy I. Jack.)*

TABLE 3-1
Cytopathic Effects of Some Viruses in Cell Culture

Cytopathic effect	Virus
Cell lysis[a]	Adenoviruses[b]
	Alphaherpesviruses
	Poxviruses
	Enteroviruses
	Rhinoviruses[c]
	Togaviruses[c]
	Reoviruses
Cell fusion (syncytium formation)	Herpesviruses
	Paramyxoviruses
Minimal	Parvoviruses
	Orthomyxoviruses[d]
	Morbilliviruses
	Coronaviruses
	Retroviruses
	Arenaviruses

[a]Rounding, pyknosis, then detachment.
[b]Some types cause aggregation of cells, and some cause foci of CPE.
[c]Often produce incomplete cytopathic effect.
[d]In most cell types.

pler methods that are more commonly used for diagnostic work (see Chapter 13), as outlined below.

Cytopathic Effects. Many viruses kill the cells in which they replicate, so that infected cell monolayers gradually develop visible evidence of cell damage, as newly formed virions spread to involve more and more cells in the culture. These changes are known as *cytopathic effects* (CPE), and the responsible virus is said to be *cytopathogenic*. Most cytopathic effects can be readily observed in unfixed, unstained cell cultures, under low power of the light microscope with the condenser racked down and the iris diaphragm partly closed to obtain the required contrast.

A trained virologist can distinguish several types of cytopathic effects, even in unstained, living cultures (Plate 3-2, Table 3-1). Fixation and staining of the cell monolayer reveals further diagnostic details, notably *inclusion bodies* (Plate 6-1, Fig. 6-1) and *syncytia* (Plate 3-2C). These morphological consequences of viral infection are discussed in Chapter 6.

Hemadsorption and Hemagglutination. Cultured cells infected with orthomyxoviruses, paramyxoviruses, or togaviruses, all of which bud

from cytoplasmic membranes, acquire the ability to adsorb erythrocytes. This phenomenon, know as *hemadsorption,* is due to the incorporation into the plasma membrane of newly synthesized viral protein that binds red blood cells (Plate 3-2D). Hemadsorption can be used to demonstrate infection with noncytopathogenic as well as cytocidal viruses, and can be demonstrated very early, e.g., after 24 hours, when only a small number of cells in the culture are infected. *Hemagglutination* is a different, though related phenomenon, in which erythrocytes are agglutinated by free virus (see below). Virions or hemagglutinin may thus be demonstrated in the supernatant fluid of an infected culture.

Immunofluorescence. Newly synthesized intracellular viral antigen can be detected by staining the fixed cell monolayer with specific antiviral antibody which has been labeled with a fluorescent dye, or with an enzyme such as peroxidase. Full details of these techniques are given in Chapter 13.

Interference. The replication of one virus in a cell usually inhibits the replication of another virus (see Chapters 6 and 8). The viruses of rubella and of the common cold were first discovered by showing that infected cell cultures, which showed no cytopathic effect, were nevertheless resistant to challenge with an unrelated enterovirus. Cell lines have now become available in which these viruses produce cytopathic effects, but interference is still used for the diagnosis of bovine virus diarrhea virus.

EMBRYONATED EGGS

Prior to the 1950s, when cell culture began to be widely adopted for the cultivation of viruses, the standard host for the cultivation of many viruses was the embryonated hen's egg (developing chick embryo). The technique was devised by Goodpasture in 1930 and was extensively developed by Burnet over the ensuing years. Nearly all of the viruses that were known at that time could be grown in the cells of one or another of the embryonic membranes, namely the amnion (plus the lung of the chick within the amniotic sac), allantois, chorion, or yolk sac.

Although now largely supplanted by cultured cells, embryonated hen's eggs are still used for the isolation and cultivation of many avian viruses (Table 3-2). The chorioallantoic membrane and the amniotic sac are convenient and sensitive substrates for the growth of many poxviruses and influenza viruses, respectively, and intravenous inoculation is used for the isolation of bluetongue viruses. Furthermore, the allantois produces such high yields of certain viruses, notably influenza virus

TABLE 3-2
Some Examples of Viruses Grown in Embryonated Eggs

Route of inoculation	Viruses	Signs of growth
Yolk sac	Avian infectious bronchitits virus	Dwarfing of embryo
	Avian encephalomyelitis virus	Encephalitis
Chorioallantoic membrane	Orthopoxviruses	Pocks
Amniotic	Influenza virus	Hemagglutination
Allantoic	Influenza virus Newcastle disease virus	Hemagglutination
Intravenous	Bluetongue viruses	Death of embryo

and Newcastle disease virus, that this system is still used by research laboratories and for vaccine production.

LABORATORY ANIMALS

Like embryonated eggs, laboratory animals have almost disappeared now from diagnostic laboratories, since cell cultures are so much simpler to handle and much more versatile. Nevertheless, infant mice are still used for the isolation of arboviruses, and the natural host animal is still used for agents that do not grow in cell culture, or when a positive isolation is of critical importance.

Animals are still essential for many kinds of virological research. Experiments on pathogenic mechanisms and the immune response are commonly carried out in the natural animal host, or in inbred strains of mice. Hamsters and other rodents are widely used in tumor virology, because they are highly susceptible to tumor production by a number of tumorigenic viruses. Finally, since serology looms large in much virological research, laboratory animals, usually rabbits, are extensively used for producing antisera. Mice are also commonly used for intraperitoneal implantation of hybridomas which secrete monoclonal antibody as an ascitic fluid.

ASSAY OF VIRAL INFECTIVITY

All scientific research depends on reliable methods of measurement, and with viruses the property we are most obviously concerned with

measuring is infectivity. The content of infectious virions in a given suspension can be titrated by infecting cell cultures (or, more rarely, chick embryos, laboratory animals, or the natural host) with dilutions of viral suspensions, then observing for evidence of viral replication. Two types of infectivity assays should be distinguished: quantitative and quantal.

Quantitative Assays

A familiar example of this type of assay is the bacterial colony count on an agar plate. Each viable organism multiplies to produce a discrete clone, recognized as a colony, and the colony count therefore represents a direct estimate of the number of viable bacteria originally plated. The parallel in virology is the *plaque assay*, using monolayers of cultured cells.

Plaque Assays. In 1952 Dulbecco introduced a modification of the bacteriophage plaque assay into animal virology, and this is now the standard procedure for the quantitation of most animal viruses. A series of 10-fold dilutions of a viral suspension is inoculated onto monolayers of cultured cells for an hour or so to allow the virions to adsorb to the cells. The infected cells are then overlaid with medium in an agar or methylcellulose gel, to ensure that the spread of viral progeny is restricted to the immediate vicinity of the originally infected cell. Hence, each infective particle gives rise to a localized focus of infected cells that becomes, after a few days, large enough to see with the naked eye as an area of cytopathology. To render the plaques more readily visible, the cell monolayers are usually stained with neutral red or crystal violet; the living (uninfected) cells take up the stain and the plaques appear as clear areas against a red or purple background (Plate 3-3). Some viruses, e.g., herpesviruses and poxviruses, will produce plaques even in cell monolayers maintained in liquid medium, because most of the newly formed virions remain cell-associated and plaques form by direct spread of virus to adjacent cells. Infection with a single virus particle is sufficient to form a plaque; the infectivity titer of the original viral suspension is expressed in terms of plaque-forming units (PFU) per milliliter.

Transformation Assays. Some oncogenic viruses do not kill cells but "transform" them (see Plate 6-3), so that they display reduced contact inhibition, and grow in an unrestrained fashion to produce a heaped-up "microtumor" that stands out conspicuously against the background of normal cells in the monolayer. Like malignant cells excised from a tumor, the transformed cells have also acquired the ability to grow in medium in semisolid agar or methylcellulose. Both properties have been exploited by tumor virologists to assay tumorigenic viruses.

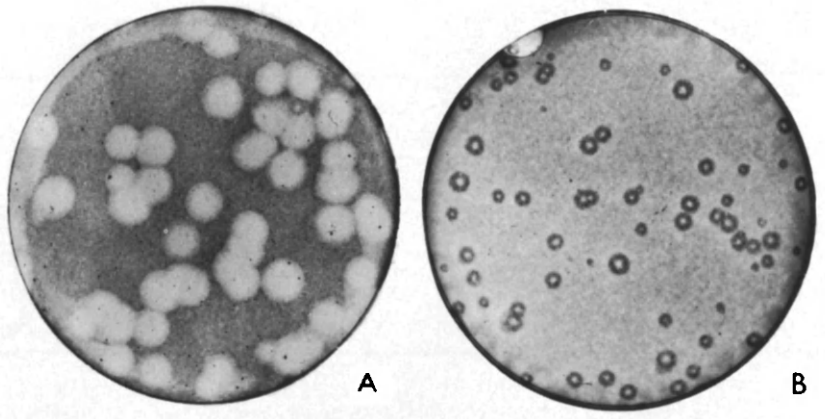

PLATE 3-3. *Plaques produced by influenza virus in monolayers of a continuous cell line derived from human conjunctival cells (Chang). Each plaque is initiated by a single infectious virus particle and yields a clone. (A) Normal plaques, seen as clear areas in monolayer stained with neutral red. (B) "Red" plaques, characteristic of certain strains of influenza virus, and some other viruses. [From E. D. Kilbourne,* In *"Fundamental Techniques in Virology" (K. Habel, ed.), pp. 154, 155. Academic Press, New York; courtesy Dr. E. D. Kilbourne.]*

Pock Assays. A much older assay, still occasionally used for the poxviruses, is the titration of viruses on the chorioallantoic membrane of the chick embryo. Newly synthesized virus escaping from infected cells spreads mainly to adjacent cells, so that each infecting particle eventually gives rise to a localized lesion, known as pock. The morphology and color of the pock is often characteristic of a particular group of viruses or even a particular mutant.

Quantal Assays

The second type of infectivity assay is not quantitative but quantal, i.e., it does not measure the exact number of infectious virus particles in the inoculum, but only whether there are any at all. Being an all-or-none assay, it is not as precise as a quantitative assay. Serial (e.g., 10-fold) dilutions of virus are inoculated into several replicate cell cultures, eggs, or animals. Adequate time is allowed for virus to replicate and spread to destroy the whole cell culture, or kill the embryo or animal, as the case may be. Hence, each host yields only a single piece of information, namely, whether or not it was infected by that particular dilution of virus.

The results of typical quantal and quantitative infectivity titrations are

TABLE 3-3
Comparison of Quantitative and Quantal Infectivity Titrations

Virus dilution[a]	Quantitative assay (plaque count)	Quantal assay[b] (cpe or death of host)
10^{-2}	C, C, C, C, C[c]	+++++
10^{-3}	50, 42, 54, 59, 45	+++++
10^{-4}	5, 7, 3, 6, 4	++−++
10^{-5}	0, 0, 1, 0, 1	−+−−−
10^{-6}	0, 0, 0, 0, 0	−−−−−
Titer	$10^{5.4}$ PFU per ml	$10^{5.2}$ $TCID_{50}$ per ml

[a]Inoculum: 0.2 ml.
[b]Each symbol (+ or −) represents the result in one tissue culture tube or animal.
[c]C, Confluent (uncountable).

given in Table 3-3. The end point of a quantal titration is taken to be that dilution of virus which infects (or kills) 50% of the inoculated hosts; the titer of the original virus suspension is then expressed in terms of 50% infectious doses (ID_{50} or $TCID_{50}$ in tissue cultures) or 50% lethal doses (LD_{50}) per milliliter. Statistical procedures must be used to calculate the end point of quantal titrations. Commonly used procedures are those introduced by Reed and Muench, and Karber, details of which can be found in laboratory manuals.

ASSAYS BASED ON OTHER PROPERTIES OF VIRIONS

Hemagglutination

Many viruses contain, in their outer coat, virus-coded proteins capable of binding to erythrocytes (Table 3-4). Such virions can, therefore, bridge red blood cells to form a lattice. This phenomenon, known as *hemagglutination* (HA), was first described in 1941 by Hirst, who then went on to analyze the mechanism of hemagglutination by influenza virus. The *hemagglutinin* of influenza is a glycoprotein, which projects from the envelope of the virion (see Plate 26-1). The virus will attach to any species of erythrocyte carrying complementary receptors, which are glycoproteins of a different sort. Hemagglutination by influenza virus and the paramyxoviruses is complicated by the fact that the virions also carry an enzyme, neuraminidase, which destroys the glycoprotein receptors on the erythrocyte surface (by removing terminal neuraminic acid) and allows the virus to elute, unless the test is carried out at

TABLE 3-4
Hemagglutination by Viruses

Virus		Erythrocytes
Family	Genera or species	
Adenoviridae	Most species	Monkey and/or rat, 37°C
Poxviridae	*Orthopoxvirus*	Chicken, 37°C
Parvoviridae	Most species	Guinea pig, hamster, pig, human, monkey, 4°C
Togaviridae	*Alphavirus*	Goose, pigeon, chick; pH and temperature critical
Flaviviridae	*Flavivirus*	
Orthomyxoviridae	Influenza A	Chick, human, guinea pig, 4°C
Paramyxoviridae	Parainfluenza Newcastle disease	Chick, human, guinea pig, 4°C
Coronaviridae	Several species	Rat, mouse, chick
Bunyaviridae	Several species	Goose; pH critical
Rhabdoviridae	Rabies	Goose, 4°C
Reoviridae	*Reovirus* *Rotavirus*	Human

ambient or lower temperature. About 10^7 influenza virions are needed to cause agglutination of sufficient erythrocytes to permit the test to be read with the naked eye. Thus, hemagglutination is not a sensitive indicator of the presence of small numbers of virions, but because of its simplicity it provides a very convenient assay if large amounts of virus are available (see Plate 13-3).

Counting Virions by Electron Microscopy

Negative staining with potassium phosphotungstate makes it possible to count the number of particles in viral suspensions by electron microscopy. There are three main approaches. The viral suspension can be mixed with a known concentration of polystyrene latex particles (or with a morphologically distinct virus of known particle count), to provide an easily recognizable marker; the ratio of "unknown" virus particles to latex beads enables its concentration to be determined. Alternatively, a known volume of viral suspension is deposited on the grid by ultracentrifugation. Another method is to remove water and salts from a drop of viral suspension hanging from the underside of an ultrathin carbon-coated plastic ("Formvar") film mounted on a copper grid, by diffusion downwards into agar; thus the number of particles in a known volume may be counted.

TABLE 3-5
Comparison of Assays for Influenza Virus

Method	Titer (per ml)
Electron microscope count	10^{10} virions
Quantal infectivity assay in eggs	10^{9} egg ID_{50}
Quantal infectivity assay in cultured cells[a]	$10^{7.8}$ $TCID_{50}$
Quantitative infectivity assay by plaque formation[a]	10^{8} PFU
Hemagglutination assay	10^{3} HA units

[a]In the same cell line.

Comparison of Different Assays

If a given preparation of viral particles were to be assayed by all of the methods described above, the "titer" would be different in every case. For example, an influenza virus suspension may provide the data set out in Table 3-5. The difference between the electron microscope and hemagglutination titers reflects merely the difference in sensitivity between the two assays. On the other hand, the ratio between the infectivity titer and the particle count, known as the infectivity to particle ratio, requires deeper analysis. In part, it is explained by the fact that most of the virions visible by electron microscopy are noninfectious, having being inactivated by heat (even at 37°C) or by other mechanisms during extraction and purification, or having been assembled unsatisfactorily in the first place (typically having a defective genome, hence known as "defective" or "incomplete" virus—see Chapter 5). However, the situation is complicated by another factor, namely, the susceptibility of the cell system in which viral infectivity is assayed; here one speaks of the *plating efficiency* of the virus. Even a fully infectious virion has only a certain chance of successfully negotiating all the barriers it may encounter in the course of entering and infecting a cell. The susceptibility of one cell system may be much lower (i.e., resistance much higher) than that of another, hence a given viral preparation may have a lower efficiency of plating in one system than in the other; for example, there is a 1.2 $\log_{10}$ difference between egg ID_{50} and $TCID_{50}$ in Table 3-5.

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CHAPTER 4

Viral Replication

Viral replication is the central focus of much experimental virology and a significant part of molecular biology. Studies with *bacteriophages* in their prokaryotic host cells in the 1940s and 1950s provided the first insights into the complexities of viral replication. With the development of mammalian cell culture procedures (see Chapter 3), the techniques used for the study of bacteriophages were adapted to animal viruses. Progress has been such that the basic mechanisms of transcription, translation, and nucleic acid replication have been characterized for all the major families of animal viruses and the strategy of gene expression and its regulation clarified. Many important biochemical phenomena, such as splicing and other types of posttranscriptional processing of RNA, posttranslational cleavage and glycosylation of proteins, replication of RNA, reverse transcription, integration, and transposition of

viral genes and cellular oncogenes, were first elucidated by virologists and have general application in cell biology.

Our knowledge of viral replication is now very detailed and is expanding rapidly. Every viral family has a different strategy of replication, and for each family several reviews have been published since 1980. It is neither possible nor appropriate to deal comprehensively with the subject in this book. This chapter provides a general overview; some additional information on particular viral families is provided in Part II. An understanding of viral replication provides a basis for understanding pathogenesis, immunity, chemotherapy, and the role of viruses in cancer.

THE VIRAL REPLICATION CYCLE

The One-Step Growth Curve

Following the pattern established in experiments with bacteriophages, studies of the replication of animal viruses began with the one-step growth experiment. In such experiments, all cells in a culture are infected simultaneously, i.e., at high *multiplicity of infection*. Unadsorbed input virus is removed or neutralized, usually after 1 hour, and the increase in infectious virions over time is followed by titrating cell-free and cell-associated infectivity. Shortly after infection, the inoculated virus "disappears"; infectious particles cannot be demonstrated, even intracellularly. This *eclipse period* (Fig. 4-1) continues until the first progeny virions become detectable some hours later. Nonenveloped viruses mature within the cell and are detectable for some hours as intracellular virions before they are released by cell lysis. Many enveloped viruses, on the other hand, mature by budding from the plasma membrane and are thus immediately released into the medium. The eclipse period ranges from 5 to 15 hours for the various DNA viruses and from 3 to 10 hours for RNA viruses (see Table 4-2).

Early studies, relying on quantitative electron microscopy and assay of infectivity, provided a limited amount of information about the early and the late events in the replication cycle (attachment, penetration, intracellular maturation, and budding) but could not tell us anything about what happened during the eclipse period. Investigation of the expression and replication of the viral genome became possible only with the development of molecular methods, and during the last two decades all the sophisticated techniques of molecular biology have been applied to this problem.

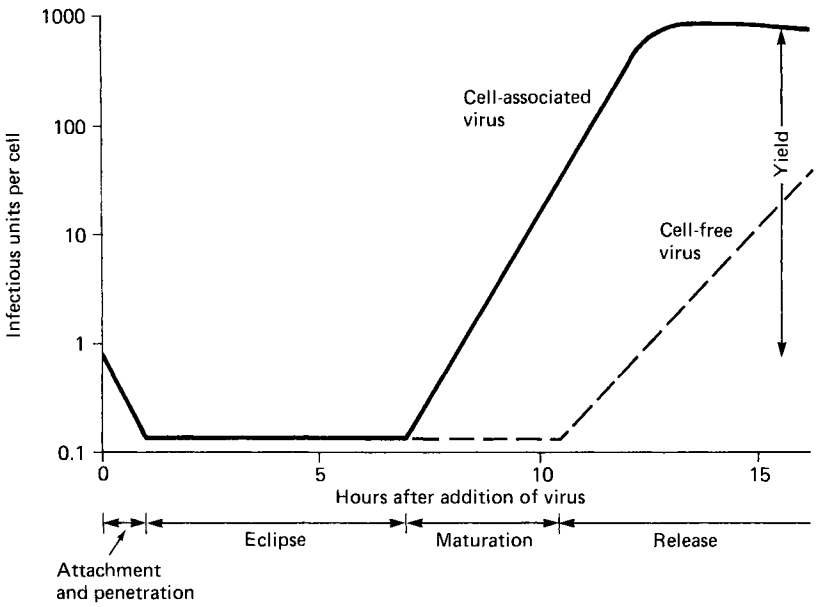

FIG. 4-1. *One-step growth curve of a nonenveloped virus. Attachment and penetration are followed by an eclipse period of 3 to 15 hours (see Table 4-2) during which cell-associated infectivity cannot be detected. This is followed by a period of several hours during which maturation occurs. Virions of nonenveloped viruses are often released late and incompletely, when the cell lyses. Release of enveloped virions occurs concurrently with maturation by budding from the plasma membrane.*

The Replication Cycle

The complete cycle, including what happens during the eclipse period, is illustrated diagrammatically in Fig. 4-2, using an icosahedral DNA virus as an example and ignoring the precise location of intracellular events. Following attachment, the virion penetrates the host cell and is partially uncoated to expose the viral genome. Certain *early viral genes* are transcribed into RNA which may then be processed in a number of ways, including splicing. The early gene-products translated from this *messenger RNA* (*mRNA*) are of two main types: proteins that regulate the expression of the viral and cellular genomes, and enzymes required for the replication of viral nucleic acid (Table 4-1). Following viral nucleic acid replication, *late viral genes* are transcribed. The late proteins are

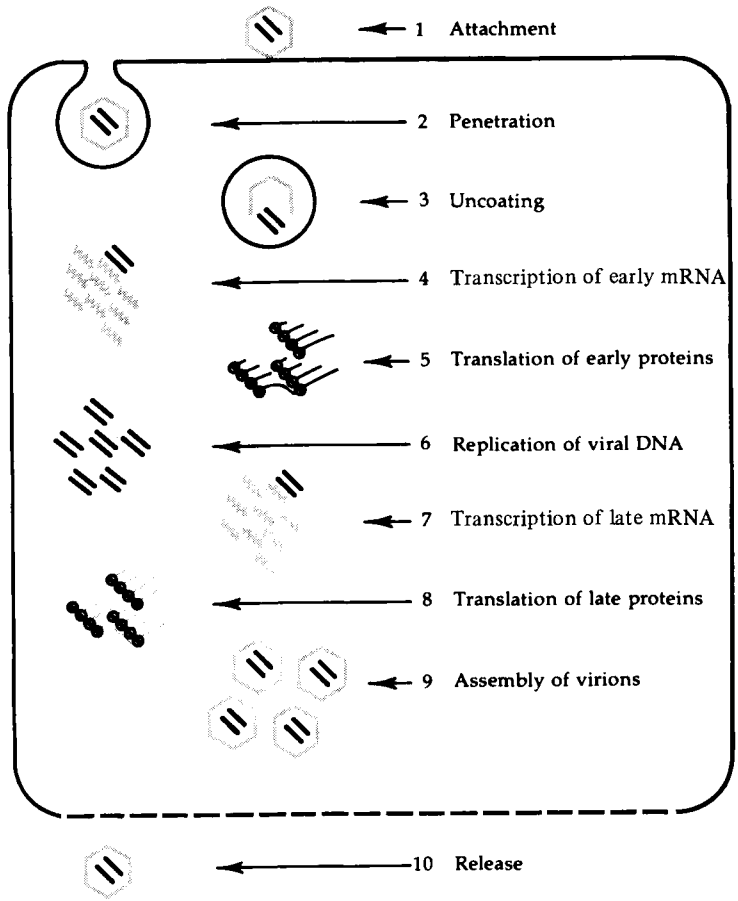

FIG. 4-2. *General features of the viral replication cycle, using a nonenveloped icosahedral DNA virus as a model. No topographical location for any step is implied. One step grades into the next such that, as the cycle progresses, several of these processes are proceeding simultaneously. Release occurs by cell lysis.*

principally viral structural proteins; some of these are subject to post-translational modification, such as glycosylation and/or cleavage. Assembly of icosahedral virions occurs in the nucleus or cytoplasm, depending on the particular family. Enveloped viruses are completed by "budding" through cellular membranes. Each infected cell yields several thousand new virions over a period of several hours.

TABLE 4-1
Kinds of Proteins Coded by the Viral Genome

Kind of gene-product	Description
Early	Nonstructural proteins, mainly enzymes, involved in transcription and replication of viral nucleic acid
Early or late	Proteins affecting cellular macromolecular synthesis Regulatory proteins controlling expression of viral genes
Late	Structural proteins of the virion Virion-associated enzymes

Differences between Families

Many of the processes that can be investigated by morphological studies and infectivity assays differ according to viral family (Table 4-2). There are three principal methods of penetration, and virions may be released by cell lysis or by budding. Some viruses acquire an envelope by budding through plasma membrane, others through nuclear membrane, and still others in the Golgi complex or the endoplasmic reticulum. Some viruses shut down the synthesis of cellular macromolecules very effectively, whereas others do not. Indeed, some viruses are noncytocidal and others actually induce the cell to divide, or even transform it to a tumor cell (see Chapter 6).

Even more significant are the differences in the strategy of expression of the viral genome. Under this heading are subsumed the key processes occurring during the eclipse period: transcription and processing of viral mRNA (Fig. 4-2, steps 4 and 7), translation and processing of viral proteins (steps 5 and 8), and replication of the viral nucleic acid (step 6). Before discussing them, we will describe the earlier events: attachment (step 1), penetration (step 2), and uncoating (step 3).

ATTACHMENT (ADSORPTION)

Because virions and cells are both negatively charged at physiological pH, they tend to repel one another, but random collisions do occur and initial (reversible) attachment may be facilitated by cations. Firm binding requires the presence of specific receptors for the virus on the plasma membrane, to which specific molecules on the surface of the virion

TABLE 4-2
Characteristics of Replication of Selected Viruses

Family	Example	Site of nucleic acid replication	Eclipse period (hours[a])	Budding (membrane)	Cell shutdown[a,b]
Parvoviridae	Rat virus	Nucleus	6	—	+
Papovaviridae	SV40	Nucleus	15	—	+[c]
Adenoviridae	Adenovirus h2	Nucleus	12	—	+
Herpesviridae	Herpes simplex virus	Nucleus	5	Nuclear	+
Unclassified	African swine fever virus	Cytoplasm	5	Plasma	+
Poxviridae	Vaccinia virus	Cytoplasm	5	Golgi	+
Picornaviridae	Poliovirus	Cytoplasm	3	—	+
Caliciviridae	Feline calicivirus	Cytoplasm	3	—	+
Togaviridae	Sindbis virus	Cytoplasm	3	Plasma	+
Flaviviridae	Kunjin virus	Cytoplasm	3	Endoplasmic	+
Coronaviridae	Murine hepatitis virus	Cytoplasm	5	Golgi	+
Paramyxoviridae	Newcastle disease virus	Cytoplasm	4	Plasma	+
Rhabdoviridae	Vesicular stomatitis virus	Cytoplasm	3	Plasma	+
Arenaviridae	Pichinde virus	Cytoplasm	5	Plasma	−
Bunyaviridae	Snowshoe hare virus	Cytoplasm	4	Golgi	+
Orthomyxoviridae	Influenza A virus	Nucleus	3	Plasma	+
Retroviridae	Avian leukosis virus	Nucleus	10	Plasma	−
Reoviridae	Reovirus 3	Cytoplasm	5	—	+
Birnaviridae	Infectious bursal disease virus	Cytoplasm	4	—	−

[a]Differs with multiplicity of infection, strain of virus, cell type, and physiological condition.
[b]Differs markedly in degree and in rapidity, from early and profound to late and partial.
[c]No shutdown in transformation.

attach. Orthomyxoviruses and paramyxoviruses bind via the hemagglutinin, an envelope glycoprotein, to glycoprotein or glycolipid cellular receptors with oligosaccharide side chains terminating in *N*-acetylneuraminic acid. Most enteroviruses of humans, swine, and chickens are highly host cell-specific, because only the homologous cells carry receptors to which the relevant viral capsid protein attachment site can bind.

While there is some specificity about the binding of virions to particular cellular receptors, several different viruses may utilize the same receptor.

PENETRATION

Electron microscopic and other data show that virions can enter cells by at least three different mechanisms: endocytosis, fusion, and translocation. The majority of virions entering a cell fail to initiate infection, many virions taken up by endocytosis being degraded by lysosomal enzymes. However, for some viruses this may be the normal route of penetration, leading to uncoating and productive infection.

Endocytosis

The majority of mammalian cells are continuously engaged in *receptor-mediated endocytosis*, a specific process for the uptake of essential macromolecules. Viruses may use receptor-mediated endocytosis to initiate infection (Plate 4-1). Following attachment to receptors, virions move down into *coated pits*. These pits, coated with clathrin, fold inward to produce coated vesicles that enter the cytoplasm and fuse with a lysosome to form a phagolysosome. With enveloped viruses, the envelope of endocytosed virions fuses with the lysosomal membrane, releasing the viral nucleocapsid into the cytoplasm. In this way a virion can be uncoated by a lysosome but escape total degradation by the lysosome's hydrolytic enzymes. Recent studies with influenza virus have identified a pH 5-mediated conformational change in the hemagglutinin molecule which enables fusion to occur between the viral envelope and the membrane of the phagolysosome.

Fusion with Plasma Membrane

The F (fusion) glycoprotein of paramyxoviruses, in its cleaved form, enables the envelope of these viruses to fuse directly with the plasma membrane, even at pH 7. This may allow the nucleocapsid to be released directly into the cytoplasm. Although a number of other enveloped viruses display a capacity to fuse cells or to lyse erythrocytes, it is not clear whether this is the normal way in which they infect cells.

Translocation

Some nonenveloped icosahedral viruses appear to be capable of passing directly through the plasma membrane.

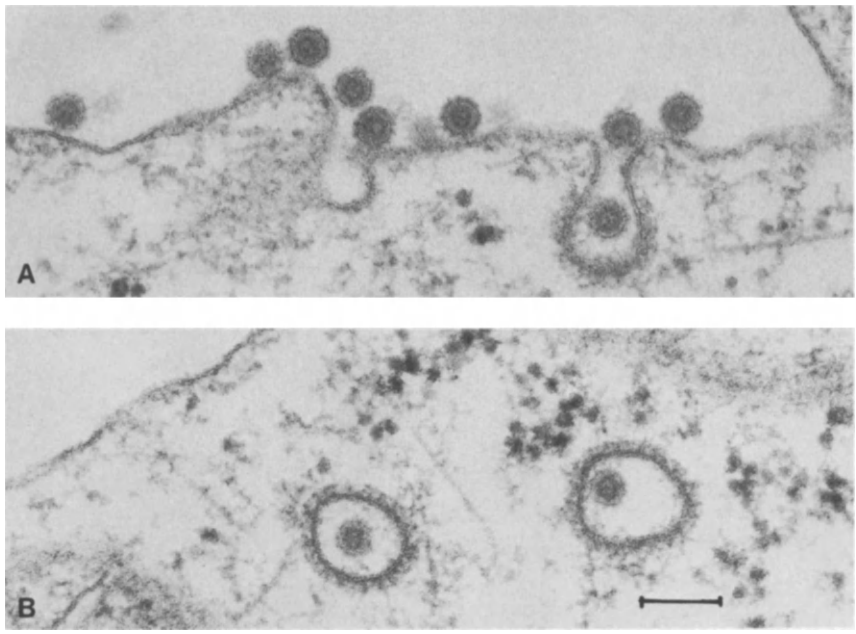

PLATE 4-1. *Penetration by a togavirus. (A) Attachment and movement into a coated pit. (B) Endocytosis, coated vesicle (bar = 100 nm). [A, from E. Fries and A. Helenius,* Eur. J. Biochem. **8,** *213 (1979); B, from K. Simons* et al., Sci. Am. **246,** *46 (1982), Courtesy Dr. A. Helenius.]*

UNCOATING

In order that at least the early viral genes may become available for transcription, it is necessary that the virion be at least partially uncoated. With viruses that enter the cell by fusion of their envelope with either the plasma membrane or the membrane of a phagolysosome, the nucleocapsid is discharged directly into the cytoplasm. In the case of viruses with helical nucleocapsids, transcription begins from viral RNA while it is still associated with nucleoprotein. In the case of the icosahedral reoviruses only certain capsid proteins are removed and the viral genome expresses all its functions, even though it is never fully released from the core ("subviral particle"; Plate 4-2). Poxviruses are uncoated in two stages: first, to a core, from which half the genome is transcribed; then completely, following the synthesis of a virus-coded uncoating protein. With the picornaviruses, the process of attachment of the virion to the cell leads to a conformational change in the capsid,

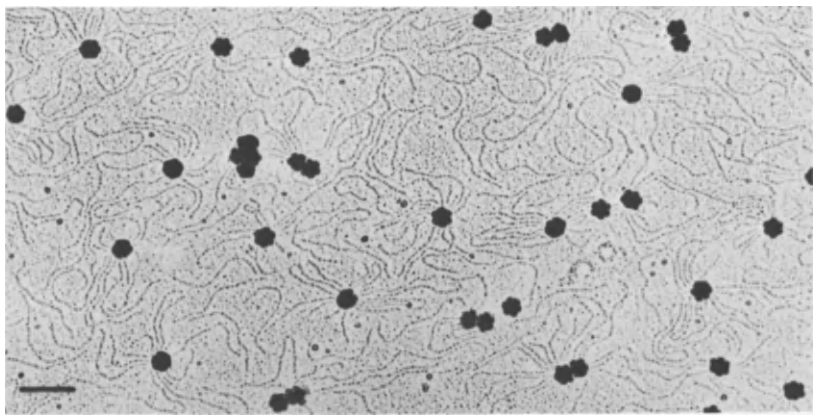

PLATE 4-2. *Reovirus messenger RNA (bar = 200 nm). Reovirus "cores" that have synthesized mRNA for 8 minutes at 37°C were prepared for electron microscopy by the Kleinschmidt technique, stained with uranyl acetate, and shadowed at a low angle with platinum–palladium, showing the fine fibrils of mRNA being extruded from the cores or occurring free around them. The results of polyacrylamide gel electrophoretic analysis of such mRNA molecules at various times during the replication cycle are illustrated in Fig. 4-6. [From N. M. Bartlett, S. C. Gillies, S. Bullivant, and A. R. Bellamy,* J. Virol. **14,** *315 (1974), courtesy Dr. A. R. Bellamy.]*

resulting in the loss of capsid proteins VP4 and VP2 and rendering the particle susceptible to proteases; the attachment step itself triggers the process of uncoating. For some viruses that replicate in the nucleus there is evidence that the later stages of uncoating occur there, rather than in the cytoplasm.

STRATEGIES OF VIRAL REPLICATION

The key events in viral replication are the synthesis of viral proteins, the replication of the viral genome, and the assembly of the new components into virions. To synthesize viral proteins, viral mRNAs must be produced in a form capable of being recognized and translated on cellular ribosomes. Eukaryotic cells synthesize their own mRNA in the nucleus by transcription of the cellular DNA followed by processing of the transcript. They lack the enzymes necessary for synthesizing mRNA off a viral RNA genome and they cannot transcribe viral DNA located in the cytoplasm. Therefore, only those DNA viruses that replicate in the nucleus utilize the cellular machinery for transcription. All other viruses

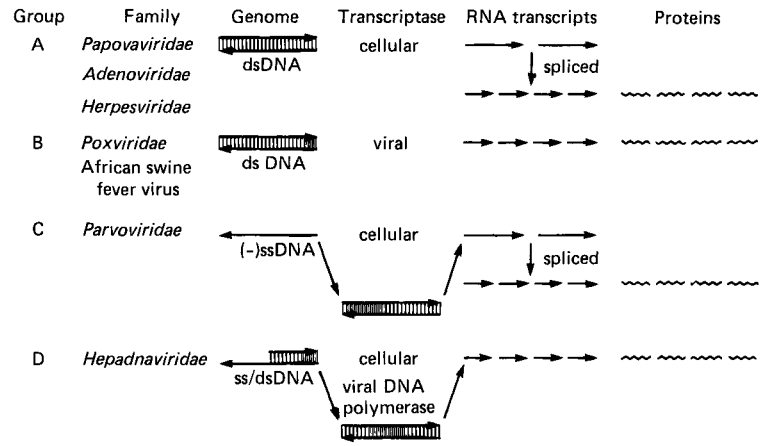

FIG. 4-3. *Simplified diagram showing essential features of the strategy of expression of the genome of DNA viruses. The sense of each nucleic acid molecule is indicated by an arrow [(+), to the right; (−) to the left]. The number of mRNA and protein species for each virus has been arbitrarily shown as four. See text for details.*

provide their own enzymes to produce mRNAs. Eukaryotic cells have a further constraint, namely, that the protein-synthesizing machinery apparently cannot recognize internal initiation sites within polycistronic mRNAs. Hence viruses must synthesize a separate (*monocistronic*) mRNA corresponding to each gene in their genome, or, alternatively, a *polycistronic* mRNA must be translated into a large precursor "polyprotein" which is then cleaved into individual proteins.

The diverse strategies followed by viruses of different families for transcription and translation are illustrated diagrammatically in Fig. 4-3 (for DNA viruses) and Fig. 4-5 (for RNA viruses). Necessarily, the processes summarized in these figures and the descriptions of them involve major oversimplifications. We will describe in turn transcription, translation, and replication of the viral nucleic acid.

TRANSCRIPTION

The viral RNA of (+) sense ssRNA viruses binds directly to ribosomes and is translated in full or in part without the need for any prior transcriptional step. With all other classes of viral genomes, mRNA must be transcribed. In the case of DNA viruses that replicate in the nucleus, the cellular DNA-dependent RNA polymerase II performs this function. All other viruses require unique and specific transcriptases which are virus-

coded and are an integral component of the virion. Cytoplasmic dsDNA viruses carry a DNA-dependent RNA polymerase, whereas dsRNA viruses have dsRNA-dependent RNA polymerase, and (−) sense ssRNA viruses carry a ssRNA-dependent RNA polymerase (see Tables 2-1 and 2-2).

DNA Viruses

For all DNA viruses, mRNA must be transcribed by a DNA-dependent RNA polymerase. Transcription of the viral DNA is programmed such that not all genes are expressed simultaneously or continuously throughout the replication cycle. Particular parts of the genome are transcribed in sequence, the so-called *early genes* first, and the *late genes* later in the cycle. Viruses of different families differ according to whether a cellular or a viral transcriptase is employed, correlating with a nuclear or cytoplasmic site of replication. There are four classes of strategy of expression of the viral genome (Fig. 4-3A–D), described below.

dsDNA; Cellular Transcriptase (Fig. 4-3A). This group comprises the papovaviruses, adenoviruses, and herpesviruses, and has in one respect the most straightforward strategy: the viral DNA is transcribed within the nucleus by a cellular DNA-dependent RNA polymerase. There are at least two temporally separated cycles for adenoviruses and herpesviruses; in each instance the structural proteins of the virion are made from mRNAs produced in the last cycle of transcription. Polycistronic but subgenomic RNA transcripts (corresponding to several genes but less than the whole genome) undergo cleavage and splicing to produce monocistronic mRNAs, introns being removed in the process.

dsDNA; Virion Transcriptase (Fig. 4-3B). The poxviruses and African swine fever virus, which replicate in the cytoplasm, carry their own transcriptase. It appears that monocistronic mRNAs are transcribed directly from the viral DNA. There are at least three cycles of transcription. The transcripts are translated directly into proteins, some of which need to undergo posttranslational cleavage to yield functional molecules.

ssDNA; Cellular Transcriptase (Fig. 4-3C). The (−) sense ssDNA of the parvoviruses requires the synthesis of a complementary strand to form dsDNA; this is then transcribed in the nucleus and the transcripts are processed to produce mRNAs, before export to the cytoplasm for translation.

ds/ssDNA; Cellular Transcriptase, Virion DNA Polymerase (Fig. 4-3D). The ssDNA portion of the genome of hepadnaviruses is first repaired by

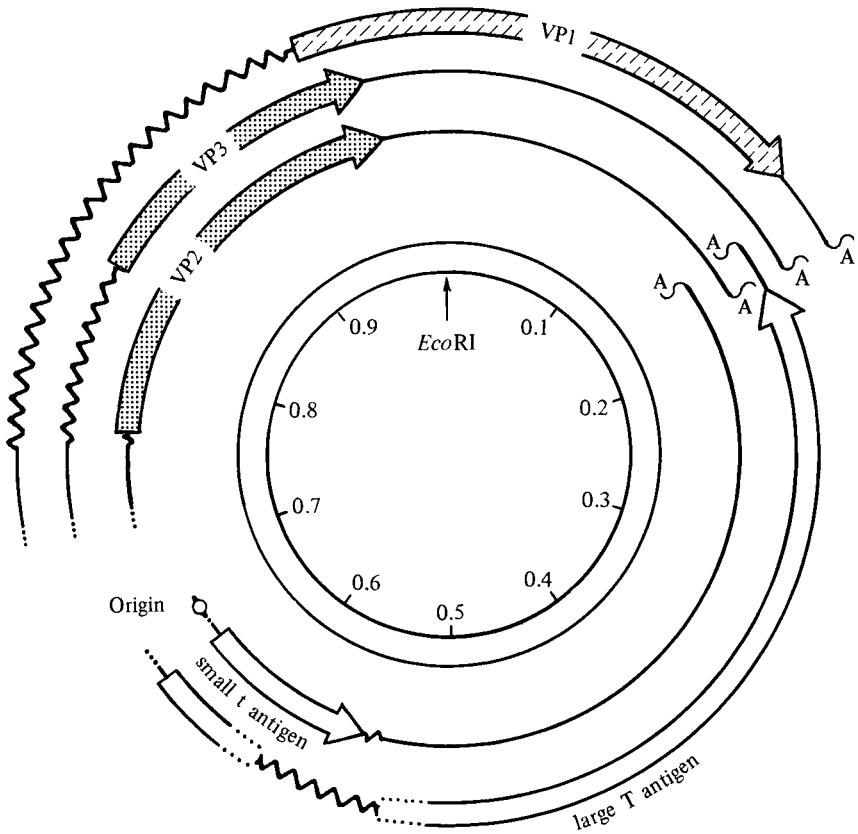

FIG. 4-4. *Transcription map of the DNA of the papovavirus SV40. The circular dsDNA is oriented with the* EcoRI *restriction endonuclease cleavage site at zero and the origin of DNA replication (origin) at map position 0.66. The direction of transcription of the early genes is counterclockwise on one DNA strand (open arrows), and that of the late genes is clockwise on the other strand (stippled and shaded arrows). The thin lines indicate regions of the primary RNA transcript that are not translated into protein, while the wavy lines indicate regions of the transcript that are spliced out (introns). The 3'-terminal poly(A) tail of each mRNA is labeled A. The coding regions of the primary transcript are shown with large arrows. The genes for the early proteins, small-t and large-T overlap, as do those for the late proteins VP1, VP2, and VP3. Large-T is coded by two noncontiguous regions of DNA. The amino acid sequence of VP3 corresponds with the C-terminal half of VP2. However, VP1 shares no part of its amino acid sequence with VP2 or VP3, even though the VP1 gene overlaps VP2 and VP3, because its mRNA is transcribed in a different reading frame. [Modified from W. Fiers* et al., Nature (London) **273,** *113 (1978).]*

a virion-associated DNA polymerase, and the DNA then converted into a supercoiled dsDNA. Transcription of mRNA by cellular RNA polymerase II then occurs.

Expression of a DNA Genome. Analysis of the 5224-bp sequence of the circular dsDNA of the papovavirus SV40 and its transcription program have provided insights into these processes (Fig. 4-4). The following points should be noted:

1. The early genes and the late genes are transcribed by the host cell RNA polymerase II in opposite directions, from different strands of the DNA.
2. Certain genes overlap and are translated in the same frame, so that their protein products have some amino acid sequences in common.
3. Some regions of the viral DNA are read in overlapping but different reading frames, so that two completely different amino acid sequences are obtained.
4. At least 15% of the viral DNA consists of *intervening sequences* (*introns*), which are transcribed but not translated, because they are excised from the primary transcript.
5. Up to three distinct proteins can be produced from mRNAs derived from a primary transcript by different splicing protocols.

Regulation of Transcription. Studies with adenoviruses have elucidated the mechanisms that regulate the expression of viral genomes, which operate principally, but not exclusively, at the level of transcription. Because of the complications arising from posttranscriptional cleavage of mRNA and posttranslational cleavage of precursor proteins in eukaryotic cells, it is no longer adequate to talk of a "gene" and its "gene-product." More appropriate perhaps is to think in terms of a *transcription unit*, i.e., that region of the genome beginning with the transcription initiation site, extending right through to the transcription termination site, and including all introns and exons in between. "Simple" transcription units may be defined as those encoding only a single protein, whereas "complex" transcription units code for more than one. There are many adenovirus transcription units. At different stages of the viral replication cycle—"pre-early," "early," "intermediate," and "late"—the various transcription units are transcribed in a given temporal sequence. A product of the early-region E1A induces the other early regions including E1B, but following viral DNA replication, there is a 50-fold increase in the rate of transcription from the major late promoter relative to early promoters such as E1B, and a decrease in E1A mRNA levels. A second control operates at the point of termination of transcription.

Transcripts that terminate at a particular point early in infection are read through this termination site later in infection to produce a range of longer transcripts with different polyadenylation sites.

Processing of RNA Transcripts. Primary RNA transcripts from eukaryotic DNA are subject to a series of posttranscriptional alterations in the nucleus, known as *processing,* prior to export to the cytoplasm as mRNA. First, a *cap,* consisting of 7-methylguanosine (m^7Gppp) is added to the 5′ terminus. The function of this *poly(A) tail* is uncertain, but it may act as a recognition signal for processing and for transport of mRNA from the nucleus to the cytoplasm, and it may stabilize mRNA against degradation in the cytoplasm. Third, a methyl group is added at the 6 position to about 1% of the adenylate residues throughout the RNA (methylation). Fourth, introns are removed from the primary transcript and the *exons* are linked together in a process known as *splicing;* the precise mechanism is not known but may involve excision of the introns by endonucleases, followed by ligation. Splicing is an important mechanism for regulating gene expression in nuclear DNA viruses. A given RNA transcript can have two or more splicing sites and be spliced in several different ways to produce a variety of mRNA species coding for distinct proteins; both the preferred poly(A) site and the splicing pattern may change in a regulated fashion as infection proceeds. The rate of degradation of mRNA provides another level of regulation. Not only do different mRNA species have different half-lives, but the half-life of a given mRNA species may change as the replication cycle progresses.

RNA Viruses

Transcription is more complicated for the RNA viruses than for rhe DNA viruses, which is perhaps not surprising, since they are the only forms of life that utilize RNA as the repository of genetic information. There are, broadly, three main strategies: (1) the virion RNA of most viruses with (+) sense RNA is itself infectious, because it functions as mRNA, (2) viruses with (−) sense ssRNA, or with dsRNA, carry a virion-associated RNA-dependent RNA polymerase which transcribes mRNA from the viral RNA, and (3) the (+) sense virion RNA of retroviruses is transcribed into DNA, which serves as a template for transcription of viral mRNAs by a cellular transcriptase. These three general strategies can be further subdivided on the basis of more subtle differences to give seven groups (Fig. 4-5A–G).

ssRNA; (+) Sense (Fig. 4-5A,B,C). In these groups the (+) sense virion RNA is itself infectious. In the picornaviruses and flaviviruses the ge-

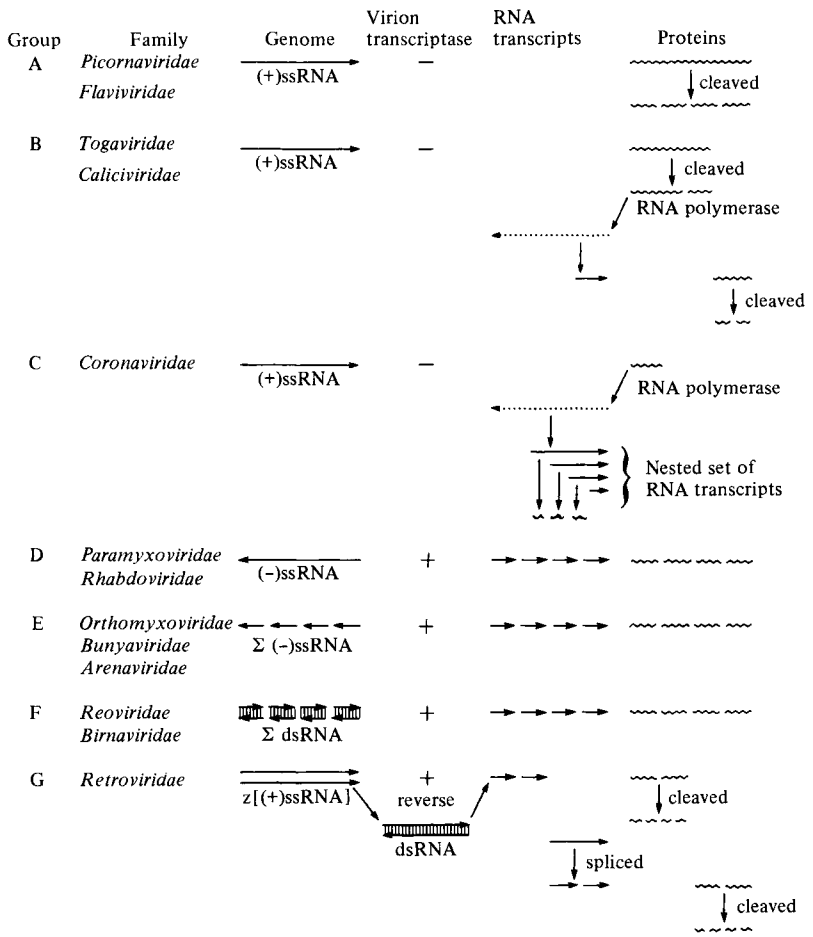

FIG. 4-5. *Simplified diagram showing the essential features of the strategy of expression of the genome of RNA viruses. The sense of each nucleic molecule is indicated by an arrow [(+) to the right; (−) to the left]. The number of segments of segmented genomes, mRNA molecules, and protein molecules has been arbitrarily shown as four. See text for details.*

nome, acting as a single polycistronic mRNA, is translated into a single polyprotein which is subsequently cleaved to give the individual viral polypeptides (Fig. 4-5A). Togaviruses of the genus *Alphavirus* also contain a single polycistronic (+) sense ssRNA molecule, but only about two-thirds of the viral RNA (the 5′ end) is translated; the resulting polyprotein is cleaved into four nonstructural proteins, two of which form the RNA polymerase. This enzyme then copies a full-length (−)

sense strand, from which two species of (+) sense strand are copied: full-length virion RNA destined for encapsidation, and a one-third length RNA, which is colinear with the 3′ terminus of the viral RNA and is translated into a polyprotein from which three or four structural proteins are produced by cleavage. The caliciviruses have not been so extensively studied, but also produce both genome-length and subgenomic mRNA species.

Flaviviruses were recently accorded the status of a family separate from the togaviruses. They do not produce subgenomic mRNAs, and translation of the (+) sense virion RNA initiates with the capsid protein near the 5′ end of the genome and proceeds sequentially through the genome to produce one precursor polyprotein. This is rapidly cleaved during the process of translation, so that the complete polyprotein is never seen.

Coronaviruses have a unique strategy. Initially, in a step about which little is known, part of the virion RNA acts as mRNA and is translated to produce an RNA polymerase, which then synthesizes a genome-length (−) sense strand. From this, a "nested set" of overlapping subgenomic RNAs is transcribed, of which only the unique (nonoverlapping) sequence in each is translated (see Chapter 28).

ssRNA; (−) Sense; Virion Transcriptase (Fig. 4-5D,E). Primary transcription from the (−) sense ssRNA viruses occurs in the cytoplasm, when the virion RNA is still within the helical nucleocapsid, in association with the nucleoprotein as well as the transcriptase. Particular sequences of 10 to 20 nucleotides, located at or near the termini of each RNA molecule, may serve as recognition signals for transcriptase binding.

The paramyxoviruses and rhabdoviruses have similar transcription strategies, as well as similar consensus sequences at the 3′ and 5′ termini of their viral RNA, suggestive of a common ancestry. The (−) sense virion RNA is copied in two distinct ways: the replication mode and the transcription mode. Copying in the replication mode produces a full-length (+) sense strand which is used as a template for the synthesis of new virion RNA. In the transcription mode, five subgenomic (+) sense RNAs are produced; each is capped and polyadenylated and serves as a monocistronic mRNA. It is still not certain what dictates whether the polymerase reads right through from 3′ to 5′ end of the (−) sense RNA template (replication mode), ignoring internal termination signals which are obeyed in the transcription mode to produce the family of five monocistronic mRNAs. There is some evidence that the polymerase may have only a certain probability of "falling off" its template as it reaches a termination codon; the five mRNAs are made in decreasing molar amounts, reading from the 3′ end of the parental RNA.

The orthomyxoviruses, bunyaviruses, and arenaviruses have segmented genomes, and each segment is transcribed to yield an mRNA which is translated into one or more proteins (Fig. 4-5E). In the case of the orthomyxoviruses, most of the segments can be regarded as single genes, for they encode single proteins. Special mention needs to be made of a phenomenon known colloquially as "cap-snatching," which is required by orthomyxoviruses for the initiation of mRNA synthesis. A virion-associated endonuclease enters the nucleus and removes a short segment from the capped 5′ terminus of cell mRNA; this is transported back to the cytoplasm, where it binds to the virion RNA and serves as a primer to initiate transcription.

In general, each viral RNA segment of the genomes of the bunyaviruses and arenaviruses codes for more than one protein. Furthermore, the S segment, at least, of arenaviruses and the *Phlebovirus* genus of bunyaviruses is ambisense. The replication strategy of ambisense RNA viruses, like the sense of their genomes, is mixed, with features of both (+) sense and (−) sense ssRNA viruses (see Chapters 29 and 34). Bunyavirus mRNAs also carry nonviral sequences at their 5′ termini, presumably derived from cellular mRNA primers.

dsRNA; Virion Transcriptase (Fig. 4-5F). The two families of viruses with dsRNA (*Birnaviridae* and *Reoviridae*) have segmented genomes and each segment is separately transcribed in the cytoplasm by a virion-associated RNA-dependent RNA polymerase. With reoviruses, each of the 10, 11, or 12 dsRNA segments corresponds to a single gene. Monocistronic mRNAs are transcribed from each segment within the partly uncoated subviral particle (see Plate 4-2); these RNAs complex with a protein before each is copied to produce a dsRNA, which serves as the template for further mRNA transcription.

ssRNA; (+) Sense; Virion Reverse Transcriptase (Fig. 4-5G). In the retroviruses the viral RNA is (+) sense, but instead of functioning as mRNA it is transcribed into DNA by a viral RNA-dependent DNA polymerase, and the resulting RNA–DNA hybrid molecule is converted to dsDNA and integrated into the cellular DNA. Transcription of RNA then occurs from the integrated viral DNA via the cellular transcriptase, followed by splicing of the RNA transcript as well as cleavage of the resulting proteins (see Chapters 12 and 31).

Regulation. Transcription from RNA viral genomes is generally not as rigorously regulated as with DNA viruses. In particular, the temporal separation into early genes transcribed before the replication of viral nucleic acid and late genes transcribed thereafter is not nearly so clear. Figure 4-6 shows that although the rate of synthesis of viral mRNAs

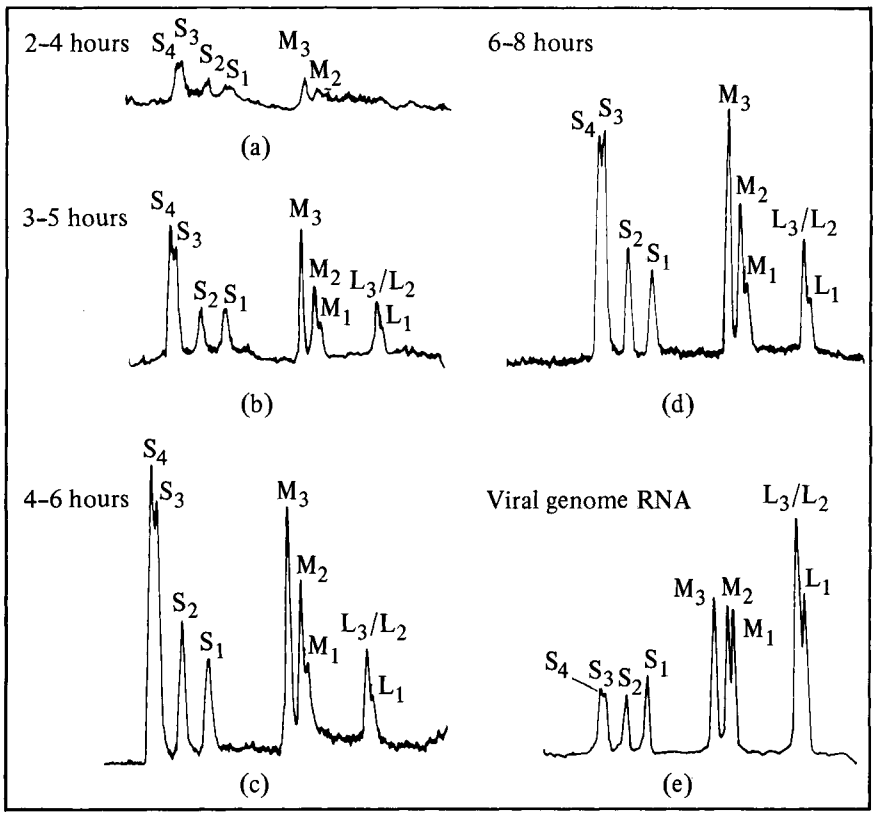

FIG. 4-6. *Transcription of mRNAs from dsRNAs of reovirus by the viral transcriptase. Tracings from polyacrylamide gel autoradiograms showing relative rates of formation of reovirus mRNA species during the replication cycle (a–d), as determined by hybridizing labeled mRNAs extracted from the cytoplasm of infected cells to genome RNAs; compared with the tracing of genome RNAs derived from virions (e). [From H. J. Zweerink and W. K. Joklik,* Virology **41,** *501 (1970).]*

increases steadily during the first 6 hours of reovirus infection as more template becomes available, the relative amounts of each of the 10 mRNA species remain unchanged. With some viruses, however, a subtle form of control can modulate the relative abundance of mRNAs for different proteins. For instance, in the case of the (−) sense ssRNA rhabdoviruses and paramyxoviruses, where the whole genome is transcribed into five monocistronic mRNA species, each coding for one of the five structural proteins, the "polarity" of the linear transcription by

the viral transcriptase, described earlier, results in favored synthesis of mRNA for the proteins coded by the 3′ end of the viral RNA.

TRANSLATION

Capped, polyadenylated, and processed monocistronic viral mRNAs bind to ribosomes and are translated into protein in the same fashion as cell mRNAs. The sequence of events has been closely studied for reovirus. Each monocistronic mRNA molecule binds via its capped 5′ terminus to the 40 S ribosomal subunit, which then moves along the mRNA molecule until stopped at the initiation codon. The 60 S ribosomal subunit then binds, together with methionyl tRNA and various initiation factors, after which translation proceeds. Despite the fact that mRNA is transcribed from each of the 10 monocistronic dsRNA reovirus segments in equimolar amounts, there are pronounced differences in the amounts of each protein made, indicating the existence of a regulatory mechanism at the level of translation.

Early Proteins

The proteins translated from the early transcripts of DNA viruses include enzymes and other proteins required for the replication of viral nucleic acid, as well as proteins that suppress host cell RNA and protein synthesis. However, the function of most early viral proteins of the large DNA viruses is still unknown.

Late Proteins

The late viral proteins are translated from late mRNA, most of which is transcribed from progeny viral nucleic acid molecules. Most of the late proteins are viral structural proteins, and they are often made in considerable excess. Some of them also double as regulatory proteins, modulating the transcription or translation of cellular or early viral genes.

Regulation

The temporal order and amount of synthesis of particular proteins of DNA viruses is regulated mainly at the level of transcription. With RNA viruses it is also usual for nonstructural proteins to be made early and structural proteins later, but the control is generally not as rigorous as for the DNA viruses and occurs at the level of translation. For instance, in the case of caliciviruses, coronaviruses, and togaviruses, only the 5′ end of the (+) sense viral RNA, which codes for the nonstructural pro-

teins, including the RNA polymerase, is translated early, hence production of complementary (−) sense RNA can commence. This then serves as the template for transcription of subgenomic RNA corresponding to the 3′ end of the viral RNA, from which are translated the structural proteins required in abundance later in infection.

Posttranslational Cleavage of Polyproteins

In the picornaviruses, the polycistronic viral RNA is translated directly into a single polyprotein which carries protease activity. This virus-coded protease cleaves the polyprotein at defined recognition sites into smaller proteins. The first cleavage steps are carried out while the polyprotein is still bound to the polyribosome. Some of the larger intermediates exist only fleetingly; others are functional but are subsequently cleaved to smaller proteins with alternative functions.

Posttranslational cleavage occurs in several other RNA virus families but is a less prominent feature in the overall production of individual proteins. In the case of the togaviruses and caliciviruses, polyproteins corresponding to only part, albeit a large part, of the genome are translated from polycistronic mRNA and then cleaved. With viruses of several other families, cleavage of particular proteins late in the replication cycle is essential for the production of infectious virions.

Migration of Proteins

Newly synthesized viral proteins must migrate to the various sites in the cell where they are needed. e.g., back into the nucleus in the case of viruses that replicate there. The mechanisms controlling such migration are unknown, but presumably resemble those used for cellular proteins and possibly involve the cytoskeleton. Migration is doubtless intimately dependent on the structural features of particular proteins. In the case of glycoproteins, the polypeptide is translated on membrane-bound ribosomes, i.e., on rough endoplasmic reticulum; various co- and posttranslational modifications, including acylation, proteolytic cleavage, and addition and subtraction of sugars, occur sequentially as the protein moves in vesicles to the Golgi complex and thence to the plasma membrane (see below).

REPLICATION OF VIRAL NUCLEIC ACID

DNA Replication

Different mechanisms of DNA replication are employed by each family of DNA viruses. We can give only a brief overview here.

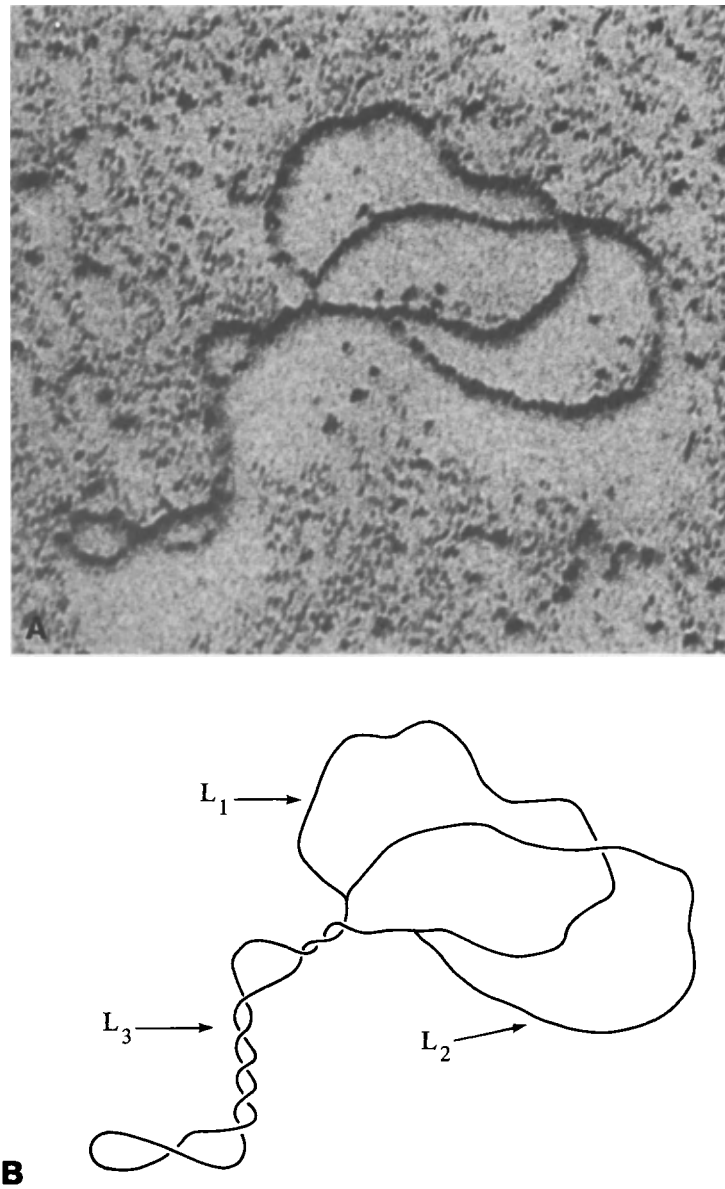

FIG. 4-7. *Replication of circular viral DNA. (A) Electron micrograph of a replicating molecule of the papovavirus SV40 DNA (Magnification: 1.5 × 10^5). In an interpretative drawing of the molecule (B) the two replicating branches are designated L_1 and L_2. The superhelical unreplicated section is designated L_3. [From E. D. Sebring* et al., J. Virol. **8,** *478 (1971).]*

Papovaviridae. Little is known about the replication of papillomavirus DNA, but the polyomaviruses, especially SV40, have been studied in great detail. The SV40 genome, with its associated cellular histones, morphologically and functionally resembles cellular DNA and utilizes host cell enzymes, including DNA polymerase α, for its replication. An early viral protein, large-T, binds to three sites in the regulatory sequence of the viral DNA, thereby initiating DNA replication. Replication of this circular dsDNA commences from a unique palindromic sequence and proceeds simultaneously in both directions at the same rate (Fig. 4-7). As in the replication of mammalian DNA, both continuous and discontinuous DNA synthesis occurs (on leading and lagging strands, respectively) at the two growing forks. The discontinuous synthesis of the lagging strand involves repeated synthesis of short oligoribonucleotide primers, which in turn initiate short nascent strands of DNA (*Okazaki fragments*), which are then covalently joined to form one of the growing strands.

Adenoviridae. Adenovirus DNA is linear, the 5′ terminus of each strand being a mirror image of the other (terminally repeated inverted sequences), and each is covalently linked to a protein. The primer for adenoviral DNA synthesis is not, as is usual, another nucleic acid, but a precursor to this protein, referred to as adenovirus preterminal protein. DNA replication proceeds from both ends, continuously but asynchronously, in a 5′ to 3′ direction, using a virus-coded DNA polymerase. It does not require the synthesis of Okazaki fragments.

Herpesviridae. Unlike other DNA viruses that replicate in the nucleus, herpesviruses specify a large number of enzymes involved in DNA synthesis. Analysis of herpesvirus DNA replication is incomplete, but it appears that a rolling-circle mechanism operates, at least in the later stages. The replicating DNA initially consists of circles and linear forked forms, which are later replaced by large bodies of tangled DNA. There are three origins of replication, two on the S component and one on the L component (see Fig. 1-3), the latter being near the genes that specify the DNA polymerase and the major DNA-binding protein. Newly synthesized viral DNA appears to be cleaved to unit lengths during the process of packaging into newly formed capsids.

Poxviridae. The special features of poxvirus DNA replication are that it occurs in the cytoplasm and depends entirely on virus-coded proteins; it can occur in enucleated cells. Replication appears to begin at each end of the genome and involves a strand displacement mechanism, with the formation of small DNA fragments covalently linked to RNA primers. The discovery of the loop structure at the ends of the vaccinia virus

genome (see Fig. 1-3) suggested a model whereby nicks near the ends of the genome allow self-priming by the 3′ ends thus generated.

Parvoviridae. In the autonomous parvoviruses (genus *Parvovirus*), DNA replication occurs in close association with cellular chromatin and is dependent on cellular functions provided in the S phase of the cell cycle, i.e., when cellular DNA synthesis is occurring, a feature that is correlated with the pathogenic potential of these viruses (see Chapter 22). The virion (−) sense DNA is copied to give a dsDNA replicative form. Further DNA synthesis requires the binding of a virus-coded protein to the 5′ termini. Production of viral ssDNA appears to occur after nicks at the 5′ end and repeated rounds of synthesis.

Hepadnaviridae. Replication occurs in the nucleus by a unique process. The viral DNA polymerase converts the viral ss/dsDNA into a complete circular dsDNA. The (−) sense strand of this molecule is then transcribed by the cellular RNA polymerase to produce a full-length "pregenome" RNA. This (+) sense RNA is then encapsidated in viral cores together with newly synthesized DNA polymerase, which also carries reverse transcriptase activity. Minus-strand DNA is then synthesized by reverse transcription of the pregenome RNA; the template is degraded to leave a full-length (−) sense DNA strand. A small RNA fragment from the 5′ end of the pregenome is then used to prime the synthesis of the (+) sense DNA strand. Complete synthesis of this strand is not necessary for maturation of the virus, hence infectious particles contain dsDNA with a single-stranded region.

RNA Replication

The replication of RNA is a phenomenon restricted to viruses. Transcription of RNA from an RNA template requires an RNA-dependent RNA polymerase, a virus-coded enzyme not normally found in cells. It is not known whether the polymerase required to transcribe (+) sense RNA from (−) sense RNA is different from that needed to transcribe (−) sense RNA from (+) sense RNA. Both processes are essential because the replication of virion RNA requires first the synthesis of complementary RNA, which then serves as a template for making more virion RNA.

Where virion RNA is of (−) sense the complementary RNA is of (+) sense and the RNA polymerase is the virion-associated transcriptase used for transcription of subgenomic RNAs. However, whereas the primary transcripts from such (−) sense virion RNA are subsequently cleaved (in most cases) to produce mRNAs, some must remain uncleaved to serve as a full-length template for virion RNA synthesis.

In the case of (+) sense virion RNA, the complementary RNA is of (−)

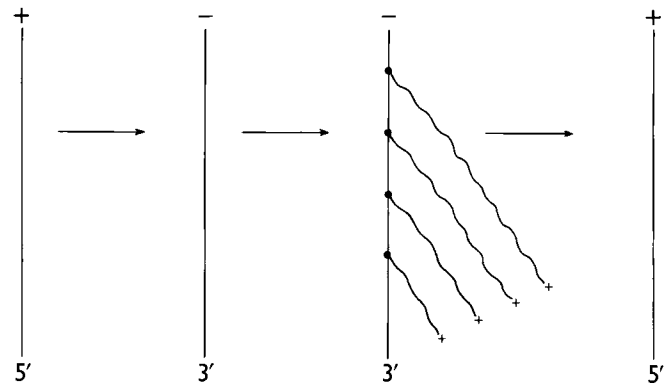

FIG. 4-8. *Mechanism of replication of ssRNA. The "replicative intermediate" (third from left) consists of several (+) sense strands being copied simultaneously from one (−) sense strand by separate molecules of RNA-dependent RNA polymerase (shown as dots).*

sense. Several RNA molecules can be transcribed simultaneously from a single complementary RNA template, each RNA transcript being the product of a separately bound polymerase molecule. The resulting structure, known as the *replicative intermediate,* is therefore partially double-stranded, with single-stranded tails (Fig. 4-8). Initiation of replication of picornavirus and calicivirus RNA, like that of adenovirus DNA, requires a protein, rather than a ribonucleoside triphosphate, as primer. This small protein, VPg, is covalently bound to the 5′ terminus of nascent (+) and (−) RNA strands, as well as virion RNA, but not to mRNA.

Replication of Retrovirus RNA. Retroviruses have a genome consisting of (+) sense ssRNA. Unlike other RNA viruses, they replicate via a DNA intermediate. A virion-associated RNA-dependent DNA polymerase (reverse transcriptase), using a tRNA molecule as a primer, makes a ssDNA copy. The reverse transcriptase, functioning as a ribonuclease, then removes the parental RNA molecule from the DNA–RNA hybrid. The free (−) sense ssDNA strand is then converted into linear dsDNA, which contains an additional sequence known as the *long terminal repeat* at each end. This linear dsDNA then migrates to the nucleus and becomes integrated into cellular DNA. Transcription of the viral RNA can then occur from this integrated (*proviral*) DNA (see Chapter 12).

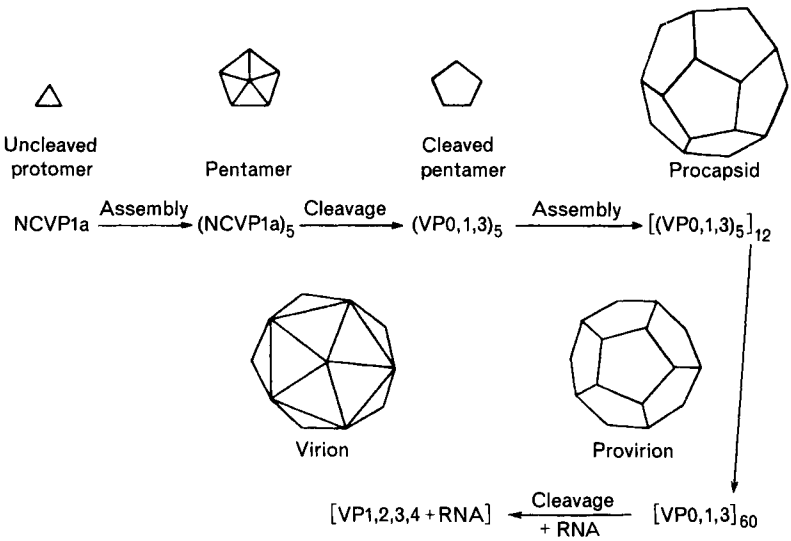

FIG. 4-9. *Assembly of picornavirions.*

MATURATION OF THE VIRION

Icosahedral Viruses

Structural proteins of nonenveloped icosahedral viruses associate spontaneously to form capsomers, which self-assemble to form empty procapsids, into which viral nucleic acid is packaged. Completion of the virion often involves proteolytic cleavage of one or more species of capsid protein. The best-studied examples among animal viruses are the picornaviruses (Fig. 4-9). The capsomer precursor protein (noncapsid viral protein, NCVP1a) aggregates to form pentamers; each of the 5 NCVP1a molecules is then cleaved by virus-specific proteases into VP0, VP1, and VP3. Twelve such pentamers aggregate to form a procapsid. A final proteolytic event, which cleaves the VP0 molecule into VP2 and VP4, is required for RNA incorporation. The mature virion is a dodecahedron with 60 capsomers, each of which is made up of one molecule each of VP1, 2, 3, and 4. There are also one or two uncleaved molecules of VP0 in the virion. X-Ray crystallography shows that the assembling units are not just rigid preformed "bricks"; they have extensions that reach across adjacent units to form second- and third-nearest neighbor

relationships. Such studies have also shown that there is no fixed way in which RNA interacts with ordered parts of the protein.

The mechanism of packaging viral nucleic acid into a preassembled empty procapsid has been elucidated for adenovirus. One terminus of the viral DNA is characterized by a nucleotide sequence referred to as the packaging sequence, which enables the DNA to enter the procapsid bound to basic core proteins, after which some of the capsid proteins are cleaved to make the mature virion.

Enveloped Viruses

All mammalian viruses with helical nucleocapsids, as well as some with icosahedral nucleocapsids, acquire an envelope by budding through cellular membranes. Since such envelopes always contain viral glycoproteins, we begin by discussing the mechanism of glycosylation of these proteins.

Glycosylation of Envelope Proteins. Much of our understanding of the glycosylation of viral proteins comes from studies with vesicular stomatitis virus (a rhabdovirus), Semliki Forest virus (a togavirus), and the orthomyxoviruses and paramyxoviruses. The essential steps appear much the same for all enveloped viruses, hence a general overview is presented (Fig. 4-10). Viruses exploit existing cellular pathways normally used for the synthesis of membrane-inserted and exported secretory glycoproteins.

The amino-terminus of viral envelope proteins initially contains a sequence of 15 to 30 hydrophobic amino acids, known as the *signal sequence,* which characterizes the protein as one destined for insertion into membrane and/or export from the cell. The hydrophobicity of the signal

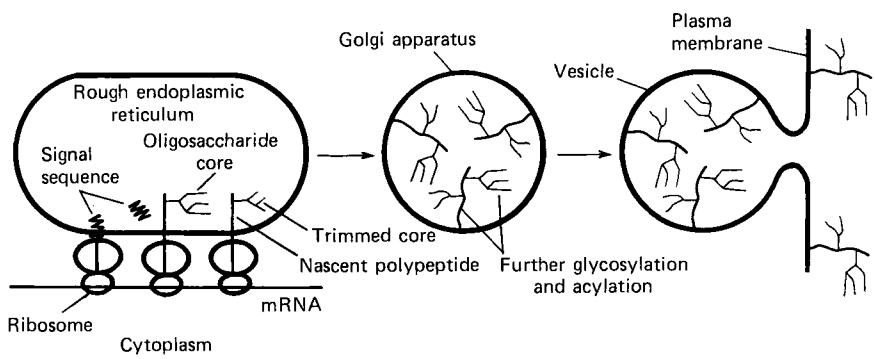

FIG. 4-10. *Glycosylation of viral protein.*

sequence facilitates binding of the growing polypeptide chain to a receptor site on the cytoplasmic side of the rough endoplasmic reticulum and its passage through the lipid bilayer to the luminal side. A signal peptidase then removes the signal sequence. Oligosaccharides are added to asparagine residues of the nascent polypeptide in the lumen of the rough endoplasmic reticulum by *en bloc* transfer of a mannose-rich core of preformed oligosaccharides from a lipid-linked intermediate, an oligosaccharide pyrophosphoryldolichol. Glucose residues are then removed by glycosidases, a process known as "trimming" of the core. The viral glycoprotein is then transported from the rough endoplasmic reticulum to the Golgi complex, probably within a coated vesicle. Here the core carbohydrate is further modified by the removal of several mannose residues and the addition of further *N*-acetylglucosamine, galactose, and the terminal sugars, sialic acid and fucose. The completed side chains are a mixture of simple ("high-mannose") and complex oligosaccharides. While in the Golgi complex the glycoprotein may become acylated, by the covalent attachment of fatty acids such as methyl palmitate to the hydrophobic membrane attachment end of the molecule. Another coated vesicle then transports the completed glycoprotein to the cellular membrane from which the particular virus buds.

Transport of Glycoproteins. Different viruses bud from different sites in the plasma membrane (orthomyxoviruses, paramyxoviruses, rhabdoviruses, arenaviruses, togaviruses, and retroviruses), some from the apical and others from the basolateral surface. Others bud from intracytoplasmic smooth endoplasmic reticulum (flaviviruses, bunyaviruses, coronaviruses) or from the nuclear membrane (herpesviruses). Presumably some structural feature of the glycoprotein serves as the "zip code" ensuring delivery to the correct location in the cell.

Cleavage of Envelope Proteins. With the orthomyxoviruses and paramyxoviruses, which bud through the plasma membrane, a cellular protease cleaves the envelope protein at the time of its insertion into the membrane into two polypeptide chains, which remain covalently linked by disulfide bonds. Cleavage is not required for viral release and does not occur in certain types of host cells, but it is essential for the production of infectious virions in the orthomyxoviruses (cleavage of the hemagglutinin) and paramyxoviruses (cleavage of both the hemagglutinin-neuraminidase and the fusion protein). Following fusion of the coated vesicle with the plasma membrane, the hydrophilic N-terminus of the glycoprotein projects from the external surface of the membrane, while the hydrophobic domain, which is near the C-terminus, remains anchored in the lipid bilayer.

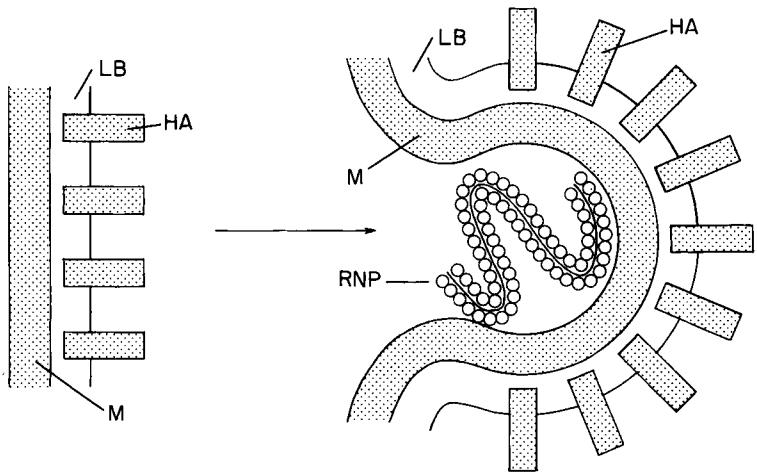

FIG. 4-11. *Budding of virus from plasma membrane. LB, Lipid bilayer; HA, hemagglutinin; M, matrix protein; RNP, ribonucleoprotein.*

Budding. Budding may be regarded as a nonphysiological form of exocytosis (Fig. 4-11). The process begins with the insertion of the completed viral glycoprotein into the appropriate cellular membrane. Because proteins are free to move laterally in the "sea of lipid" that constitutes the lipid bilayer of the plasma membrane, cellular proteins are displaced from the patch of membrane into which viral glycoproteins are inserted. It is not known whether there is selection of particular lipids for incorporation into the viral envelope, but the ratio of phospholipids to glycolipids and cholesterol is essentially the same as that of the membrane of the particular host cell.

The monomeric, cleaved viral glycoprotein molecules associate into oligomers, to form the typical rod-shaped peplomer with a prominent hydrophilic domain projecting from the external surface of the membrane; the hydrophobic domain near the C-terminus spans the membrane and a short hydrophilic domain at the C-terminus projects slightly into the cytoplasm. In the icosahedral togaviruses (Plate 4-3A), each protein molecule of the nucleocapsid binds directly to the C-terminus of a glycoprotein oligomer of the envelope. Multivalent attachment of numerous peplomers, each to an underlying molecule on the surface of the icosahedron, molds the envelope around the nucleocapsid, forcing it to bulge progressively outward until finally the nucleocapsid is completely enclosed in a tightly fitting envelope and the new virion buds off. The capsid proteins of most enveloped viruses with helical nucleocapsids do

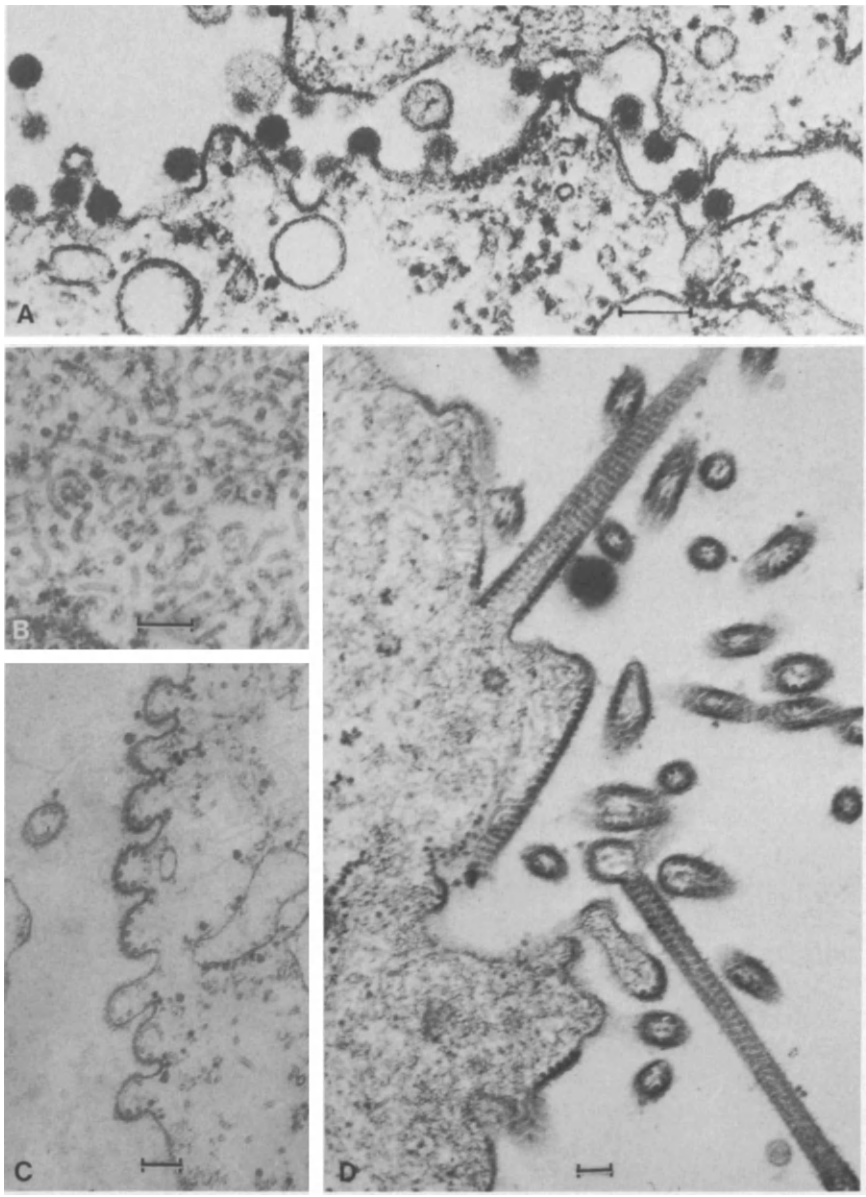

PLATE 4-3. *Viruses budding from the plasma membrane. (A) Togavirus. (B) Accumulation of paramyxovirus SV5 nucleocapsids. (C, D) Budding of SV5 from the plasma membrane, with some filamentous forms (bars = 100 nm). [A, courtesy Dr. A. Helenius; B, C, and D, from R. W. Compans* et al., Virology **30,** *411 (1966), courtesy Dr. P. W. Choppin.]*

not bind directly to envelope glycoprotein but to a *matrix protein* which is bound to the cytoplasmic side of the plasma membrane beneath patches of viral glycoprotein (Fig. 4-11).

Coronaviruses and bunyaviruses bud from rough endoplasmic reticulum and the Golgi complex; orthopoxviruses may acquire an envelope in the Golgi, but enveloped forms are released from the plasma membrane. The envelope of the herpesviruses is acquired by budding through the inner lamella of the nuclear membrane; the enveloped virions then pass directly from the space between the two lamellae of the nuclear membrane to the exterior of the cell via the cisternae of the endoplasmic reticulum.

RELEASE

There are basically two mechanisms for the release of mature virions from the infected cell. With most nonenveloped viruses that accumulate within the cytoplasm or nucleus, release occurs only when the cell lyses. This may occur shortly after the completion of viral replication; e.g., cells infected with picornaviruses lyse as soon as assembly of virions is completed, with immediate release of the progeny virions. On the other hand, parvoviruses accumulate within the cell nucleus and are not released until the cell slowly degenerates and dies. Most enveloped viruses, on the other hand, are released by budding, a process which can occur over a prolonged period without much damage to the cell, hence many such viruses (e.g., arenaviruses, retroviruses) are noncytopathogenic and are associated with persistent infections. However, some enveloped viruses that are released by budding are cytolytic, e.g., the alphaherpesviruses. Orthopoxviruses may be released as enveloped forms by budding from the plasma membrane or as nonenveloped forms, by cell lysis; both forms are infectious.

INHIBITION OF VIRAL REPLICATION: ANTIVIRAL CHEMOTHERAPY

If this had been a book about bacterial diseases of domestic animals, there would have been at least one chapter on antibacterial chemotherapy. However, of the hundreds of antibiotics and other antibacterial compounds now available, not one has the slightest effect on any virus, and there are no specifically antiviral chemotherapeutic agents in common use. The reason is that viruses are absolutely dependent on the metabolic pathways of the host cell for their replication, hence most

agents that interfere with viral replication are toxic to the cell. Increased knowledge of the biochemistry of viral replication has led to a more rational approach to the search for antiviral chemotherapeutic agents.

Strategy for Development of Antiviral Agents

Several steps in the viral replication cycle represent potential targets for selective attack. Theoretically, all virus-coded enzymes are vulnerable, as are all processes (enzymatic or nonenzymatic) that are more essential to the replication of the virus than to the survival of the cell. Obvious examples include: (1) transcription of viral mRNA (or copy DNA, in the case of the retroviruses) by the viral transcriptase, (2) replication of viral DNA or RNA by the virus-coded DNA polymerase or RNA-dependent RNA polymerase, (3) posttranslational cleavage of protein(s) by (virus-coded) protease(s). Less obvious at first sight, but proven points of action of currently known antiviral agents are: (4) penetration/uncoating, (5) polyadenylation, methylation, or capping of viral mRNA, (6) translation of viral mRNA into protein, and (7) assembly/maturation of the virion.

A logical approach to the discovery of new antiviral chemotherapeutic agents is to isolate or synthesize substances that might be predicted to serve as an inhibitor of a known virus-coded enzyme. Analogs (congeners) of this prototype are then synthesized with a view to enhancing activity and/or selectivity. The discovery of a class of nucleoside analogs which selectively inhibit herpesvirus DNA synthesis has led to a realization that virus-coded enzymes with a broader (or different) substrate specificity than their cellular counterparts can be exploited to convert an inactive precursor ("prodrug") to an active antiviral agent. Because the viral enzyme occurs only in infected cells, such drugs are nontoxic for uninfected cells. Exploitation of this principle may revolutionize antiviral chemotherapy.

Acycloguanosine (Acyclovir) and Homologs

Acycloguanosine, now commonly known as acyclovir, is a guanine derivative with an acyclic side chain, the full chemical name being 9-(2-hydroxyethoxymethyl)guanine (Fig. 4-12). Its unique advantage over earlier nucleoside derivatives is that it requires the herpesvirus-specified enzyme, deoxythymidine-deoxycytidine kinase, to phosphorylate it intracellularly to acycloguanosine monophosphate; a cellular GMP kinase then completes the phosphorylation to the active agent, acycloguanosine triphosphate (Fig. 4-12). Acycloguanosine triphosphate inhibits the herpesvirus-specified DNA polymerase. Since activation of the prodrug

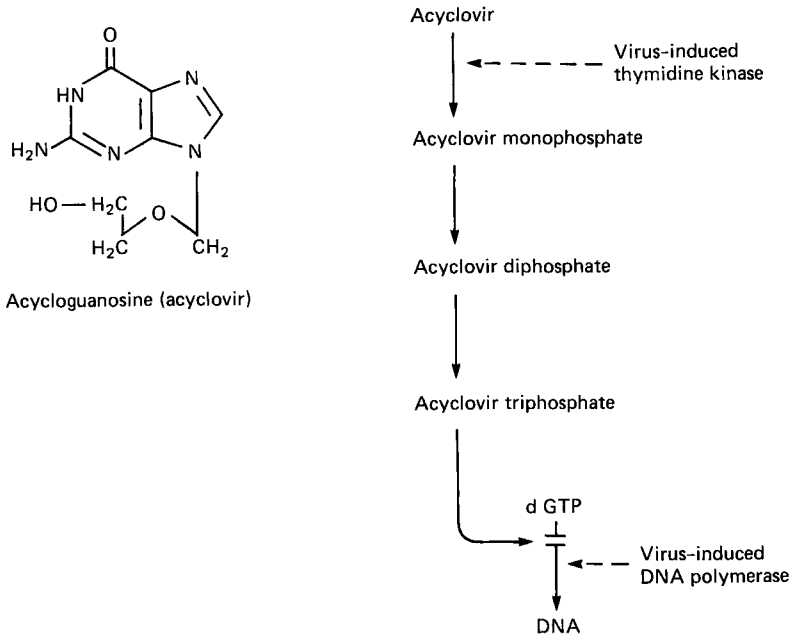

FIG. 4-12. *Structure of acyclovir and its mode of action, which is dependent on the presence in the cell of herpesvirus thymidine kinase.*

needs the viral thymidine kinase, acyclovir is essentially nontoxic to uninfected cells but is powerfully inhibitory to viral DNA synthesis in infected cells.

Acyclovir and various derivatives, as well as other nucleoside analogs dependent on viral enzymes for conversion to the active form, are beginning to be used in human medicine for the treatment of herpesvirus infections. It is a small start, but it does demonstrate that antiviral chemotherapy may have a future. Such drugs find limited use in veterinary medicine, e.g., for treatment of feline herpesvirus 1 corneal ulcers.

Other Antiviral Agents

A few other antiviral agents are in use in human medicine. For example, rimantadine and amantadine can prevent the uncoating of influenza virus, and several compounds known to inhibit reverse transcriptase are undergoing clinical trials against AIDS.

Interferons

In theory at least, interferons are the ideal antiviral antibiotics. They are naturally occurring, relatively nontoxic, and display a broad spectrum of activity against essentially all viruses (see Chapters 6 and 8). However, clinical trials in humans have been disappointing. Currently, it appears that they have a demonstrable effect on infections with papillomaviruses, herpesviruses, and rhinoviruses. It is now possible to produce large amounts of various human and other interferons using cloned interferon genes, but it is still uncertain whether they will be of clinical value in humans. Their use for therapy in viral diseases of domestic animals is even further away.

Perspective

Overall, in spite of decades of effort and massive expenditure by the pharmaceutical industry, the yield of useful antiviral drugs has been meager. Only a handful of marginally effective agents have found a place in human medicine, and very few are used in veterinary practice. Nevertheless, it is important to be aware of the continuing research in this field, for antiviral chemotherapy may one day come to constitute an integral part of veterinary medicine.

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CHAPTER 5

Viral Genetics and Evolution

Animal virology developed largely from the need to control viral diseases in humans and their domesticated animals. Since direct experimentation using the natural host is often expensive or impossible, much effort has been devoted to two ends: (1) adaptation of viruses of veterinary and medical importance to small laboratory animals and cell culture, and (2) attenuation of viruses by serial passage in an unnatural host to obtain attenuated live-virus vaccines. These processes of adaptation and attenuation operate via spontaneous mutation or more rarely. by genetic recombination, followed by selection. As is usual in science, useful practical results were obtained empirically long before the genetic or molecular mechanisms involved in these phenomena were understood. This is well illustrated by the development of an effective attenuated rabies vaccine by Pasteur a hundred years ago. Today, however, it is possible and important to understand what these processes are and how they occur.

Following the spectacular advances in molecular genetics that emerged from the study of bacterial viruses, and later, tumorigenic viruses, efforts have been made to understand the genetic properties and processes of

other animal viruses. This work has involved the selection of appropriate mutants, the construction of genetic maps by recombination and complementation tests, and study of the functions of the products of the genes in which mutations occur.

In the 1970s several discoveries were made which have revolutionized the study of genetics, including viral genetics. The exploitation of these discoveries resulted in the development of recombinant DNA technology, or what is popularly called genetic engineering. As outlined in Chapter 4, our understanding of the structure and function of viral genomes has expanded greatly in recent years, and genetic engineering has already begun to provide important practical results in the form of advances in the diagnosis, prevention, and treatment of viral infections.

MUTATION

The most important and universal changes in the nucleic acid sequences of viral genomes are due to *mutations.* In every viral infection of an animal or a cell culture, one or a small number of virus particles replicates to produce millions of progeny. In such large populations, errors in copying the nucleic acid (i.e., mutations) inevitably occur. Many such mutations are lethal. Whether a particular nonlethal mutation survives in the genotype of virus depends on two factors: (1) whether the resultant change in the polypeptide gene-product affords the mutant virus some selective advantage, or whether it is neutral or disadvantageous, and (2) whether the mutation happens to occur early or late in the course of the infection.

In the laboratory, reasonable genetic constancy of viral stocks (e.g., those used for making viral vaccines or retained as reference strains) is achieved by: (1) isolating a *clone,* i.e., a population of viral particles originating from a single particle, usually by growth from a single plaque in cell culture, followed by replaquing, then (2) growing "seed" stock from this clone, and (3) as far as practicable avoiding or strictly limiting further passage of the virus. If passage is unavoidable, a single plaque is again selected to grow up a new seed stock. Sometimes, however, mutations are so common that there is a significant chance that the selected clone will be a mutant virus rather the parental type.

Mutation Rates

Rates of mutation involving base substitutions (*point mutations*) are probably the same in DNA viruses as they are in DNAs of prokaryotic

and eukaryotic cells, since replication usually occurs in the nuclei of such cells and is subject to the same "proofreading" exonuclease error correction as operates in cells. Such errors are estimated to occur at a rate of 10^{-8} to 10^{-11} per incorporated nucleotide (i.e., per base bair per replication).

Point mutations in the third nucleotide of a triplet often do not result in an altered amino acid, because of *coding redundancy,* and some point mutations are lethal, because they produce a stop codon or other aberrant regulatory sequences. Viable mutations that are neutral or deleterious in one host may provide a positive selective advantage in a different host.

RNA is not the repository for genetic information in eukaryotic cells, so that there is no a priori reason why cells should contain a "proofreading" mechanism for RNA. So far no RNA exonuclease (an essential component of a proofreading mechanism) has been discovered, hence the error rate in the replication of viral RNA is much higher than that of viral or cellular DNA. For example, the base substitution rate per incorporated nucleotide in the 11-kb genome of vesicular stomatitis virus is 10^{-3}–10^{-4}, so that nearly every progeny genome will be different from its parent and sisters in at least one base. Evidence from studies of several different RNA viruses suggests that the rate of base substitutions per average site per year in viral RNA is greater than 10^{-3}, which is about a million times higher than the average rate of evolution of chromosomal DNA in their eukaryotic hosts. Of course, most of these base substitutions would be deleterious and the genomes containing them would be lost. Even so, there is growing evidence that nonlethal mutational changes can be incorporated in the genome of RNA viruses very rapidly. For example, outbreaks of human poliomyelitis in the years 1978–1979 were traced from the Netherlands to Canada and then to the United States; oligonucleotide mapping of the RNAs obtained from successive isolates of the virus showed that over a period of 13 months of epidemic transmission about 100 base changes (in a genome of 7441 bases) had become fixed in the poliovirus genome.

Types of Mutations

Mutations can be classified according to the kind of change in the nucleic acid; the most common are nucleotide substitutions (point mutations), small deletions, or large deletions (Table 5-1). The physiological effects of mutations depend not only on the kind and location of the mutation but also on the activity of other genes. The phenotypic expression of a mutation in one gene may be reversed not only by a back-

TABLE 5-1
Types of Mutations

Type	Change in nucleic acid	Effect on amino acid sequence	Effect on polypeptide function
Silent mutation	Nucleotide substitution	None (e.g., redundancy in third base of triplet code)	None
Missense mutation	Nucleotide substitution	Amino acid substitution	Variable (depends on amino acid and its location in polypeptide)
Nonsense mutation	Nucleotide substitution (extragenic suppression may occur)	Premature termination	Usually lost
Frame-shift mutation	Small deletion (intragenic suppression common)	Premature termination	Usually lost
Deletion mutation	Large deletion (does not revert)	Premature termination	Lost

mutation in the substituted nucleotide but, alternatively, by a *suppressor mutation* occurring elsewhere in the same, or even in a different gene. For example, temperature-sensitive mutants of influenza virus developed as potential attenuated live-virus vaccines reverted to virulence by virtue of a quite independent suppressor mutation in an apparently unrelated gene, which by some unknown mechanism negated the effect of the original mutation.

Mutations can be classified by their phenotypic expression—hence, temperature-sensitive, cold-adapted, host range, plaque size mutants have been described, among others. Each of these kinds of mutants has been used for the analysis of viral functions, temperature-sensitive mutants being particularly useful (see below). Cold-adapted and temperature-sensitive mutants have been used extensively in attempts to produce attenuated live-virus vaccines (see Chapter 14). Mutations affecting antigenic sites (epitopes) on virion surface proteins may be strongly favored when viruses replicate in the presence of antibody, and are of importance both in persistently infected animals (e.g., in vis-

na/maedi and equine infectious anemia viruses; see Chapter 11) and epidemiologically, as in vesicular exanthema of swine virus in sea lions (see Chapter 24) and human influenza virus (see below). Space does not permit a description of all types of mutants, but two that are of particular importance will be described: temperature-sensitive *conditional lethal mutants* and *defective interfering mutants.*

Conditional Lethal Mutants. These are produced by mutations that so affect a virus that it cannot grow under certain conditions, determined by the experimenter, but can replicate under "normal" or permissive conditions. Their importance is that a single selective test can be used to obtain and analyze mutants in which mutations may be present in any one of several different genes. The conditional lethal mutants most commonly studied are those whose replication is blocked at certain defined temperatures, i.e., *temperature-sensitive mutants;* the selective condition used is a high temperature of incubation of infected cells. A point mutation in the genome, leading to an amino acid substitution in the translated polypeptide product, results in a structurally abnormal protein which though functional at the *permissive temperature* cannot maintain its structural integrity and functional conformation when the temperature is raised by a few degrees.

Defective Interfering (DI) Mutants. Mutants of this class have been demonstrated in all families of RNA viruses and in some DNA viruses. They occur when viruses are passed at high multiplicity of infection, and the ratio of defective interfering to infectious particles increases dramatically on serial passage. The properties that all defective interfering virus particles have in common are (1) they are *defective* (i.e., they cannot replicate alone, but can in the presence of parental wild-type virus), and (2) they decrease the yield of wild-type virus (*interference*).

All defective interfering particles studied are deletion mutants. In the case of influenza viruses and reoviruses, which have segmented genomes, the defective virions lack one or more of the larger segments and contain instead smaller segments consisting of an incomplete portion of the encoded gene(s). In the case of viruses with a nonsegmented genome, defective interfering particles contain RNA which is greatly shortened—as little as one-third of the original genome remains in the defective interfering particles of vesicular stomatitis virus (a rhabdovirus). Morphologically, defective interfering particles resemble the parental virions, having a comparable envelope or capsid, but they are sometimes smaller. Sequencing studies of the RNA of defective interfering particles reveal simple deletions and a great diversity of structural rearrangements.

Defective interfering particles tend to increase preferentially with serial passage in cultured cells because the shortened RNA molecules that characterize them (1) require less time to be replicated, (2) are less often diverted to serve as templates for transcription of mRNA, and (3) have enhanced affinity for the viral replicase, so giving them a competitive advantage over their full-length counterparts from infectious virus. These features also explain why on passage the defective interfering particles interfere with the replication of full-length parental RNA with progressively greater efficiency.

Mutagenesis

Spontaneous mutations occur because of chance errors (base changes) during replication, the occurrence of which is probably influenced by natural background ionizing radiation. Mutation frequency can be enhanced by treatment of virions or isolated viral nucleic acid with physical agents such as UV or X irradiation or with chemicals such as nitrous acid, hydroxylamine, ethylmethyl sulfonate, or nitrosoguanidine. Base analogs, such as 5-fluorouracil or 5-bromodeoxyuridine, are active only when virus is grown in their presence because they are mutagenic only when incorporated into the viral nucleic acid. Chemical mutagenesis is an important tool for laboratory studies in viral genetics, since the mutation rate is greatly increased, thereby making it possible to select mutants that would otherwise occur too rarely.

Site-directed mutagenesis, which enables the experimenter to introduce mutations at a selected site in a DNA molecule (a DNA genome or copy DNA transcribed from an RNA genome), is effected in the following way. A nick made with an appropriate restriction endonuclease is extended with exonuclease to expose a short single-stranded region, which is mutagenized using a single-strand-specific mutagen, and the gap is then repaired with DNA polymerase and ligase. This technique has opened up a new research area; for example: (1) the function of individual genes and the proteins for which they code, or of particular regions of these genes and proteins, can be dissected; (2) mutations can be introduced into particular genes, e.g., those concerned with viral virulence, to produce mutants suitable for use as attenuated live-virus vaccines.

Pleiotropism

Mutants selected for a certain phenotypic alteration are often found to be changed in other properties, reflecting the relevance of a single protein to several properties of the virus. For example, a single change in

the hemagglutinin of influenza virus can affect properties such as hemagglutination, susceptibility to glycoprotein inhibitors, antigenicity, and ability to grow in particular hosts.

Such pleiotropic effects are useful in certain applications of viral genetics, e.g., the selection of an attenuated variant for a vaccine. Initial selection and characterization can be based on *in vitro* tests, reserving more expensive animal testing for final characterization of the selected strain. Thus, attenuated mutants can be selected on the basis of their ability to grow better at low than at high temperatures before being subjected to tests for virulence in animal or human hosts.

Adaptation to New Hosts

Although much experimentation with viruses of veterinary importance can be carried out in natural hosts, major advances usually depend on the growth of the viruses in a laboratory animal or in cultured cells. This requirement for laboratory adaptation applies even more stringently with viruses of humans. Some viruses produce clinical signs of disease the first time that they are inoculated into an experimental animal, e.g., eastern equine encephalitis virus in suckling mice. In other cases minimal signs of infection are observed initially, but after serial passage, sometimes prolonged, clinical disease is regularly produced, as, for example, in the adaptation of bluetongue and bovine ephemeral fever viruses to mice. Likewise, newly isolated viruses at first often fail to grow in certain kinds of cultured cells, but can be adapted by serial passage, e.g., street rabies virus adapted to neuroblastoma cells. Most modern virological research is performed with strains of virus adapted to grow rapidly to high yield and to produce plaques or cytopathic changes in continuous cell lines. A frequent by-product of such adaptation to a new experimental host is the coincident attenuation of the virus for its original host; e.g., infectious canine hepatitis virus (an adenovirus) is attenuated for the dog after adaptation to pig kidney cell cultures.

Although growth in cell culture is a great advantage, it is not essential for the study of viral nucleic acid and proteins, as long as reasonable amounts of highly purified virions can be obtained from other sources. For example, the complete sequences of the DNAs of several papillomaviruses have been determined even though these viruses are very difficult or impossible to grow in cultured cells. Once viral DNA is available, recombinant DNA technology makes it possible to produce virtually unlimited quantities of any required nucleic acid or any polypeptide for chemical and biological analysis.

ABORTIVE INFECTIONS AND DEFECTIVE VIRUSES

Viral infection is not always *productive,* i.e., does not always lead to the synthesis of infectious progeny; some or even all viral components may be synthesized but not assembled properly. This is called *abortive infection.* It may be due to the fact that certain types of cells are nonpermissive, i.e., lack some enzyme or other requirement essential for the replication of a particular virus, whether it be the wild type or a host-dependent conditional lethal mutant.

Abortive infections due to genetically defective viruses are of considerable theoretical interest. For example, temperature-sensitive mutants are defective at high temperatures, but can be rescued (i.e., helped to yield infectious progeny) by coinfection of the cell with a *helper virus,* which is usually but not necessarily a related virus. Defective interfering particles have already been described.

Adeno-associated viruses (family *Parvoviridae*), which can be recovered from the throats of various animals, appear to be absolutely defective, in that they fail to replicate unless an adenovirus (or experimentally a herpesvirus) is also replicating in the same cells. In nature, they are invariably recovered with an adenovirus, from animals concurrently infected with both viruses.

The extreme example of defectiveness is seen in cells transformed by certain papillomaviruses (family *Papovaviridae,* see Chapter 12). Here part or all of the viral genome is integrated into the cellular genome and replicates with it, or persists in the cell as an episome. The genetic information in part of the viral genome may be expressed, for virus-specified proteins are often synthesized, but ordinarily no infectious virus is produced.

GENETIC RECOMBINATION BETWEEN VIRUSES

When two different virions simultaneously infect the same cell, several kinds of genetic recombination may occur between the newly synthesized nucleic acid molecules: intramolecular recombination, reassortment, reactivation (when one of the virions has been inactivated), and marker rescue.

Intramolecular Recombination

Intramolecular recombination involves the exchange of nucleic acid sequences between different but usually closely related viruses (Fig. 5-1A; Table 5-2). It occurs with all dsDNA viruses, presumably because

TABLE 5-2

Nucleic Acid Interactions: Genetic Recombination and Reactivation[a]

Phenomenon	Parent 1	Parent 2	Progeny	Comment
Intramolecular recombination	A**B**C	AB**C**	ABC, A**BC**	With mutants of DNA viruses and *Picornaviridae*
	ABC	AST	ABT, ASC	With different strains of DNA viruses
	ABCD	XYZ	ABCZ	Loss of adenovirus genes (D), addition of SV40 genes (Z)
	ABC	123	12ABC3	Tumorigenic viruses; integration of viral genes (ABC) into genome of cell (123)
	ABC	*onc*	A*onc*BC	Transduction of cellular oncogene by retroviruses
Gene reassortment	A/**B**/C	A/B/**C**	A/**B**/**C**	RNA viruses with segmented genomes
	A/B/C	A/S/T	A/B/T, A/S/C	
Cross-reactivation				
Between UV-inactivated virus and virus of a different but related strain	~~A~~~~B~~C	AST	ASC	Rescue of gene C from inactivated parent, by intramolecular recombination or gene reassortment
	~~A~~/~~B~~/C	A/S/T	A/S/C	
Multiplicity reactivation				
Between virions of same virus inactivated in different genes	AB~~C~~	A~~B~~C	ABC	Recombination or reassortment of genes B and C to yield viable virus
	A/B/~~C~~	A/~~B~~/C	A/B/C	

[a] A, etc., active viral genes; 1, etc., active cellular genes; **B**, etc. (boldface) mutant genes; ~~A~~, etc. (slashed) inactivated genes; *onc*, cellular oncogene; ABC, continuous linear genome; A/B/C, segmented genome.

of strand-switching by the viral DNA polymerase. Except for retroviruses, where recombination of proviral DNA occurs with high frequency, it has been demonstrated among RNA viruses only with foot-and-mouth disease virus and poliovirus.

In rare cases, intramolecular recombination occurs between unrelated viruses; the best example is between SV40 (a papovavirus) and adenoviruses. Both SV40 and adenovirus DNAs become integrated into cellular DNA, so that it is perhaps not surprising to find that when rhesus monkey cells which harbor a persistent SV40 infection are superinfected with an adenovirus, not only does complementation occur (see below), the SV40 acting as a helper in an otherwise abortive adenovirus infection, but recombination occurs between SV40 and adenovirus DNAs to yield hybrid (recombinant) DNA which is packaged into adenovirus capsids.

Integration of proviral DNA into the cellular DNA is an essential part of the replication cycle of retroviruses. Although the genome of these viruses is (+) sense ssRNA, replication does not occur until this is transcribed into DNA by the virion-associated reverse transcriptase and the resultant copy DNA is integrated into the cell's DNA. Furthermore, retroviruses may pick up *cellular oncogenes* by recombination; such oncogenes, after incorporation into proviral DNA and subsequent transcription to virion RNA, become *viral oncogenes*, which confer the property of rapid oncogenicity on the retrovirus (see Chapter 12). Integration of viral DNA into cellular DNA by intramolecular recombination is an essential part of the transformation process in cells transformed by adenoviruses, hepadnaviruses, and polyomaviruses, but not by papillomaviruses or certain herpesviruses, in which transformation occurs while the viral DNA remains episomal (see Chapter 12).

Reassortment

What was once called high-frequency recombination, but is best described as *reassortment* (Fig. 5-1B; Table 5-2), occurs with viruses that have segmented genomes, whether these are ss or dsRNA and consist of 2 segments (*Arenaviridae, Birnaviridae*), 3 (*Bunyaviridae*), 8 (*Influenzavirus* A and B), or 10–12 (*Reoviridae*). In cells infected with two related viruses, there is an exchange of segments with the production of various stable reassortants. Reassortment occurs in nature, and is an important source of genetic variability.

Reactivation

The term multiplicity reactivation is applied to the production of infectious virus by a cell infected with two or more virions of the same strain,

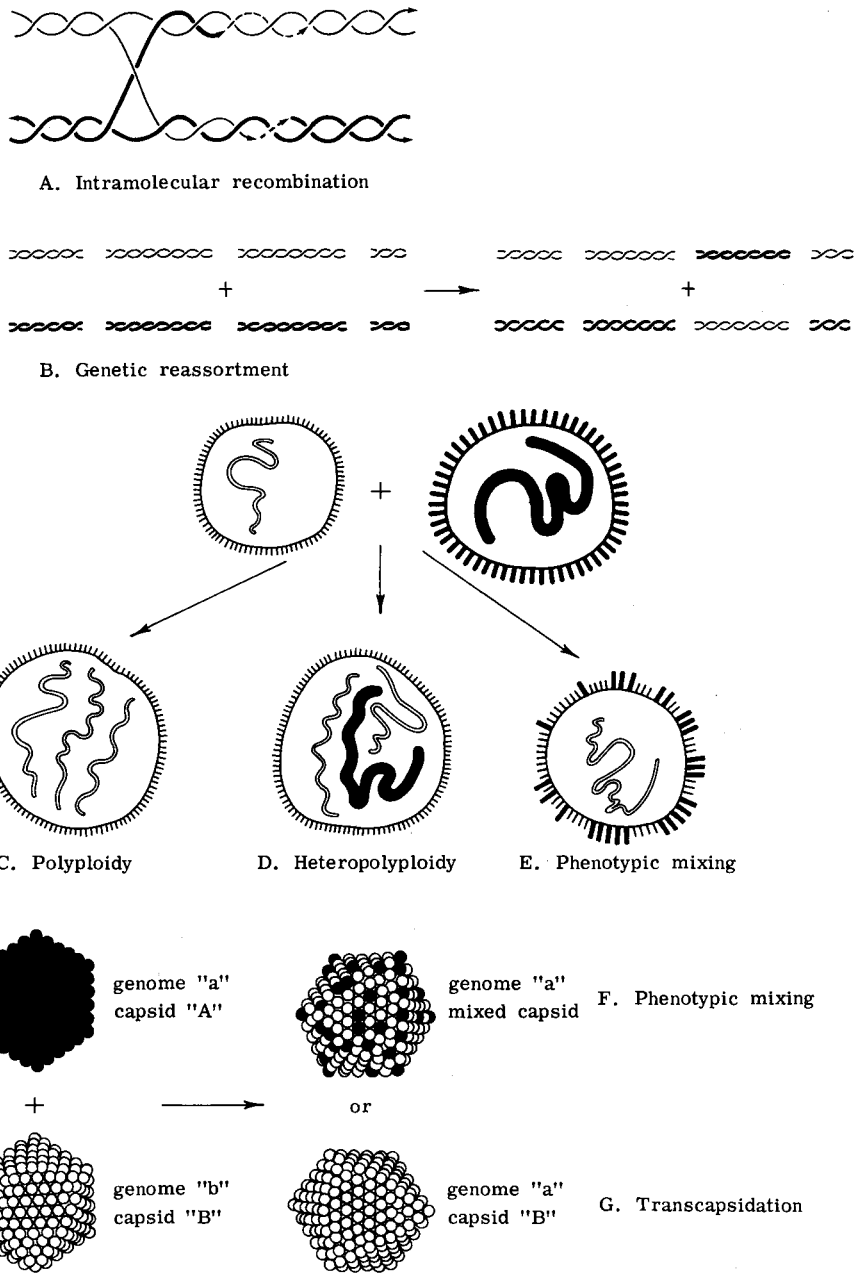

FIG. 5-1. *Genetic recombination, polyploidy, phenotypic mixing, and transcapsidation. (A) Intramolecular recombination. (B) Reassortment of genome fragments, as in reoviruses and orthomyxoviruses. (C) Polyploidy, as seen in unmixed infections with paramyxoviruses. (D) Heteropolyploidy, as may occur in mixed infections with paramyxoviruses and other enveloped RNA viruses. (E–G) Phenotypic mixing: (E) with enveloped viruses; (F) viruses with icosahedral capsids; (G) extreme case of transcapsidation or genomic masking.*

each of which has suffered a lethal mutation in a different gene, e.g., after exposure to UV irradiation (Table 5-2). The phenomenon by definition implies the production of viable recombinants. It has been demonstrated with viruses of several families: *Poxviridae, Orthomyxoviridae,* and *Reoviridae.* Multiplicity reactivation could theoretically lead to the production of infectious virus if animals were to be inoculated with UV-irradiated vaccines; accordingly, this method of inactivation is not used for vaccine production.

Cross-reactivation or marker rescue are terms used to describe genetic recombination between an infectious virus and an inactivated virus of a related but distinguishable genotype. By the same processes of exchange of nucleic acid described above (intramolecular recombination or genetic reassortment), some infectious virions are produced which contain active genes from both viruses (Table 5-2).

A special kind of marker rescue is now extensively used for correlating functional and physical maps of viral DNA. Called fragment rescue, it involves the introduction into a susceptible cell by *transfection* of a fragment of DNA containing a specified mutation, produced by recombinant DNA techniques (see below), together with the parental virus or its complete genome. Thus the desired mutation can be introduced, by recombination, into a proportion of the progeny virions (see Fig. 14-1). Alternatively, complementation tests with SV40, for example, can be carried out by transfecting cells, under restrictive conditions, with dsDNA produced by annealing a single strand of SV40 DNA containing a conditional lethal mutation with various known single-stranded fragments of wild-type SV40 DNA. If the wild-type fragment corresponds to the site of the mutation in the intact mutant strand, DNA replication and segregation of daughter molecules leads to plaque production and thus identification of the site of the mutation.

INTERACTIONS BETWEEN VIRAL GENE-PRODUCTS

Complementation

Complementation is the term used to describe all cases in which interaction between viral gene-products (structural proteins, enzymes, etc.) in doubly infected cells results in an increased yield of one or both viruses (Table 5-3). By definition, complementation does not involve recombination or reassortment, but reflects the fact that one virus provides a gene-product which the other cannot make, thus enabling the latter to replicate in the mixed virus-infected cell. In experimental virology, complementation tests between related viral mutants are useful as a prelimi-

TABLE 5-3
Gene-Product (Protein) Interactions: Complementation and Phenotypic Mixing[a]

BYZ byz Phenomenon	Parent 1	Parent 2	Progeny	Comment
Complementation				
(a) Between conditional lethal mutants of the same virus (under restrictive conditions)	A **B** C ↓ ↓ ↓ a **b** c	A B **C** ↓ ↓ ↓ a b **c**	A**B**C, AB**C**, (ABC)	Reciprocal; both mutants rescued; sometimes recombination also
(b) Between defective virus and unrelated helper virus	A $\not{B}$ C ↓ ↓ ↓ a c	B Y Z ↓ ↓ ↓ b y z	A$\not{B}$C and BYZ	Defective virus is rescued by gene-product "b" of helper BYZ
Phenotypic mixing				
(a) Enveloped viruses	$\underline{\text{ABC}}$ a	$\underline{\text{XYZ}}$ x	$\underline{\text{ABC}}$, $\underline{\text{XYZ}}$ ax ax	Mixed peplomers in envelopes, genomes unaltered; also parental phenotypes
(b) Nonenveloped viruses	$\underline{\text{ABC}}$ a	$\underline{\text{XYZ}}$ x	$\underline{\text{ABC}}$, $\underline{\text{XYZ}}$ ax ax	Mixed capsomers in capsids
	$\underline{\text{ABC}}$ a	$\underline{\text{XYZ}}$ x	$\underline{\text{ABC}}$, $\underline{\text{XYZ}}$ x a	Genome of one parent with capsid of the other (transcapsidation). Not always reciprocal

[a] A, etc., active viral genes, **B**, etc., mutant genes; $\not{B}$, defective gene B; a, etc., product of gene A, etc.; **b**, etc., product of mutant gene **B**; ABC/ax = genome/proteins in envelope (or capsid).

nary step to genetic mapping, to sort out mutants into groups. Temperature-sensitive mutants, for example, can usually be complemented by other mutants of the same virus which are not temperature sensitive, and different temperature-sensitive mutants can be allocated to functional groups by testing which pairs of *ts* mutants can complement one another.

Complementation can occur between unrelated viruses, e.g., between

an adenovirus and adeno-associated virus (a parvovirus), or between SV40 and an adenovirus in monkey cells.

Phenotypic Mixing

Following mixed infection by two viruses which share certain common features, some of the progeny may acquire phenotypic characteristics from both parents, although their genotype remains unchanged. For example, when cells are coinfected with antigenically different strains of influenza virus, or with influenza virus and a paramyxovirus, the envelopes of some of the progeny particles contain viral antigens characteristic of both parents. However, each virion contains the nucleic acid of only one parent; on passage it produces only virions resembling that parent (Fig. 5-1E,F; Table 5-3). Phenotypic mixing is very common in cells infected with defective retroviruses, progeny virions being *pseudotypes* with the genome of the defective parental virus but the envelope glycoproteins of the helper retrovirus.

With nonenveloped viruses phenotypic mixing can take the form of what has been called transcapsidation (Fig. 5-1G), in which there is partial or usually complete exchange of capsids. For example, poliovirus nucleic acid may be enclosed within a coxsackievirus capsid, or adenovirus 7 genome may be enclosed within an adenovirus 2 capsid.

Polyploidy

With the exception of the retroviruses, which are *diploid*, all viruses of vertebrates are *haploid*, i.e., they contain only a single copy of each gene. However, among viruses that mature by budding from the plasma membrane, e.g., paramyxoviruses, it is commonly found that several nucleocapsids (and thus genomes) are enclosed within a single envelope (*polyploid*, Fig. 5-1C). If cells are doubly infected with recognizably different strains of such viruses, many of the multiple genome progeny particles are heteropolyploid (Figure 5-1D) and they may also have phenotypically mixed envelope antigens.

GENETIC ENGINEERING

Restriction Endonucleases

During the 1960s, it was shown that a phenomenon that had been called "restriction" in bacteriophages, whereby certain bacteriophages failed to replicate in particular species of bacteria, was due to the rapid

degradation of the bacteriophage DNA by specific bacterial endonucleases, which were therefore called *restriction endonucleases*. The enzymes presumably provide a defense mechanism; bacterial DNA is not degraded because it is extensively methylated. Subsequently several hundred of these enzymes have been identified and purified from various bacteria. Each recognizes a unique short, *palindromic sequence* of nucleotides (a sequence that reads the same backwards as forwards), generally four to six nucleotide pairs long. Depending on the location and frequency of the specific unique sequence in a particular DNA molecule, a particular restriction endonuclease cleaves the DNA into a precise number of fragments of precise sizes. Other endonucleases, recognizing different sequences, cleave the same DNA into different numbers and sizes of fragments. These DNA fragments, produced by a panel of endonucleases, are analyzed by gel electrophoresis; different viruses, often even closely related strains of the same virus, yield characteristically different fragment patterns. Analysis of these patterns can be used to identify viral subtypes and strains and to follow chains of transmission ("molecular epidemiology," see Chapter 15). One practical application of this technique has been the separation of equine herpesvirus 4 from equine herpesvirus 1 (see Chapter 19). However, the greatest importance of restriction endonucleases lies in the construction of recombinant DNA molecules.

Recombinant DNA

The identification of the recognition sequences and cleavage sites of the restriction endonucleases, and the development of knowledge of other enzymes involved in DNA synthesis (polymerases, ligases, transferases), opened up the possibility of deliberately introducing specific foreign DNA sequences into DNA molecules. When these recombinant molecules replicate, there is a corresponding amplification of the foreign DNA. The process is called *molecular cloning*. When the replicating recombinant molecules are placed in a situation where their genetic information can be expressed, the polypeptide specified by the foreign DNA is produced. These results are usually achieved by incorporating the foreign DNA into a bacteriophage or a plasmid (Fig. 5-2), which serves as a *cloning vector* for introducing the foreign DNA into bacterial or other cells. Vectors that replicate in bacteria, yeasts, animal cells, and even intact animals are available. The development of recombinant DNA methodology has been facilitated by great improvements in the techniques of sequencing DNA. Using reverse transcriptase, it is also possible to make *copy DNA*, from either viral RNA or mRNA (Fig. 5-3).

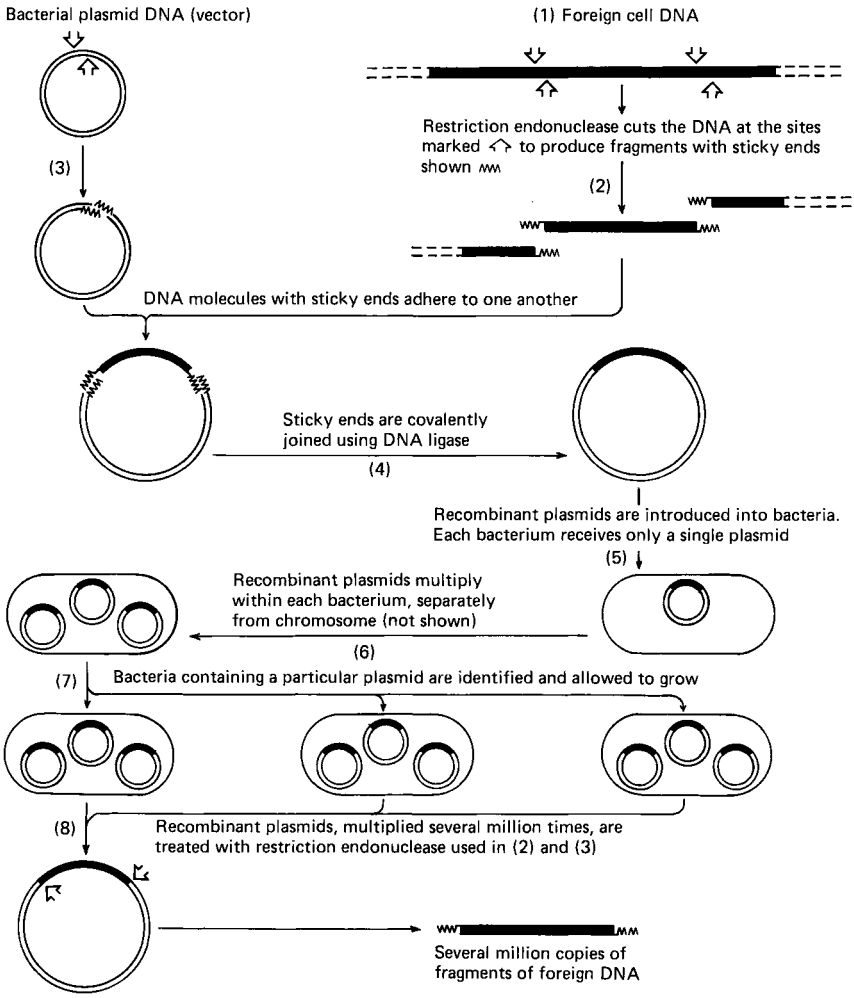

FIG. 5-2. *The steps in obtaining recombinant DNA. In parallel, DNA (genome DNA or copy DNA from virion or mRNA) from a virus (1) is cut into fragments by a selected restriction endonuclease (2), and the circular DNA molecule of the plasmid vector is cut with the same endonuclease (3). The viral DNA is then inserted and ligated into the plasmid DNA, which is thus circularized again (4). The plasmid is then introduced into the host bacterium by transfection (5). Replication of the plasmid as an episome may produce many copies per bacterial cell (for small plasmids), or there may be only one (for large plasmids) (6). Bacteria containing the desired plasmid are identified, cloned, and allowed to grow (7). The plasmids are then isolated from the bacteria and the viral DNA fragment excised (8) using the same restriction endonuclease as was employed in steps (2) and (3). In this way a specified gene may be replicated several millionfold.*

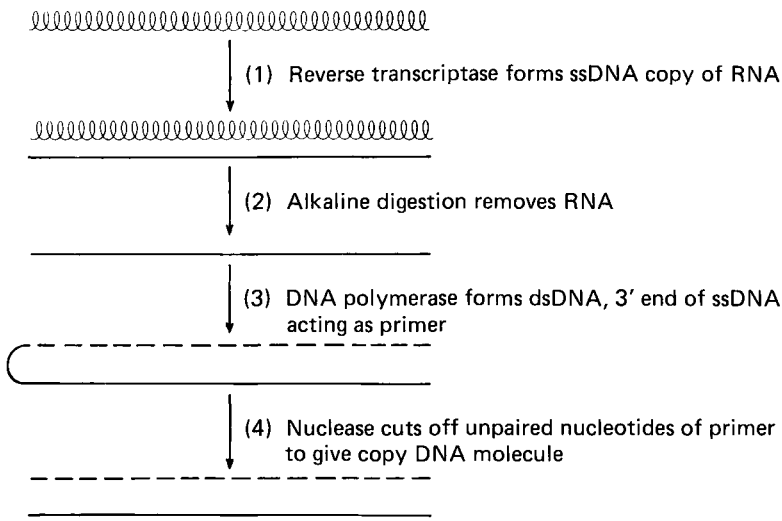

FIG. 5-3. *Preparation of copy DNA. The appropriate virion RNA or mRNA is treated with reverse transcriptase to produce a ssDNA copy (1). The RNA is removed by alkaline digestion (2). The ssDNA is treated with DNA polymerase to form dsDNA (3), the 3' end of the first DNA strand acting as a primer. A nuclease is used to cut off the unpaired nucleotides of the primer to give a "blunt-end" copy DNA molecule (4), which may be treated with terminal transferase to provide the molecule with "sticky ends" for insertion into a vector, as illustrated in Fig. 5-2.*

Uses of Genetic Engineering

Practical applications of genetic engineering to animal viruses include the development of nucleic acid probes for diagnosis by nucleic acid hybridization (see Chapter 13) and novel methods for the production of vaccines (see Chapter 14). One of the most promising new approaches to vaccination involves the use of vaccinia or fowlpox virus as a vector. One or several genes that specify protein antigens relevant to the production of protective immunity can be incorporated into the genome of a single vector-poxvirus, thus providing a means for immunizing animals against a number of different viral diseases by means of a single inoculation.

Combined with the availability of simple and fast methods of sequencing nucleic acids, genetic engineering has also led to studies of animal virus genomes that could not be contemplated before it became possible to produce large quantities of selected fragments of viral nucleic acid.

Among the achievements so far are:

1. Complete sequencing of the genome of several DNA viruses, including the 172-kbp genome of EB virus (a herpesvirus), and parts of the very large genome of vaccinia virus.
2. Complete sequencing of copy DNA corresponding to the entire genome of several RNA viruses.
3. Recognition of the number and sequence of viral or proviral DNAs that are integrated into the DNA of transformed cells.
4. Marker rescue by transfection with gene fragments, as a method of genetic mapping.
5. Production of proteins coded by specific viral genes—in bacteria, yeast, and animal cells, or by cell-free translation.
6. Synthesis of peptides based on DNA sequence data.

Methodology for Obtaining a Viral Protein by Molecular Cloning

A desired gene, or copy DNA transcribed from an RNA gene, is recombined with a suitable vector, such as a plasmid or bacteriophage. It is not essential to know the identity of the nucleotide sequence required; one can simply clone a random population of nucleic acid fragments ("shotgun" cloning), then screen isolated cloned colonies of the transfected bacteria for the gene-product. This can be done by radioimmunoassay, ELISA, or immunoblotting of the transformed bacterial cell lysate, using a labeled antibody. The particular recombinant plasmid found to carry the desired viral gene can then be manipulated further to optimize yield, for example, by the use of multicopy vectors or high-efficiency promoters, or by linking to a gene whose protein is normally excreted from the cell. It may not even be necessary to produce the protein in its native form; commonly, the viral gene is inserted into the vector adjacent to all or part of the gene for a plasmid- or bacteriophage-coded protein that is made in large amounts, causing the production of a "fusion protein" containing part of the vector protein and the viral protein in question. There are some technical problems when such proteins are expressed in bacteria: (1) if the protein is made in too high a concentration it may be toxic for the host bacteria, (2) some proteins are unstable, (3) bacteria cannot glycosylate proteins, and (4) it may be very difficult to solubilize the clumped polypeptide. Some fusion protein products, once formed, may be vulnerable to proteases or may not adopt their native conformation. Ways of circumventing these and other problems are rapidly being developed.

More recently, cloning has been accomplished in eukaryotic cells, both in yeast and animal cells. The viral gene may first be incorporated,

for convenience, into a bacterial plasmid, then into a "dual" (or "shuttle") vector able to replicate autonomously in eukaryotic as well as prokaryotic cells. The circular viral DNAs of bovine papilloma virus and SV40 are now widely used as eukaryotic cloning vectors. A useful method for cloning and selecting cells containing a viral gene of interest is to cotransfect with the gene of an enzyme that is deficient in the cell; only cells containing the enzyme grow in a selective medium. For instance, if a viral gene is cloned into a vector plasmid alongside the gene for dihydrofolate reductase (DHFR), and then a DHFR$^-$ Chinese hamster ovary cell line is transfected with this dual vector, in the presence of methotrexate, only transfected cells grow. The yield of the viral protein may reach 20% of the cells' total protein output. At this level of expression it is realistic to think of vaccine development.

THE GENETIC BASIS OF VIRULENCE

Unraveling the genetic basis of virulence has long been one of the major goals of animal virology, and one of the most difficult to achieve, since many genes, both viral and host, are involved. With advances in molecular genetics it has been possible to dissect the problem in a more precise way.

The most detailed studies of this kind are those carried out with retroviruses and tumorigenic DNA viruses to determine the genetic basis of cellular transformation (see Chapter 12). Experiments with other viral infections are less advanced, but a start has been made. Viruses with segmented genomes provide an easier experimental model, since each segment of the genome of influenza viruses and reoviruses, for example, is in general equivalent to one gene, and reassortants can be readily obtained. Study of a number of reassortants involving different genome segments enables the functions that relate to virulence to be assigned to particular genes. In addition, a detailed understanding of the basis of virulence at the molecular level has been obtained with human poliovirus, and to a lesser extent with foot-and-mouth disease virus, where it has been possible to compare the sequences of the genomes of strains of high and low virulence, including vaccine strains.

Influenza Virus

Experiments with influenza virus in mice have confirmed the view advanced by the pioneer in the field, Sir Macfarlane Burnet, that virulence is multigenic, i.e., that in general, and for influenza virus in particular, no one gene determines virulence. Recent studies show that one essential requirement for virulence in the chicken and neuroviru-

lence in the mouse is that the hemagglutinin protein must be cleaved. In nonpathogenic strains of avian influenza virus, the HA1 and HA2 moieties of the hemagglutinin are linked by a single amino acid, whereas in virulent strains the linker is a sequence of several amino acids and is more readily cleaved. Virulent strains thus contain HA which is activated (cleaved) in a wide spectrum of different types of cell, hence they can replicate and spread throughout the host organism. Nonpathogenic strains, on the other hand, soon reach a barrier of cells which lack an appropriate protease.

However, virulence is only partly explained by this factor, for when reassortants were made between highly pathogenic and avirulent strains of avian influenza virus, exchange of any one of the eight RNA segments modified the virulence. Studies with a variety of reassortants showed that for each reassortant, an optimal combination of genes (the optimal "gene constellation") was selected which favored survival in nature and determined virulence.

Reovirus

Detailed analyses of the virulence of reoviruses in mice have been carried out with reassortant viruses. The fact that the protein product of

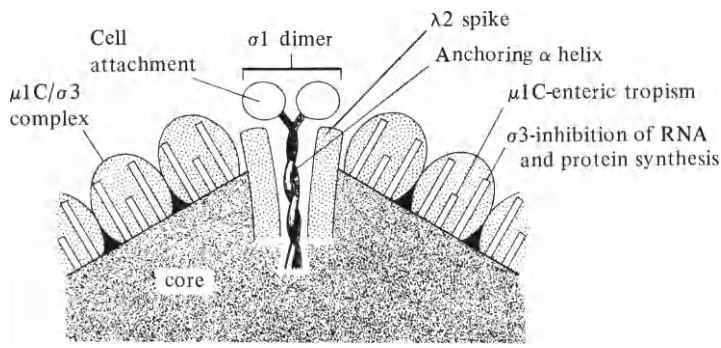

FIG. 5-4. *One apex of a reovirus capsid, showing the location of the polypeptides that play major roles in virulence (see Table 5-4). σ1 is located at the vertices of the icosahedron and consists of two components: a globular dimer at the surface, which is responsible for hemagglutination and cell attachment, and an α-helical region which anchors the hemagglutinin by interaction with the λ2 spike protein; μ1C and σ3 are associated with each other, in the ratio of one molecule of μ1C to two molecules of σ3, on the surfaces of the icosahedral capsid. [Modified from R. Basel-Duby, A. Jayasuriya, D. Chatterjee, N. Sonenberg, J. V. Maizel, Jr., and B. N. Fields,* Nature (London) **315,** *421 (1985).]*

TABLE 5-4
Correspondence between Certain Reovirus Genes and Capsid Polypeptides: Effects of Mutations of These Genes on Virulence[a,b]

Gene segment	Polypeptide product	Location in virion	Virulence function	Result of attenuating mutations
M2	μ1/μ1C	Capsid	A. Growth at mucosal surfaces	Decreased capacity to grow in intestine
			B. Immune induction	Loss of immune stimulation of suppressor T cells
			C. Growth in target tissue	Relative decrease in neurovirulence
S1	σ1	Capsid	Hemagglutinin; binds to receptors	Loss of neurovirulence
			A. Nerve cells	Altered specificity in humoral and cytotoxic T cell responses
			B. Lymphoid cells	
S4	σ3	Capsid	Inhibition of RNA and protein synthesis	Persistent infection Interference

[a]Based on B. N. Fields and M. I. Greene, *Nature (London)* 300, 19 (1982).

[b]Genes L1, L2, L3, M1, and S2 specify core polypeptides λ3, λ2, λ1, μ2, and σ2; λ2 is the core spike that projects from each vertex. Genes M3 and S3 specify nonstructural polypeptides.

each of the 10 genome segments has been isolated and characterized has allowed determination of their functions and effects on virulence (Table 5-4). Three genes (S1, M2, and S4) encode the three polypeptides that are found on the outer capsid (Fig. 5-4). Each plays a role in determining virulence. Gene S1 specifies the hemagglutinin (protein σ1), which is located on the vertices of the icosahedron, and is responsible for cellular and tissue tropism. Gene M2 specifies polypeptide μ1C, which determines sensitivity of the virion to chymotrypsin and thus growth in the intestine. Gene S4 specifies polypeptide σ3, which inhibits protein and RNA synthesis in infected cells; mutations in this gene play a role in establishing persistent infection in cultured cells, This sort of structure–function analysis is immediately applicable to the other dsRNA viruses, notably the bluetongue viruses and rotaviruses, where studies now are in progress which will influence vaccine development.

Poliovirus

Although useful sequencing studies have been carried out with vaccine strains of foot-and-mouth disease virus, the best analyzed example of the determinants of virulence in a picornavirus is with human poliovirus. Its genome is a single molecule of ssRNA, and the entire nucleotide sequence of several strains has been determined. Comparison of the nucleotide sequence of the poliovirus type 1 vaccine strain with that of the virulent parental strain (Mahoney) from which Sabin derived it shows that there are 57 base substitutions in the 7441 bases and these are scattered along the entire genome. Twenty-one of these substitutions resulted in amino acid changes; these involve several of the viral proteins. On the other hand, with poliovirus type 3 vaccine strain, which reverts to virulence with a frequency at least 10 times that of type 1 vaccine, there are only 10 nucleotides different from the parental strain, of which only 3 result in amino acid changes, one in each of three viral proteins.

GENETIC VARIATION IN NATURE

As well as providing the basis for the development of attenuated live-virus vaccines, genetic variation by mutation, reassortment, and intramolecular recombination is the mechanism which, under the influence of natural selection, has produced the great variety of viruses found in nature. There is growing evidence that reassortment is an important mechanism for producing sudden major genetic changes in nature, among the bunyaviruses, the orbiviruses, the rotaviruses, and the influenza A viruses. Mutation is also a potent mechanism for changing the properties of viruses, especially with the RNA viruses, which, because there is no proofreading mechanism, have a much higher base substitution rate during replication than the DNA viruses. If the process of natural selection is sufficiently rigorous, even DNA viruses can undergo relatively rapid genetic changes. We will conclude this chapter with a brief examination of how one DNA virus, myxoma virus, and one RNA virus, influenza A virus, have evolved over the past few decades, the one in rabbits and the other in humans, swine and birds.

Genetic Changes in Virus and Host in Myxomatosis

Observations made on myxomatosis in wild European rabbits in Australia and Britain illustrate how rapidly evolutionary changes in a virus and its mammalian host may occur.

Myxomatosis, caused by a poxvirus, occurs naturally as a mild infection of rabbits in South America and California (*Sylvilagus* spp.). It produces a skin tumor from which virus is transmitted mechanically by biting insects. Occasionally, lethal disease due to infection with this virus occurred in laboratory (European) rabbits in South America (see Plate 21-5). Recognition of its lethal effects led to its use for biological control of wild European rabbits.

The wild European rabbit was introduced into Australia in 1859 and rapidly spread over the southern part of the continent, where it became the major animal pest of the agricultural and pastoral industries. Myxoma virus from South America was successfully introduced into Australia in 1950 in an effort to control the rabbit pest. When originally liberated in Australia, the virus produced case fatality rates of over 99%. This highly virulent virus was readily transmitted by mosquitoes during the summer, when their populations were high. Farmers operated "inoculation campaigns" to introduce virulent myxoma virus into the wild rabbit populations in the spring and summer.

It might have been predicted that the disease would disappear at the end of each summer, due to the greatly diminished numbers of susceptible rabbits and the greatly lowered opportunity for transmission by mosquitoes during the winter. This must often have occurred in localized areas, but not over the continent as a whole. The capacity of virus to survive the winter conferred a great selective advantage on viral mutants of reduced lethality. Rabbits infected by such mutants survived in an infectious condition for weeks instead of a few days, as happened when they were infected by the original highly virulent strains. Within 3 years such "attenuated" mutants became the dominant strains throughout Australia. Some inoculation campaigns with the virulent virus produced localized highly lethal outbreaks, but in general, the viruses that spread through the rabbit populations each year were the "attenuated" strains, which because of the prolonged illness in their hosts provided a greater opportunity for mosquito transmission. The much better transmission of these strains, even during summer outbreaks, was verified by field experiments; their capacity for overwintering was also an obvious advantage. Thus the original highly lethal virus was progressively replaced by heterogeneous strains of lower virulence. It should be noted that "attenuated" is a relative term; the strains that became dominant in Australia killed 70–90% of rabbits that had not been genetically selected for resistance. Experimental studies suggested that highly attenuated strains that killed very few rabbits were very poorly transmitted and could not be expected to survive in nature.

Rabbits that recover from myxomatosis are immune to reinfection for

the rest of their lives. However, since most wild rabbits have a life span of less than a year, herd immunity is not as critically important in the epidemiology of myxomatosis as it is in infections of longer lived species.

Even in the initial outbreaks some rabbits recovered from the infection, suggesting that selection for genetically more resistant animals operated from the outset. Sometimes the severity of the disease was lessened and recovery rates improved by environmental factors, e.g., high ambient temperature. In areas where repeated outbreaks occurred, the genetic resistance of the surviving rabbits progressively increased. The early appearance of the viral strains of lower virulence, which allowed 10% of genetically unselected rabbits to recover, was an important factor in allowing the number of genetically resistant rabbits to increase rapidly. In areas where annual outbreaks occurred, the genetic resistance of the rabbits changed such that the case fatality rate after infection under laboratory conditions with a particular strain of virus fell from 90% to 25% within 7 years.

Genetic resistance increased more rapidly in areas where annual outbreaks occurred than in areas where several years elapsed between successive outbreaks. Strains of myxoma virus recovered in areas where the genetic resistance of rabbits was high were more virulent than those recovered from areas where the genetic resistance increased more slowly, since strains of higher virulence were required to produce lesions of adequate transmissibility.

Genetic Changes in Influenza A Virus

Influenza viruses produce important diseases in humans, swine, horses, and poultry. Because of the significance of human influenza and the longevity of humans, the most detailed long-term study of the evolution of influenza viruses has been carried out with influenza A in humans. The general principles derived from these studies will be described first. Relevant findings from an outbreak of avian influenza, in which there was a rapid change in virulence, are then described.

Evolutionary Changes in Influenza A Virus in Humans, 1933–1983. Human influenza virus was first isolated in 1933. Since that time influenza viruses have been recovered from all parts of the world and their antigenic properties have been studied in considerable detail, thus providing an opportunity for observing continuing evolutionary changes in the influenza viruses. The mortality from influenza is too low, and the 30-year generation time of humans too long, for any significant observable change to have occurred in the genetic resistance of human beings.

Influenza A virus periodically causes epidemics in humans, swine,

horses, birds, and occasionally in other animals such as seals. Subtypes are classified according to the two envelope antigens, the hemagglutinin (HA or H) and neuraminidase (NA or N). All of the 13 subtypes of the H molecule have been found in birds, three of them also in humans and pigs and two in horses. The 9 N subtypes show a similar distribution.

The outstanding feature of influenza A virus is the antigenic variability of the envelope glycoproteins, HA and NA, which undergo two types of changes, known as *antigenic drift* and *antigenic shift*. Antigenic drift occurs within a subtype and involves a series of minor changes, usually point mutations, producing strains each antigenically slightly different from its predecessor. Antigenic shift involves the acquisition of a gene for a completely new HA or NA. A new human subtype of virus and a pandemic in humans may result. In theory, at least, antigenic shift could produce pandemic disease among horses, swine, and chickens.

Antigenic Drift. After antigenic shift introduces a new pandemic strain, drift begins as mutations subsequently accumulate in all its RNA segments. Mutations in the gene that codes for the HA include some which alter antigenic determinants. When antiserum against the previously prevalent strain no longer neutralizes the variant, a new strain has emerged. Most of the significant changes in the HA are clustered in four regions of the molecule which are thought to be important antigenic sites (see Fig. 9-5). Substitution of a single amino acid in a critical antigenic site may totally abolish the capacity of the prevalent antibody to bind to that site. On the other hand, some regions of the HA protein are conserved in all human and avian strains that have been sequenced, presumably because they are essential for the maintenance of the structure and function of the molecule. The important feature of antigenic drift in human influenza viruses is that in immune populations the new strains have a selective advantage over their predecessors such that they tend to displace them. Although minor variants can cocirculate, the general rule is that the novel strain supplants previous strains of that subtype.

Antigenic Shift. Since influenza A virus was first isolated in 1933, sudden replacement of the prevalent human subtype with a new subtype occurred in 1957 (H1N1 to H2N2) and 1968 (H2N2 to H3N2). After the H2N2 strain ("Asian flu") first appeared in China in 1957 it rapidly spread round the world, as did the H3N2 strain ("Hong Kong flu") after 1968, each displacing the then prevalent subtype. In 1977 the H1N1 subtype mysteriously reappeared, and since then the two subtypes H3N2 and H1N1 have cocirculated (Fig. 5-5).

The HA glycoprotein molecules of different subtypes are, by definition, quite distinct serologically. Further information about their degree

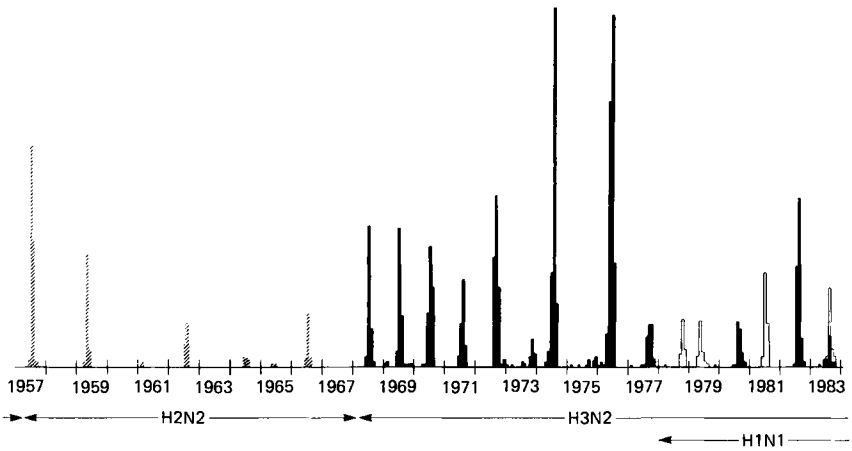

FIG. 5-5. *Epidemiology of influenza in humans. The histograms show the monthly isolations of influenza A viruses from human patients admitted to Fairfield Hospital for Infectious Diseases, Melbourne, from 1957 to 1983. Note annual or biennial epidemics in the Australian winter (June to August). The major peak in 1957 represents the country's first experience of the H2N2 ("Asian flu") subtype (hatched bars). The next major peak corresponds with the 1968–1969 pandemic caused by the H3N2 ("Hong Kong flu") subtype (closed bars). The sporadic winter outbreaks since then, sometimes quite extensive as in 1974 and 1976, have been due to a succession of H3N2 strains arising as point mutations (antigenic drift). However, almost all cases in 1978, 1979, and 1981 were of the H1N1 subtype (open bars), which reemerged after being totally absent since 1950. Most of the H1N1 cases were in young people, born after 1957. The 1983 outbreak was a mixture of H3N2 and H1N1. (Data courtesy of A. A. Ferris, F. Lewis, N. Lehmann, and I. D. Gust.)*

of relatedness, and thus possibly their evolutionary history, has been derived from the nucleotide sequences of the HA genes of different subtypes. The preservation of cysteine residues and certain other amino acids in all sequences indicates that the 13 known subtypes evolved from a common ancestor and share a common basic structure. However, there is only about 30% homology between the amino acid sequences of H3 and H2, whereas the homology between strains within each subtype is usually over 90%. In other words, the many strains that emerged by antigenic drift within the H2 subtype between 1957 and 1968 are closely related genetically, and within the H3 subtype, strains which have become prevalent since 1968 are also closely related to one another, but there are major differences between the two groups. Clearly, sharp discontinuities in the evolutionary pattern occurred with the emergence of the new subtype H2 in 1957 and again with the appearance of H3 in 1968.

What is the mechanism of this abrupt change in subtypes, which is referred to as antigenic shift? The answer is derived by comparing the sequences of the HA proteins from human influenza subtypes with those isolated from birds and animals. Such studies revealed much closer homology between, say, H3 (human) and any avian or equine influenza H3, than between H3 (human) and H2 (human). Since laboratory studies have clearly demonstrated the ease with which genetic reassortment can occur in animals or birds, as well as in embryonated eggs or cultured cells, it seems reasonable to suppose that new subtypes of human influenza virus are derived by naturally occurring genetic reassortment between influenza viruses infecting humans and animals.

An exception to this general pattern appears to be the reemergence of H1N1 in 1977. The strain recovered in May 1977 resembled the subtype recognized in swine in the Midwest of the United States in 1931, which circulated in humans from 1931 to 1956. It resembled very closely indeed a strain that circulated in humans in 1950. Since 1977 this subtype has circulated worldwide among persons born after the H1N1 subtype was replaced by H2N2 (1957). The explanation of the reemergence of H1N1 twenty years after its disappearance from the human population is still a mystery; a laboratory deep-freezer seems the most likely source, but no one has yet claimed responsibility. Meanwhile H3N2 has continued to be common, so that for the first time since virological studies have been possible two subtypes are circulating contemporaneously in human beings.

Avian Influenza in Pennsylvania in 1983. In April 1983 an avian influenza virus appeared in chickens in Pennsylvania, in the United States, producing a mortality of less than 10%. Its serotype was H5N2 [A/-Chick/Penn/83(H5N2)], and comparison of the genome segments of this isolate with other influenza A isolates by RNA–RNA hybridization indicated that all its genes were closely related to the genes of H5N2 isolates from wild birds made in the eastern United States at the time. In October 1983 a change occurred in its virulence, such that the mortality rate in infected chickens suddenly rose to over 80%.

Comparison of individual RNA segments of the April and October isolates by oligonucleotide mapping showed that genetic reassortment had not occurred, suggesting that the change in virulence had been due to the accumulation of a small number of point mutations (i.e., genetic drift). Previous studies of reassortants between virulent avian influenza virus (fowl plague virus) and avirulent avian or human influenza viruses had shown that virulence for birds is polygenic but that the HA gene is of major importance. For example, the virulence of another virulent avian serotype, H7, is linked to the ready cleavage of the HA protein in

cultured cells. Sequencing of the HA genes from the mild and virulent H5N2 strains revealed seven nucleotide differences which resulted in four amino acid changes in the HA protein. One of these changes affects a glycosylation site near the site of cleavage between the HA1 and HA2 subunits of the HA molecule. Cleavage of the HA molecule occurs more readily in the virulent strain. The two strains also differ in the extent to which they produce defective interfering particles, which are much more frequent in the original less virulent strain. The practical significance of the critical single-base change in the RNA of the HA genome segment of this H5N2 avian influenza virus that occurred in October 1983 is underlined by the fact that control measures, which involved the slaughter of millions of chickens because of the threat posed to the poultry industry of the United States, cost more than $60 million.

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CHAPTER 6

Pathogenesis: Virus-Induced Changes in Cells

Four of the previous five chapters have been concerned with viruses as such, and the other one (Chapter 3) with their cultivation and assay, which are prerequisites for such investigations. The next group of seven chapters deals with the interactions of viruses with the animals that they infect. However, virus-induced changes at the subcellular and molecular levels are best studied in cultured cells; observations at this level can then be used to interpret changes found in whole animals. Viral cytopathology is as complex as cell biology itself, hence it is not surprising that the subject is still largely at the descriptive level of understanding. The analysis of viral replication has been simplified at a biochemical level by the concept of strategies of viral replication (see Chapter 4); there is as yet no such unifying theme as to how DNA or RNA viruses redirect cellular metabolism and kill or transform infected cells.

The various types of interactions that can occur between virus and cell are summarized in Table 6-1. Viruses may be categorized as *cytocidal* (*lytic*) and *noncytocidal* (*nonlytic*). Not all infections, whether cytocidal or

TABLE 6-1
Virus–Cell Interactions

Type of infection	Effect on cell	Production of infectious virions
Cytocidal (lytic)	Morphological changes in cells (cytopathic effects); inhibition of protein, RNA, and DNA synthesis; cell death	Usually; but cytopathology can occur in nonproductive (abortive) infections
Persistent noncytocidal (productive)	No cytopathic effect; little metabolic disturbance; cells continue to grow and divide; may be associated with loss of the special functions of differentiated cells	Always, although often with reduced yield
Persistent noncytocidal (nonproductive)	Usually nil	Nil. Viral genome persists as episome or integrated. Normally virus is not expressed but may be induced by cocultivation with permissive cells, irradiation, or chemical mutagens
Transformation	Alteration in cell morphology; cells survive and can be passaged indefinitely; transformed cells may produce tumors in experimental animals	Rarely by oncogenic DNA viruses and usually by oncogenic RNA viruses

noncytocidal, necessarily lead to the production of new virions. Cell changes of a profound nature, leading to cell death in some cases and cell transformation in others, may also occur in *nonproductive (abortive) infections*. Looked at from the point of view of the cell rather than the virus, certain kinds of cells are *permissive*, i.e., they support complete replication of a particular virus, while others are *nonpermissive*, i.e., replication is blocked at some point. Cytopathic changes can occur in both productive and nonproductive infections and in permissive and nonpermissive cells.

CYTOPATHIC EFFECTS OF VIRUSES

The gross and histological appearance of the damage produced in cultured cells by particular cytocidal viruses, known as the *cytopathic*

effect, is an important diagnostic criterion, since many viruses cause a characteristic cytopathic effect in cells commonly used in diagnostic laboratories (see Chapter 13). Many biochemical changes occur in cells infected with cytocidal viruses. Early in infection, virus-specified proteins often shut down the cell's protein synthesis, an event which is incompatible with its survival. Late in infection, large numbers of viral macromolecules accumulate; some of these, particularly certain capsid proteins, are directly toxic. Viral proteins or virions themselves are sometimes found in large crystalline aggregates or inclusions that visibly distort the cell. Viral proteins are often inserted into the plasma membrane, sometimes quite early in the replication cycle. There are also changes in membrane permeability, leading to osmotic swelling.

Thus there are numerous changes in the virus-infected cell which, individually or cumulatively, can be lethal. Cell damage by certain viruses can occur even without replication of the virus, e.g., when late stages of the expression of the viral genome are blocked, as in infection of alveolar macrophages by influenza or parainfluenza viruses.

Shutdown of Cellular Protein Synthesis

Most cytocidal viruses code for proteins that shut down the synthesis of cellular proteins; cellular RNA and DNA synthesis are usually affected secondarily. The shutdown is particularly rapid and severe in infections of cultured cells by picornaviruses, some poxviruses, and herpesviruses, all of which are rapidly cytopathogenic. With other viruses (e.g., adenoviruses) the shutdown is later and more gradual, while with noncytocidal viruses such as arenaviruses and retroviruses there is, by definition, no shutdown and no cell death. Some viruses (e.g., flaviviruses) are cytocidal, even though they do not shut down cellular protein synthesis very well, indicating that this is not the only mechanism involved.

Cytopathic Effects of Viral Proteins

Although toxin production is not a feature of viral infections, viral capsid proteins, e.g., adenovirus penton and fiber proteins, in high concentrations are often toxic and may be a principal cause of a cytopathic effect. Pathological changes in cells may follow the accumulation of viral proteins late in the replication cycle after infection at low multiplicity, or may be seen quite early, as a laboratory artifact, after the experimental use of very large inocula.

Inclusion Bodies

A characteristic, but by no means universal morphological change in virus-infected cells is the formation of *inclusion bodies* which are recog-

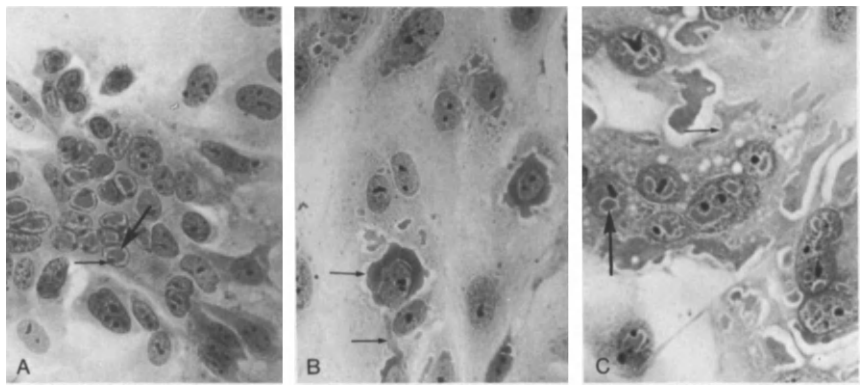

PLATE 6-1. *Types of viral inclusion bodies (H and E stain, ×200). (A) Intranuclear inclusions; cells form syncytium—herpesvirus. Small arrow, nucleolus; large arrow, inclusion body. Note also margination of chromatin. (B) Intracytoplasmic inclusions—reovirus. Arrows indicate inclusion bodies in perinuclear locations. (C) Intranuclear and intracytoplasmic inclusions; cells form syncytium—measles virus (also seen with distemper and rinderpest viruses). Small arrow, intracytoplasmic inclusion body; large arrow, intranuclear inclusion body. (Courtesy I. Jack.)*

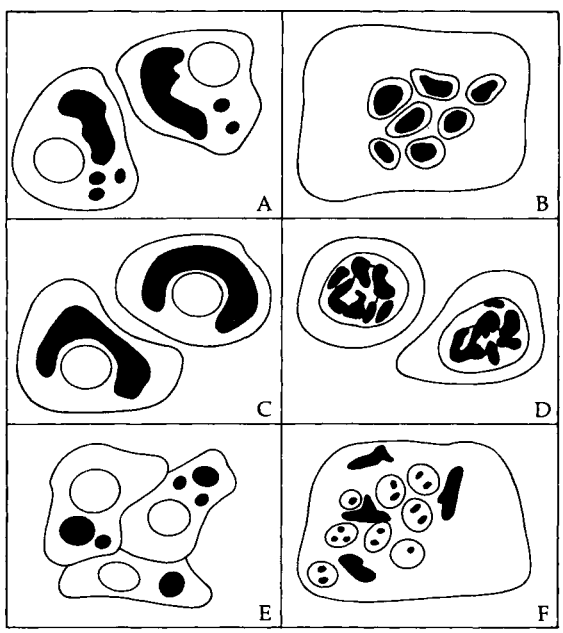

FIG. 6-1. *Inclusion bodies in virus-infected cells. (A) Vaccinia virus—intracytoplasmic acidophilic inclusion. (B) Herpesvirus—intranuclear acidophilic inclusion; cell fusion produces syncytium. (C) Reovirus—perinuclear intracytoplasmic acidophilic inclusion. (D) Adenovirus—intranuclear basophilic inclusion. (E) Rabies virus—intracytoplasmic acidophilic inclusions (Negri bodies). (F) Morbillivirus—intranuclear and intracytoplasmic acidophilic inclusions; cell fusion produces syncytium.*

nized with the light microscope by their staining behavior (Plate 6-1). Depending on the virus, such inclusions may be single or multiple, large or small, round or irregular in shape, intranuclear or intracytoplasmic, and acidophilic or basophilic.

The most striking viral inclusion bodies are the intracytoplasmic inclusions found in cells infected with poxviruses, paramyxoviruses, reoviruses, and rabies virus, and the intranuclear inclusion bodies produced by herpesviruses, adenoviruses, and parvoviruses (Fig. 6-1). Some viruses, e.g., canine distemper and rinderpest viruses, may produce both nuclear and cytoplasmic inclusion bodies in the same cell. Many such inclusions have now been shown, by fluorescent-antibody staining or electron microscopy, to be accumulations of viral structural components, e.g., the nucleocapsids of paramyxoviruses. The basophilic intracytoplasmic inclusions invariably found in cells infected with poxviruses are the sites of viral synthesis (viral "factories"). Other very prominent inclusion bodies found in the cytoplasm of cells infected with fowlpox, ectromelia, and cowpox viruses, are acidophilic; these represent accumulations of viral protein and may or may not contain numerous mature virions.

In a few instances (e.g., adenoviruses, reoviruses) inclusion bodies represent crystalline aggregates of virions. Other inclusion bodies, such as those found in the nucleus of cells infected with herpesviruses, are the result of late degenerative changes which produce margination of chromatin.

Cell Fusion

A conspicuous feature of the infection of cell monolayers by paramyxoviruses, herpesviruses, and some coronaviruses and poxviruses is the production of *syncytia*, also called *polykaryocytes* or giant cells (see Plate 27-2). Late in their replication cycle, these viruses cause changes in the cell membrane which result in the fusion of the infected cell with neighboring uninfected cells. Such syncytia are often seen in the tissues of animals infected with these viruses.

If present at high multiplicity, paramyxoviruses can also cause rapid fusion of cultured cells to form syncytia. Cell biologists have used this phenomenon to produce functional heterokaryons by fusing different types of cells; for example UV-inactivated parainfluenza virus was used to produce "hybridoma cells" by fusion of antibody-producing B lymphocytes with myeloma cells in the pioneering experiments that produced the first monoclonal antibodies.

Nonspecific Histological Changes

In addition to changes due to the specific effects of viral replication, most virus-infected cells also show nonspecific changes, very much like those induced by physical or chemical agents. The most common early and potentially reversible change is what histopathologists call "cloudy swelling," which is associated with changes in the permeability of the plasma membrane. Electron microscopic study of such cells reveals diffuse swelling of the nucleus and distention of the endoplasmic reticulum and mitochondria. Later the nucleus becomes condensed and shrunken. Further cell destruction is the autolytic consequence of the leakage of lysosomal enzymes into the cytoplasm.

PERSISTENT INFECTIONS

Noncytocidal viruses, by definition, do not kill the cells in which they replicate. They often produce persistent infection, in which the infected cells produce and release virions but cellular metabolism is little affected and the infected cells continue to grow and divide. This type of cell–virus interaction is found in vertebrate cells infected with several kinds of RNA viruses: arenaviruses, retroviruses, and some paramyxoviruses, for example. In all these infections virions are released by budding from the plasma membrane of the cell. Although such virus-yielding cells may grow and divide in culture for long periods, there are slow, progressive changes that ultimately lead to cell death, except with retroviruses. In the animal, cell replacement occurs so rapidly in most organs and tissues that the slow fallout of cells due to persistent infection may have no effect on overall function. However, persistently infected differentiated cells may lose their capacity to carry out specialized functions (see below). Also, antigenic changes are produced in the cell membrane of persistently infected cells. In the animal, such changes may provoke an immunological response, which can rapidly lead to destruction of the cells (see Chapter 10).

The existence of persistent infection in cultured cells is demonstrable by a variety of laboratory procedures, such as hybridization with nucleic acid probes, staining with fluorescent antibody, hemadsorption, interference with the replication of a superinfecting virus, or electron microscopy.

EFFECTS OF NONCYTOCIDAL VIRUSES ON FUNCTIONS OF SPECIALIZED CELLS

Although they do not immediately kill cells, infections with noncytocidal viruses often interfere with the specialized functions of cells. For example, murine neuroblastoma cells persistently infected with lymphocytic choriomeningitis virus fail to produce acetylcholine, although functions concerned with cell survival are unaffected. Viruses may also interfere with the secretion of immunoglobulins by lymphocytes and hormones by somatotrophic cells, e.g., the β cells of the islets of Langerhans, without killing the cells concerned. It is likely that these subtle effects, which can only be detected with special techniques, are quite common. Clearly, they may be of considerable importance in the infected animal.

Studies of respiratory epithelium in explant cultures have shown a specific functional response mimicking *in vivo* infection; rhinovirus infection results in cilial stasis and later in the destruction of cilia, although the cells are often not killed (Plate 6-2). This pathophysiological effect is important in the animal, because it lowers the resistance of the respiratory tract to secondary bacterial infection.

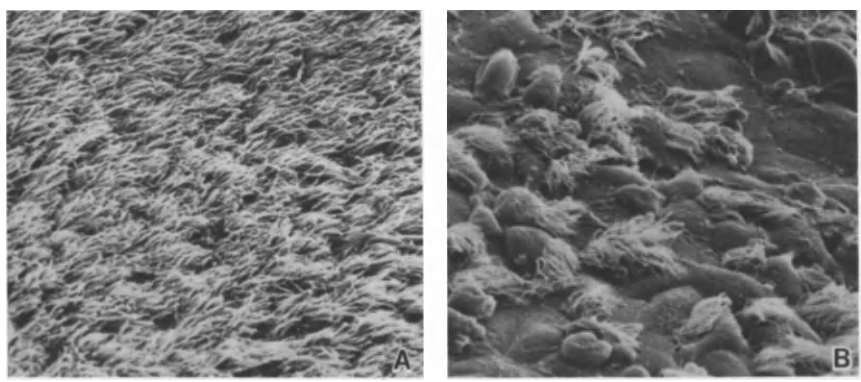

PLATE 6-2. *The direct cytopathic effect of rhinovirus on bovine tracheal epithelium, as shown by scanning electron microscopy of explant cultures. (A) Normal appearance of ciliated cells. (B) Six days after infection many cells are rounded up or detached. [From S. E. Reed and A. Boyde,* Infect. Immun. **6,** *68 (1972).]*

NEW ANTIGENS IN THE PLASMA MEMBRANE OF INFECTED CELLS

In many viral infections new virus-specified proteins are inserted into the plasma membrane of infected cells. For example, the plasma membrane of cells infected with enveloped RNA viruses (e.g., influenza virus, paramyxoviruses, and togaviruses) incorporates viral hemagglutinin, demonstrable by adsorbing red blood cells to their surface (*hemadsorption*). New virus-specified proteins appear in the plasma membrane quite early in the course of infection with many viruses.

Viral glycoproteins are not inserted at random in the plasma membrane. Cells in culture can be shown to be polar; their basolateral surface, in contact with the solid substrate, is different from their apical surface, which is in contact with the medium. In cultured canine kidney cells, influenza and parainfluenza viruses mature by budding from the free apical surface, but vesicular stomatitis virus (a rhabdovirus) and some retroviruses bud from the basolateral membrane. In each case the site of maturation is determined by the site of insertion of the viral glycoprotein into the plasma membrane, which is in turn a function specified by its nonglycosylated precursor. As discussed in Chapter 4, the precise nature of the "zip code" that determines the destination of any particular glycoprotein is unknown, but it may be of great functional significance *in vivo*. Viruses that mature at the apical surface of glandular epithelial cells are shed into the environment, whereas those maturing at the basolateral surface presumably move to other sites in the body, perhaps entering the bloodstream and establishing systemic infection. An example of this phenomenon *in vivo* is the polarized budding of rabies virus in the salivary gland, from the apical end of mucous epithelial cells into the salivary duct (see Plate 10-3).

Virus-coded antigens in the plasma membrane constitute a target for the body's specific immune mechanisms, both humoral and cellular, which may destroy the cell before significant numbers of new virions are produced and slow down the progress of the infection, hastening recovery. In some cases the host immune response may precipitate immunopathological disease (see Chapter 10). Herpesvirus-infected cells acquire a capacity to bind immunoglobulin nonspecifically, because of the synthesis of herpesvirus-coded proteins with the properties of Fc receptors in the plasma membrane; this, in turn, may lead to the destruction of the cell by antibody-dependent cellular cytotoxicity. Another class of new antigens found on cell membranes are the transplantation antigens, which are found in cells that are transformed by viruses (see below).

TABLE 6-2
Viruses That Transform Cells in Vitro, *and Induce Tumors* in Vivo

Family	Subfamily or genus[a]	Transformation *in vitro*	Tumor induction in natural host[b]	Viral genome	Production of virus
Papovaviridae	*Papillomavirus*	+ (a few species)	Papilloma (rarely carcinoma)	Episomal	+(papilloma) −(carcinoma)
	Polyomavirus	+	No, but carcinomas in newborn rodents	Integrated	−
Adenoviridae	*Mastadenovirus* *Aviadenovirus*	+	No, but carcinomas in newborn rodents	Integrated	−
Herpesviridae	*Gammaherpesvirinae*	+	Lymphoma, carcinoma	Episomal	−
Hepadnaviridae	*Hepadnavirus*	−	Carcinoma	Integrated	−
Retroviridae	*Oncovirinae*	+	Leukemia, sarcoma	Integrated	+

[a]Some members only, in most groups.
[b]See Chapter 12 and relevant chapters of Part II for further details.

TABLE 6-3
Characteristics of Cells Transformed in Vitro *by Viruses*

1. Greater growth potential *in vitro*
   (a) formation of three-dimensional colonies of randomly oriented cells in monolayer culture, usually due to loss of contact inhibition
   (b) Capacity to divide indefinitely in serial culture
   (c) Higher efficiency of cloning
   (d) Capacity to grow in suspension or in semisolid agar (anchorage independence)
   (e) Reduced serum requirement for growth
2. Altered cell morphology
3. Altered cell metabolism and membrane changes
4. Chromosomal abnormalities
5. Virus-specified antigens and DNA
   (a) New surface antigens (transplantation antigens)
   (b) New intracellular antigens (e.g., T antigens)
   (c) Some viral DNA sequences present, integrated with cellular DNA or as episomes
6. Capacity to produce malignant neoplasms when inoculated into isologous or severely immunosuppressed animals

CELL TRANSFORMATION

Viruses of several families can greatly change the growth characteristics of cultured cells, the process being called cell *transformation*. This phenomenon is correlated with the ability of the virus to induce tumors in animals (Table 6-2). Transformation by DNA viruses is always nonproductive (i.e., the transformed cells do not yield infectious progeny virus); transformation by retroviruses, on the other hand, is usually productive. Viral (or proviral) DNA in transformed cells is integrated into the cell DNA, except in the case of papillomavirus and herpesvirus DNAs, which remain episomal.

Transformed cells differ in many ways from normal cells (Table 6-3). One of the changes is an increased mitotic rate; transformed cells acquire a capacity to divide unrestrictedly, which can be demonstrated in a variety of ways (Plate 6-3), including the capacity to produce tumors in "nude" mice (which have defective cellular immunity, but do not support the growth of normal foreign cells).

Virus-Specific Antigens in Transformed Cells

Cells transformed by nondefective retroviruses express the full range of viral proteins, and new virions bud from their plasma membranes. In contrast, transformation by DNA viruses occurs only in cells undergoing

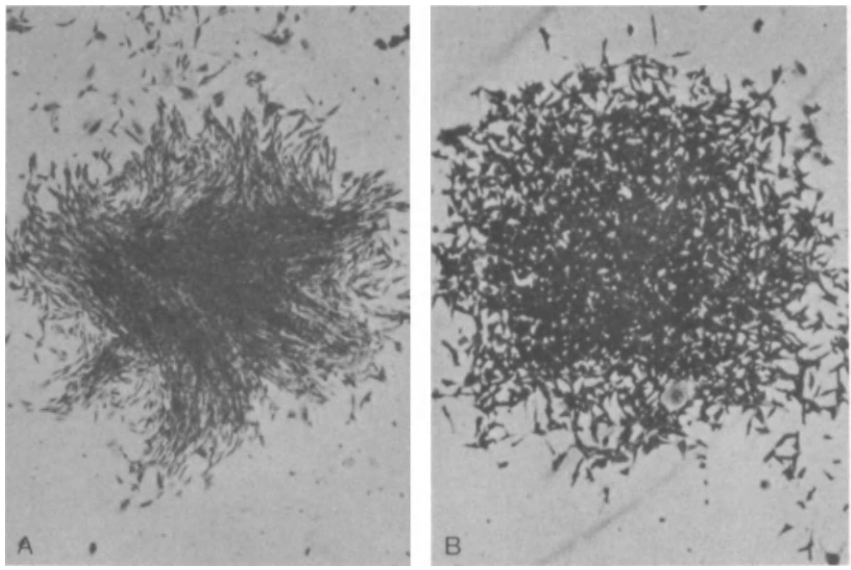

PLATE 6-3. *Transformation of cultured BHK21 cells by polyomavirus. (A) Normal colony, illustrating the regular parallel arrangement of elongated fibroblastic cells. (B) Transformed colony, illustrating the criss-cross random orientation of more rounded cells (×25). [From W. House and M. G. P. Stoker,* J. Cell Sci. **1,** *169 (1966).]*

nonproductive infection; nevertheless, certain virus-specific antigens are regularly demonstrable. *Tumor-associated transplantation antigens* are located in the plasma membrane, whereas the so-called *tumor (T) antigens* are usually found in the nucleus. The proteins that are collectively called the T antigens play an important role in transformation (see Chapter 12).

INTERFERENCE AND INTERFERONS

Viral *interference* is said to occur when a cell infected by one virus resists superinfection with the same or a different virus. The interfering virus does not necessarily have to replicate to induce interference, and the ability of the challenge virus to replicate may be completely or only partially inhibited. Two main mechanisms have been clearly demonstrated: (1) interference mediated by defective interfering mutants and operating only against the homologous virus (see Chapter 5), and (2) interference mediated by interferon.

The last category is the most important and has generated a vast amount of research since its discovery by Isaacs and Lindenmann in 1957. As this research was stimulated primarily by the possibilities of treating human viral infections and human tumors, much of the work has been done with human interferons, now produced by genetic engineering, but the principles learned are of general application. The importance of interferons in veterinary medicine is currently limited to their role in recovery from viral infections (see Chapter 8); studies are in progress to determine whether and how they might be used for treatment of animal diseases.

Interferons

Most virus-infected cells produce a set of proteins called *interferons,* which are released and react with uninfected cells so as to render them resistant to infection with viruses. Some 17 human interferons have been identified and characterized, and there are probably a comparable number in other animal species. They fall into three antigenically and chemically distinct types, known as α, β, and γ (Table 6-4). All mammalian species have complex families of genes encoding different subtypes of interferon α, and one or two interferon β genes (except in cattle, which have multiple genes for interferon β). Nonmammalian verte-

TABLE 6-4
Characteristics of Interferons[a]

Property	Type		
	α	β	γ
Produced by	Leukocytes, epithelia	Fibroblasts	Lymphocytes
Inducing agent	Viral infection or dsRNA	Viral infection or dsRNA	Mitogens (nonsensitized lymphocytes) Antigens (sensitized lymphocytes)
Number of subtypes	15	1	1
M_r (major subtypes)	15,000–25,000	20,000	20,000–25,000
Glycosylation	No[b]	Yes	Yes
Stability at pH 2	Yes[b]	Yes	No

[a]Data from human interferons.
[b]Most subtypes, but not all.

brates have interferon β genes but no interferon α genes. Interferon γ is not induced by viral infection per se, but by lymphocytes following antigen-specific (or mitogenic) stimulation. It is really a *lymphokine,* which is regularly produced during the response to viruses *in vivo* (see Chapter 9), but not by virus-infected cell cultures. Its functions are chiefly immunoregulatory, although it does have some antiviral and antitumor activity.

Interferons α and β are not made constitutively in significant amounts, but are induced by most or all viruses when they replicate, in virtually all vertebrate species. Most RNA viruses are good interferon inducers; most DNA viruses, except for poxviruses, are rather poor. Some interferons, especially β and γ, display a certain degree of host species specificity; e.g., rabbit interferons are ineffective in mice or humans. However, there is no viral specificity, e.g., interferons induced by a paramyxovirus are effective against a togavirus or any other sensitive virus. Nevertheless, it is likely that individual subtypes of interferon, purified following cloning by recombinant DNA technology, will be found to be more effective against some viruses than against others.

Biological Actions of Interferons. Interferon was discovered as an antiviral agent, defined accordingly, and generally regarded as such by virologists for two decades. It is now widely acknowledged that the interferons probably represent a family of "nonclassical hormones" which lead, under defined circumstances, not only to inhibition of viral replication, but also to modulation of the immune system and several additional phenomena.

The mechanism of antiviral action of interferon α and β has been elucidated, in part at least. Interferon binds to a specific receptor on the plasma membrane, thus triggering a cascade of biochemical events (Fig. 6-2). Three new enzymes are induced: (2′-5′) $(A)_n$ synthetase (often abbreviated to 2-5A synthetase), RNase L (endoribonuclease), and a protein kinase. By different mechanisms these enzymes inhibit protein synthesis in interferon-treated virus-infected cells.

It is also clear that interferons may stimulate or inhibit various arms of the immune response. One can think of interferons as lymphokines, induced principally by viral infection, and playing a key role in the regulation of the immunological response to that virus (see Chapter 9). Interferons secreted by virus-infected target cells, as well as by lymphocytes, macrophages, and NK cells, activate T_c lymphocytes, macrophages, and NK cells in the immediate vicinity to develop their cytotoxic potential, while also enhancing the expression of both class I and class II MHC antigens on the surface of the cells with which such

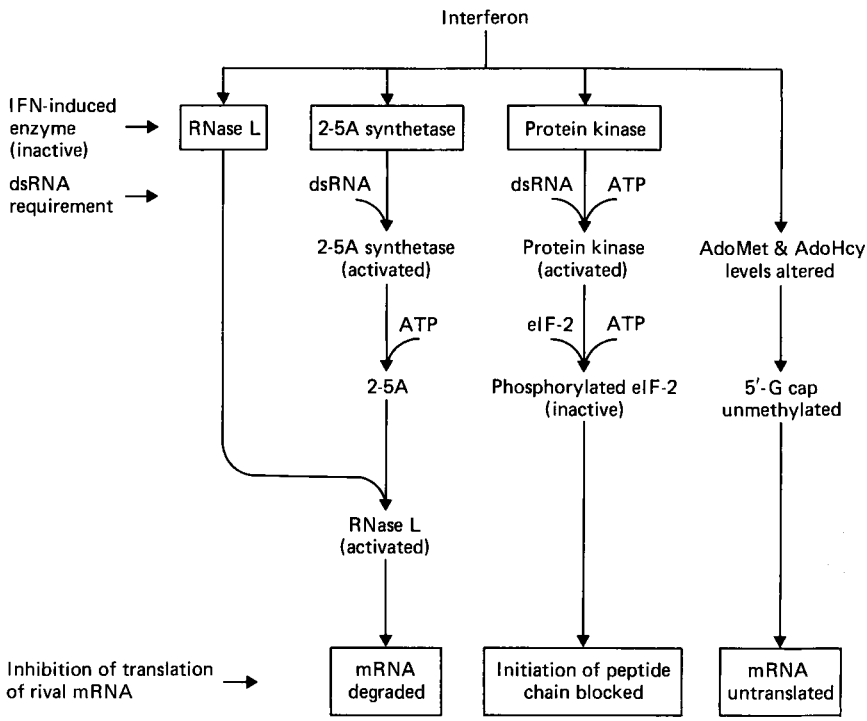

FIG. 6-2. *Antiviral action of interferon (IFN): postulated pathways (see text). (From D. O. White, "Antiviral Chemotherapy, Interferons and Vaccines." Karger, Basel, 1984.)*

leukocytes interact. In turn, these effector cells not only destroy the target but produce more interferon following contact with viral antigen on the cell surface. The consequential cascade greatly amplifies the lytic arm of the immune response. Yet it is apparent that other important arms of the immune response are depressed by interferon, perhaps reflecting the propensity of interferon to inhibit the replication of lymphocytes. A full analysis of this complicated issue must await careful *in vitro* studies of the various actions of purified preparations of each cloned subtype of interferon on cloned populations of well-characterized lymphocyte subtypes. Armed with that knowledge, it should then be feasible to go back to the whole animal to document the biological relevance of each of the diverse effects of various interferons.

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CHAPTER 7

Pathogenesis: Infection and the Spread of Viruses in the Body

At the molecular and cellular levels, viruses behave quite differently from bacteria and protozoa, but to some extent this distinction disappears when viruses are considered at the levels of the whole animal and the population. Viruses, like other infectious agents, must enter the animal body through one of its surfaces (or enter the fetus across the placenta or through the egg). They must then spread, either locally on one of the body surfaces, or through lymphatic and blood vessels to produce systemic infection. Finally, if the infection is to be maintained in nature, infectious virus must be shed into the environment, or taken up by an arthropod vector or a needle, or passed congenitally.

ROUTES OF ENTRY

In order to infect its host, a virus first attaches to and infects cells of one of the body surfaces: the skin, or the mucosa of the digestive, respiratory, or urogenital tract or the conjunctiva (Fig. 7-1). Parenteral injection, either by needle or by the bite of an arthropod or vertebrate, bypasses the body surfaces. A lesion may be produced at the site of entry, although invasion may occur without the development of any

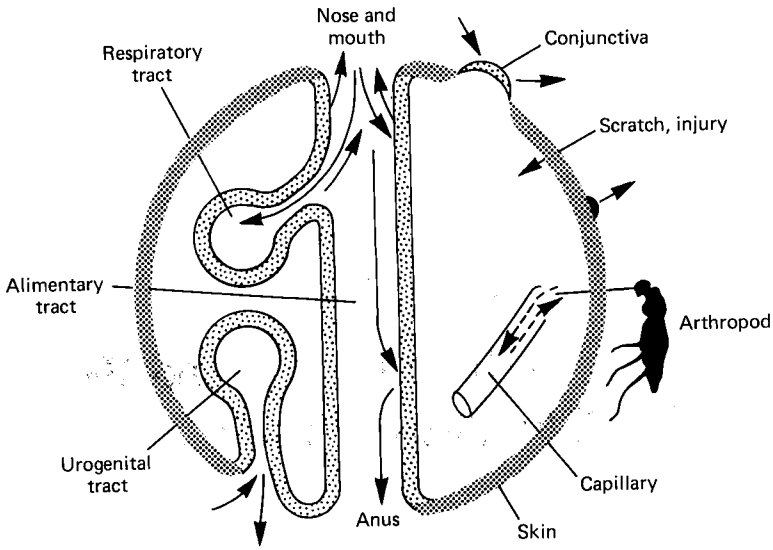

FIG. 7-1. *The surfaces of the body in relation to the entry and shedding of viruses. (Modified from C. A. Mims and D. O. White, "Viral Pathogenesis and Immunology." Blackwell Scientific Publications, Oxford, 1984.)*

local evidence of infection. The infection may remain localized in the skin, respiratory tract, or digestive tract, or it may spread through the animal via the lymphatics, blood vessels, or nerves.

Infection via the Skin

The largest organ in the body, the skin, provides a tough and impermeable barrier to the entry of viruses. However, some viruses replicate in the skin to produce local lesions, after entry through minor abrasions or by artificial puncture (Table 7-1). Several poxviruses—e.g., cowpox, orf, and fowlpox, and the papillomaviruses—cause local skin lesions. The most efficient way by which viruses are introduced through the skin is by the bite of an arthropod vector, such as a mosquito, tick, sandfly, or *Culicoides*. Such insects may be mechanical vectors (e.g., for myxomatosis and fowlpox), but most viruses introduced in this way replicate in the vector. Viruses that are transmitted by and replicate in arthropod vectors are called *arboviruses*, a term that carries no taxonomic implications. Infection can be acquired through the bite of an animal, as in rabies. Finally, introduction of a virus by skin penetration may be *iatrogenic*—the result of human intervention, e.g., transmission of

TABLE 7-1
Viruses of Animals That Initiate Infection via the Skin, Oral Mucosa, Genital Tract, or Eye

Route	Family or genus	Viruses
Minor abrasion	*Poxviridae*	Cowpox, swinepox, orf, bovine papular stomatitis, pseudocowpox, fowlpox viruses
	Herpesviridae	All viruses
	Papillomavirus	All viruses
	Picornaviridae	Swine vesicular disease virus
Arthropod bite		
Mechanical	*Poxviridae*	Fowlpox, swinepox, myxoma viruses
	Herpesviridae	Marek's disease virus
	Retroviridae	Equine infectious anemia virus
Biological	*Alphavirus*	All viruses
	Flaviviridae	All viruses
	Bunyaviridae	Rift Valley fever, Nairobi sheep disease viruses
	Rhabdoviridae	Bovine ephemeral fever, vesicular stomatitis viruses
	Orbivirus	Bluetongue, African horse sickness viruses
	Unclassified	African swine fever virus
Bite of vertebrate	*Rhabdoviridae*	Rabies virus
Contaminated needle or equipment	*Papillomavirus*	All viruses
	Togaviridae	Hog cholera, bovine virus diarrhea viruses
	Retroviridae	Equine infectious anemia, bovine leukemia viruses
Genital tract	*Herpesviridae*	Many viruses
	Papillomavirus	Bovine papilloma virus
	Togaviridae	Equine arteritis virus
Conjunctiva	*Herpesviridae*	Infectious bovine rhinotracheitis virus, equine herpesvirus 1

equine infectious anemia by a contaminated needle. Generalized infection of the skin (exanthema), such as is found in lumpyskin disease, sheeppox, and swine vesicular disease, for example, is due to viral spread via the bloodstream.

Infection via the Respiratory Tract

Though lined by cells that are susceptible to infection by many viruses, the respiratory tract is ordinarily protected by effective defense

TABLE 7-2
Some Viruses of Animals That Initiate Infection via the Respiratory Tract

Family	Viruses
Producing respiratory disease	
Herpesviridae	Most viruses
Adenoviridae	Most viruses
Parvoviridae	Feline panleukopenia virus, canine parvovirus
Picornaviridae	Rhinoviruses, aphthoviruses
Caliciviridae	Feline calicivirus
Orthomyxoviridae	Influenza viruses
Paramyxoviridae	Parainfluenza, respiratory syncytial viruses
Producing systemic disease, usually without initial respiratory signs	
Herpesviridae	Pseudorabies, bovine malignant catarrhal fever, Marek's disease viruses
Togaviridae	Hog cholera virus
Paramyxoviridae	Canine distemper, rinderpest, Newcastle disease viruses
Arenaviridae	Lymphocytic choriomeningitis virus

mechanisms. A mucus blanket and ciliary escalator line the nasal cavity and most of the lower respiratory tract. Thus, inhaled foreign particles, including viral particles, deposited on this surface are trapped in mucus and carried by ciliary action from the nasal cavity and airways to the pharynx and then swallowed. Particles greater than 10 μm in diameter are usually deposited on the nasal mucosa over the turbinate bones, which project into the nasal cavity and act as baffle plates. Particles 5–10 μm in diameter may be carried to the trachea and bronchioles, where they are usually trapped in the mucociliary blanket. Smaller particles (≤5μm) are usually inhaled directly into the lungs and some may reach the alveoli, where virions may be destroyed by alveolar macrophages, or may infect such cells, or cells of the adjacent alveolar epithelial lining.

Despite these protective mechanisms, the respiratory tract is, overall, the most important entry site of viruses into the body (Table 7-2). Not only is the respiratory tract susceptible to infection via aerosols or particles that are inhaled, but infection may occur via *fomites* (i.e., contaminated objects), a mode of transfer well exemplified by the role of fingers in transferring rhinoviruses between humans but doubtless operative in other animals also.

Many viruses remain localized in the respiratory tract, but some viruses that enter via the respiratory tract produce their principal effects

following systemic spread (e.g., foot-and-mouth disease, canine distemper, rinderpest, hog cholera, and Newcastle disease viruses).

Infection via the Digestive Tract

Many viruses, belonging to several families, are acquired by ingestion. They may either be swallowed or infect cells in the pharynx. The esophagus is rarely infected initially, probably because of its tough stratified squamous epithelium and the rapid passage of swallowed material over its surface. The intestinal tract is partially protected by mucus, which may contain secretory IgA antibodies, but the constant movement of the contents provides opportunities for virions to attach to specific receptors. Virions may also be taken up by specialized cells that overlie Peyer's patches in the ileum, from which they are passed to adjacent mononuclear cells in which they may be able to replicate.

There are other protective mechanisms in the intestinal tract, because substances involved in the digestion of food may also destroy viruses; from the stomach caudally, these are acid, bile, and proteolytic enzymes. In general, viruses that cause intestinal infection, such as enteroviruses, rotaviruses, caliciviruses, and parvoviruses (Table 7-3), are resistant to acid and bile. However, there are examples of acid- and bile-labile viruses that cause important intestinal infections. Bovine, porcine, and murine coronaviruses are protected by the buffering capacity of milk during passage through the stomach of young animals, and avian influenza viruses are assisted by the buffering capacity of food to survive passage through the upper digestive tract of birds. Because of the de-

TABLE 7-3
Some Viruses of Animals That Initiate Infection via the Intestinal Tract

Family	Viruses
Producing scours	
Coronaviridae	Some coronaviruses
Reoviridae	Rotaviruses
Unclassified	Astroviruses, toroviruses
Producing systemic disease, usually without local signs	
Adenoviridae	Some adenoviruses
Caliciviridae	Vesicular exanthema of swine virus
Picornaviridae	Enteroviruses

structive effects of acid, bile, and proteolytic enzymes on viruses, dosage is important in determining whether infection will occur.

Coronaviruses, rotaviruses, parvoviruses, and caliciviruses are now recognized as the major causes of viral diarrhea. The great majority of intestinal infections by enteroviruses and adenoviruses are asymptomatic. Some of the enteroviruses (e.g., porcine, avian, and murine encephalomyelitis viruses) are important causes of generalized infection but do not produce signs referable to the intestinal tract. Parvoviruses cause diarrhea after reaching cells of the intestinal tract via viremic spread.

Infection by Other Routes

The genital tract (in coitus) is the route of entry of several important pathogens, e.g., bovine herpesvirus 1, equine herpesvirus 3, and porcine papillomavirus. The conjunctiva, though much less resistant to viral invasion than the skin, is constantly cleansed by the flow of secretion (tears) and is wiped by the eyelids. The conjunctiva is unproven as a portal of entry of viruses in animals, although experimentally infection with a wide range of viruses can be achieved via this route.

MECHANISMS OF SPREAD IN THE BODY

Viruses may remain localized to the body surface through which they entered (i.e., skin, respiratory tract, intestine, genital tract, or conjunctiva), or they may cause generalized infections, which are usually associated with viremia and subsequent localization in particular organs.

Local Spread in Epithelia

Many viruses, e.g., poxviruses and papillomaviruses, replicate in epithelial cells at the site of entry, produce a localized or spreading infection in the epithelium, and are then shed directly into the environment. Infection spreads by sequential infection of neighboring cells. Papillomaviruses initiate infection in the basal layer of the epidermis, but maturation, with the production of virions, occurs only when the cells become keratinized as they move toward the skin surface. Since this is a slow process, taking several weeks, warts have a long incubation period. Many poxviruses produce infection via the skin. In contrast to the papillomaviruses, however, there is also subepithelial and lymphatic spread. In infection with vaccinia virus, which may become as common for domestic animals as it used to be in humans if vaccinia is used as a vector for veterinary vaccines (see Chapter 14), a few epidermal cells are

infected by scarification and virus spreads locally from cell to cell, primarily in the epidermis, before spreading to the local lymph nodes. Some poxviruses, e.g., orf, rabbit fibroma, and Yaba poxvirus, remain localized in the skin and produce a proliferative response and a localized tumor.

In contrast to the skin, viruses that enter the body via the mouth or respiratory tract can spread rapidly over the moist epithelial surfaces. After infections of the respiratory tract by paramyxoviruses and influenza virus, or the intestinal tract by rotaviruses or coronaviruses, there is little or no invasion beyond the epithelium. Although these viruses usually enter the lymphatics and thus have the potential to spread, they do not appear to replicate in the deeper tissues, possibly because the appropriate virus receptors are restricted to epithelial cells. However, restriction to an epithelial surface cannot be equated with lack of severity of clinical disease. Large areas of intestinal epithelium may be damaged by rotaviruses or coronaviruses, for example, causing severe diarrhea. The severity of localized infections of the respiratory tract depends on their location: infections of the upper respiratory tract may produce severe rhinitis but few other signs; infection of the bronchioles or alveoli produces more severe respiratory distress and may predispose to secondary bacterial invasion.

Subepithelial Invasion and Lymphatic Spread

After traversing the epithelium to reach the subepithelial tissues, virions are immediately exposed to tissue macrophages and can enter lymphatics (Fig. 7-2). Macrophages play an important role in controlling viral infections in many sites in the body and are discussed below; suffice it to say here that they phagocytose and destroy most viruses.

A network of lymphatics lies beneath the skin and all mucosal epithelia. Virions that enter lymphatics are carried to local lymph nodes. As they enter, they are exposed to macrophages lining marginal sinuses and may be engulfed. Virions may be inactivated and processed and their component antigens presented to adjacent lymphocytes in such a way that an immune response is initiated (see Chapter 9). Some viruses, however, replicate in macrophages (e.g., canine distemper virus, some adenoviruses, and some herpesviruses). Some virions may pass straight through lymph nodes to enter the bloodstream.

There is often a local inflammatory response, the extent of which depends on the extent of tissue damage. Local blood vessels are dilated and rendered more permeable, so that macrophages and lymphocytes, immunoglobulins, and complement components can be delivered di-

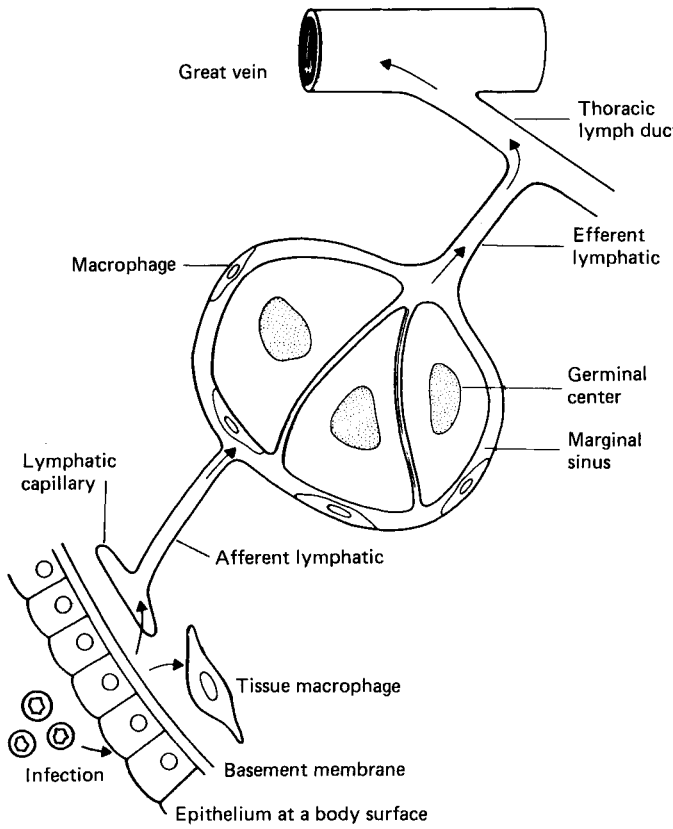

FIG. 7-2. *Subepithelial invasion and lymphatic spread of viruses. (From C. A. Mims and D. O. White, "Viral Pathogenesis and Immunology." Blackwell Scientific Publications, Oxford, 1984.)*

rectly to the site of infection, especially after the immune response has been initiated.

Spread by the Bloodstream

Once a virus has reached the bloodstream, usually via the lymphatic system (Fig. 7-2), it can localize in any part of the body within minutes. The presence of virus in the blood is called *viremia*. In some infections it is possible to distinguish a primary from a secondary viremia. A primary viremia follows directly after entry of virions via the lymphatics (Fig. 7-3); usually it is recognized only because of the invasion of distant

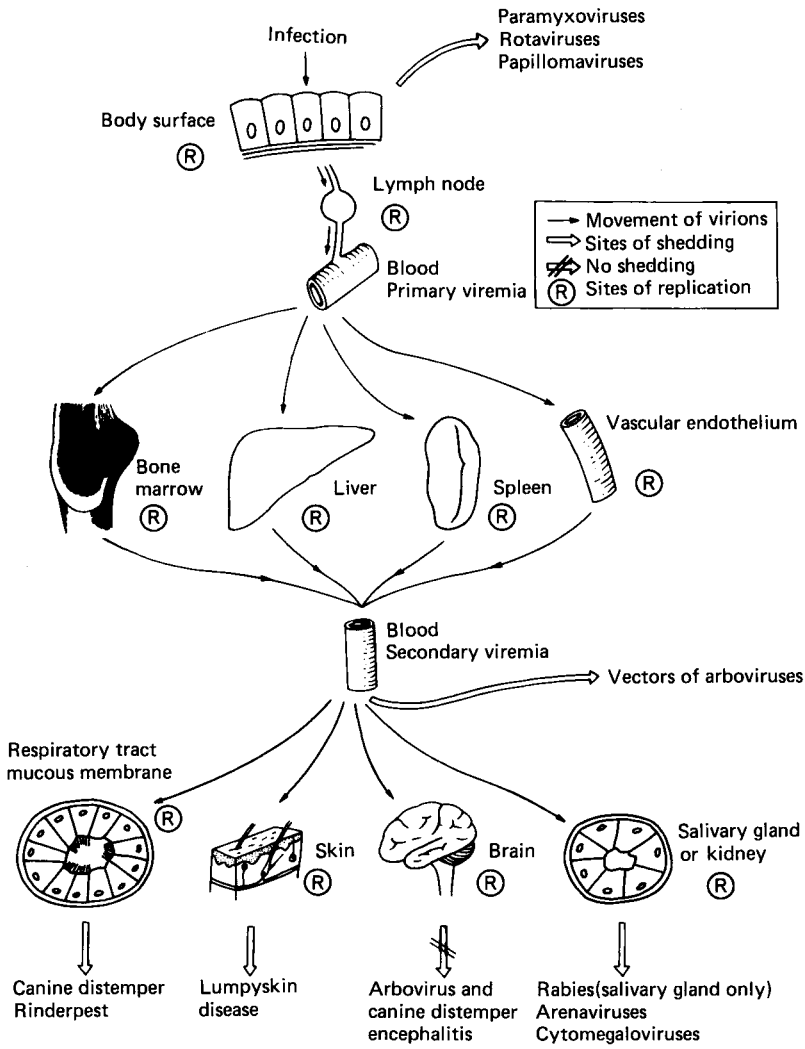

FIG. 7-3. *Spread of virions through the body, indicating sites of replication and important routes of shedding of various viruses.*

organs. Further replication in these sites leads to the sustained liberation of much higher concentrations of virus, producing a secondary viremia, which can in turn lead to the establishment of infection in yet other parts of the body.

In the blood, virions may be free in the plasma or may be associated

with particular types of leukocytes, with platelets, or with erythrocytes. Leukocyte-associated viremia is a feature of several types of infection, including canine distemper, bluetongue, and many herpesvirus infections, for example. As already mentioned, several viruses replicate in macrophages; others, e.g., Marek's virus, replicate in lymphocytes. Rarely, as in African swine fever and Colorado tick fever, virions may be associated with erythrocytes. All the togaviruses and flaviviruses, and the enteroviruses that cause viremia, circulate free in the plasma.

Virions circulating in the bloodstream encounter many kinds of cells, but two kinds play a special role in determining their subsequent fate and thus merit special consideration; macrophages and vascular endothelial cells.

Role of Macrophages. The term "reticuloendothelial system" is used to designate macrophages, fixed and circulating, throughout the body. These very efficient phagocytes are present in all compartments of the body, in alveoli, subepithelial tissues, sinusoids of the lymph nodes, free in plasma, and above all in the sinusoids of the liver, spleen, and bone marrow. The kinds of interactions that may occur between macrophages and virions can be described in relation to those found in the sinusoids of the liver, the Kupffer cells (Fig. 7-4; the numbers in the list refer to the circled numbers in this diagram).

1. Virions may resist phagocytosis by macrophages. This occurs in Venezuelan equine encephalitis virus infection, and is a factor that favors prolonged viremia.
2. Virions may be phagocytosed and destroyed. This is the usual fate of picornaviruses that circulate in the plasma. Since the macrophage system is so efficient, viremia with such viruses can be maintained only if virions enter the blood as fast as they are removed.
3. Virions may be phagocytosed and then passively transferred to adjacent cells (hepatocytes in the liver). If the virus cannot replicate in hepatocytes the situation resembles (2) above; if, like Rift Valley fever virus, the virus replicates in these cells it can cause clinical hepatitis and the virus produced in the liver can sustain a high level of viremia.
4. Virions may be phagocytosed by macrophages and then replicate in them. With some viruses, such as lactic dehydrogenase virus in mice, only macrophages are infected [Fig. 7-4 (4A)] and progeny virions enhance the viremia, which reaches an extremely high level. More commonly [Fig. 7-4 (4B)], as in infectious canine hepatitis, virus replicates in both macrophages and hepatic cells, producing severe hepatitis.

The situation *in vivo* is much more complex than this simple scheme suggests, since macrophages comprise several subpopulations that dif-

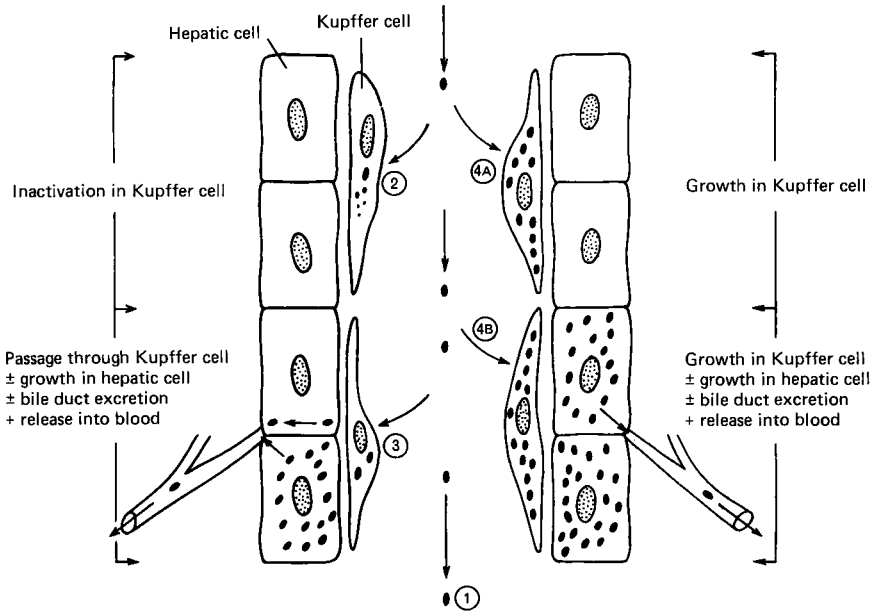

FIG. 7-4. *Types of interaction between viruses and macrophages, exemplified by the Kupffer cells lining a sinusoid in the liver. (Modified from C. A. Mims and D. O. White, "Viral Pathogenesis and Immunology." Blackwell Scientific Publications, Oxford, 1984.)*

fer in their properties according to their site in the body and depending on whether they have been activated.

Vascular Endothelial Cells. The vascular endothelium with its basement membrane and tight cell junctions constitutes the blood–tissue interface—for particles such as virions, often a barrier. Since most tissues and organs lack sinusoids, parenchymal invasion by circulating virions depends on localization in the endothelial cells of capillaries and venules, where blood flow is slowest and the barrier thinnest. Virions may move passively between or through endothelial cells and basement membrane, or they may infect endothelial cells and "grow" through this barrier. This subject has been most intensively studied in relation to viral invasion of the central nervous system (see below), but it also applies to secondary invasion of the skin, pulmonary epithelium, salivary gland epithelium, intestinal epithelium, kidney, and placenta.

Maintenance of Viremia. Since virions circulating in the blood are continuously removed by cells of the reticuloendothelial system, viremia

can be maintained only (1) if there is a continuing introduction of virus into the blood from infected tissues, or (2) if there is impairment of the reticuloendothelial system. Circulating leukocytes can themselves constitute a site for viral replication; indeed, blood leukocytes maintained in culture support replication of many viruses. However, viremia is usually maintained by infection of the parenchymal cells of organs with extensive sinusoids, like the liver, spleen, lymph nodes, and bone marrow. In some infections (e.g., hog cholera) the viremia is partly maintained by infection of endothelial cells. Striated and smooth muscle cells may be an important site of replication of some enteroviruses, togaviruses, and rhabdoviruses; virions are transferred to the blood via the lymph.

Invasion of the Skin

As well as being a site of initial infection, the skin may be invaded via the bloodstream, producing a rash. Rashes are more easily seen in humans than other animals, where fur or feathers usually restrict observation. The individual lesions in generalized rashes are described as macules, papules, vesicles, or pustules. A lasting local dilation of subpapillary dermal blood vessels produces a macule, which becomes a papule if there is also edema and infiltration of cells into the area. Primary involvement of the epidermis or separation of epidermis from dermis by fluid pressure results in vesiculation. Erosion or sloughing of the epithelium results in ulceration and scabbing, but prior to ulceration a vesicle may be converted to a pustule by cellular infiltration. More severe involvement of the dermal vessels may lead to petechial or hemorrhagic rashes, although coagulation defects and thrombocytopenia may also be important in the genesis of such lesions.

Invasion of the Central Nervous System

Because of its critical physiological importance and its vulnerability to damage by any process that damages neurons directly or via increased intracranial pressure, viral invasion of the central nervous system is always a serious matter. Viruses can spread from the blood to the brain by two routes: via the bloodstream (Fig. 7-5) or via peripheral nerve fibers (Fig. 7-6). When invasion occurs from the bloodstream, the early stages in pathogenesis (upper part of Fig. 7-5) are the same as in all generalized infections (see Fig. 7-3). Viruses can pass from the blood into the brain or cerebrospinal fluid at several anatomical sites and by several different mechanisms. Although the cerebral capillaries represent a morphological blood–brain barrier, most viruses that invade the central nervous system cross these vessels. Some viruses infect the vas-

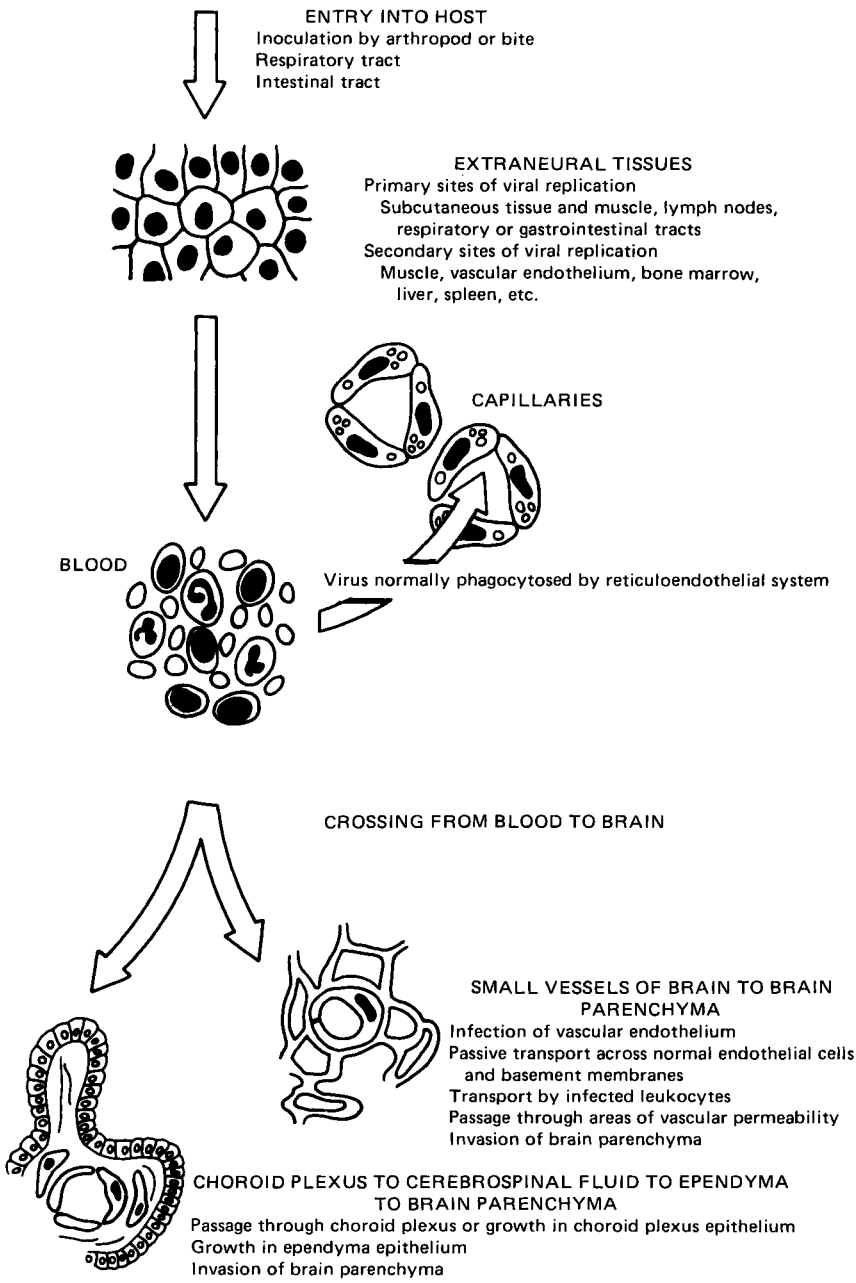

FIG. 7-5. *Steps in the hematogenous spread of virus into the central nervous system. (Modified from R. T. Johnson, "Viral Infections of the Nervous System." Raven Press, New York, 1982.)*

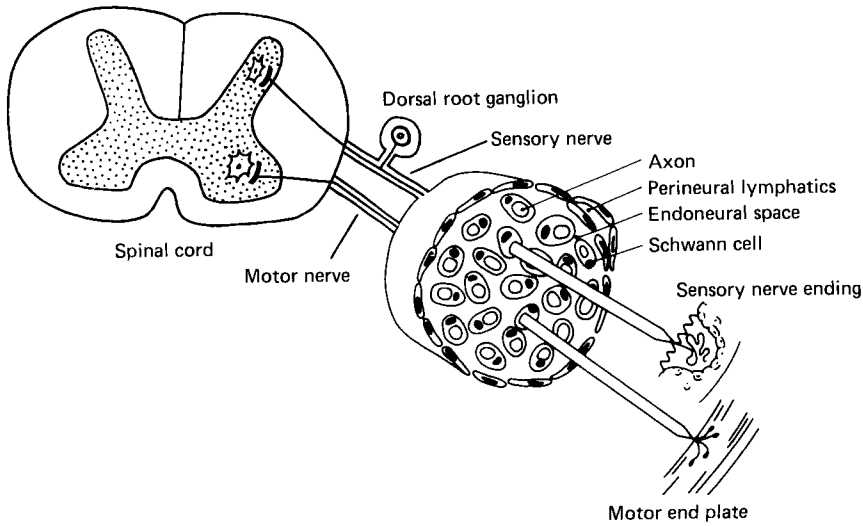

FIG. 7-6. *Neural pathways of central nervous system infection. Virions can be taken up at sensory, motor, or autonomic nerve endings and moved centripetally within axons, endoneural space, or perineural lymphatics, or by infection of Schwann cells. If transport is via axons, viruses taken up at sensory nerve endings will be delivered selectively to dorsal root ganglia neurons and then to the spinal cord; they may then move via the nerve tracts to the brain stem. Viruses taken up at motor nerve endings will be delivered to ventral nerve roots in the spinal cord and then to the brain stem. (Modified from R. T. Johnson, "Viral Infections of the Nervous System." Raven Press, New York, 1982.)*

cular endothelial cells prior to infection of the cells of the brain parenchyma; others appear to be transported across the capillary walls without endothelial cell infection. Rarely, virus may be carried across capillary walls into the brain parenchyma via infected leukocytes.

Viruses that cause meningitis rather than encephalitis may traverse the blood–cerebrospinal fluid junction in the meninges or choroid plexus or may grow in the epithelium of the choroid plexus, thus causing an alteration in the delicately balanced osmotic pressure of the interstitial spaces of the brain. Interstitial edema in the brain may, in turn, open the way to more extensive viral spread.

The other important route of infection of the central nervous system is via the peripheral nerves, as seen, for example, in rabies, herpes B virus encephalitis, and pseudorabies in secondary hosts (Fig. 7-6). Herpesvirus particles travel to the central nervous system in axon cytoplasm, but while doing so also sequentially infect the Schwann cells of

the nerve sheath. Rabies virus also travels to the central nervous system in axon cytoplasm, but usually does not infect the nerve sheath. Sensory, motor, and autonomic nerves may be involved in the neural spread of these viruses. Rabies and some togaviruses are able to use the olfactory nerve for movement to the central nervous system; they can infect olfactory neuroepithelial cells, which are exposed in the epithelium of the nares and have their axonal endings in the olfactory bulb of the brain.

Lytic infections of neurons, whether due to togaviruses, herpesviruses, or other viruses, lead to the three histological hallmarks of encephalitis: neuronal necrosis, phagocytosis of neurons by phagocytic cells (neuronophagia), and perivascular infiltration of inflammatory cells (perivascular cuffing)—the latter a manifestation of cell-mediated immunity (see Chapter 9). The cause of clinical neurological signs in other central nervous system infections is more obscure. Rabies virus infection is noncytocidal; it evokes little of the inflammatory reaction or cell necrosis found in other encephalitides, yet it is highly lethal for most mammalian species. With certain other viruses, infection of neurons causes no clinical signs; for example, the extensive central nervous system infection of mice congenitally infected with lymphocytic choriomeningitis virus, which is readily demonstrable by fluorescent-antibody staining, has no recognizable deleterious effect. Still other pathological changes are produced by some of the viruses that cause slowly progressive diseases of the central nervous system (see Chapter 11). In scrapie of sheep, for example, there is slow neuronal degeneration and vacuolization; in visna (a chronic retrovirus infection of sheep), changes in glial cell membranes lead to demyelination.

Invasion of Other Organs

Almost any organ may be infected via the bloodstream with one or another kind of virus, but most viruses have well-defined organ and tissue tropisms. The clinical importance of infection of various organs and tissues depends in part on their role in the economy of the body. Thus invasion of the liver, causing severe hepatitis, as in Rift Valley fever and infectious canine hepatitis, is a life-threatening situation. The critical importance of such organs as the brain, heart, and lungs is self-evident.

Infection of the testis or accessory sexual organs may lead to excretion of virus in the semen and the risk of transmission during coitus (see Table 7-5). The lungs may be infected via the bloodstream as well as the airways. Sometimes viremic infections reach the respiratory epithelium,

TABLE 7-4
Some Congenital Viral Infections in Domestic Animals

Animal	Viral family	Virus	Syndrome
Cattle	*Herpesviridae*	Infectious bovine rhinotracheitis virus	Fetal death, abortion
	Togaviridae	Bovine virus diarrhea virus	Fetal death, abortion, congenital defects, inapparent infection with lifelong carrier state and shedding
	Bunyaviridae	Akabane virus	Fetal death, abortion, stillbirth, congenital defects
	Retroviridae	Bovine leukemia virus	Inapparent infection, leukemia
	Reoviridae	Bluetongue viruses	Fetal death, abortion, congenital defects
Horse	*Herpesviridae*	Equine herpesvirus 1	Fetal death, abortion, neonatal disease
	Togaviridae	Equine arteritis virus	Fetal death, abortion
Swine	*Herpesviridae*	Pseudorabies virus	Fetal death, abortion
	Parvoviridae	Parvovirus	Fetal death, abortion, mummification, stillbirth, infertility
	Flaviviridae	Japanese encephalitis virus	Fetal death, abortion
	Togaviridae	Hog cholera virus	Fetal death, abortion, congenital defects, inapparent infection with lifelong carrier state
Sheep	*Togaviridae*	Border disease virus	Congenital defects
	Bunyaviridae	Rift Valley fever virus	Fetal death, abortion
		Nairobi sheep disease virus	Fetal death, abortion
	Reoviridae	Bluetongue virus	Fetal death, abortion, congenital defects
Dog	*Herpesviridae*	Canine herpesvirus 1	Perinatal death
Cat	*Parvoviridae*	Feline panleukopenia virus	Cerebellar hypoplasia
	Retroviridae	Feline leukemia virus	Inapparent, leukemia, fetal death
Mouse	*Parvoviridae*	Rat virus	Fetal death
	Arenaviridae	Lymphocytic choriomeningitis virus	Inapparent, with lifelong carrier state
Chicken	*Picornaviridae*	Avian encephalomyelitis virus	Congenital defects, fetal death
	Retroviridae	Avian leukosis–sarcoma viruses	Inapparent, leukemia, other disease

causing bronchitis and pneumonia, with shedding of virus in the respiratory secretions; such infections, like airways-derived infections, may cause an interstitial pneumonia.

Delivery of circulating virus to the salivary glands or mammary glands may lead to lesions in those organs and excretion in the saliva or milk. Infection of muscle cells occurs with several togaviruses and coxsackieviruses, while infection of the synovial cells of goats by caprine arthritis–encephalomyelitis virus produces arthritis.

Infection of the Fetus

Many viral infections of the dam have no harmful effect on the fetus, but some viruses regularly cross the placenta to reach the fetal circulation, sometimes after establishing foci of infection in the placenta. Severe cytolytic infections of the fetus cause fetal death and resorption or abortion, outcomes which are common in, for example, pseudorabies and parvovirus infections in swine. Also important are the teratogenic effects of less lethal viruses like bovine virus diarrhea virus and Akabane virus infections in cattle (Table 7-4).

Generally, infection in the first and second trimesters of gestation is most damaging. Little is known of the pathogenesis of most fetal infections, but experimental studies of bluetongue virus and Akabane virus infections in bovine fetuses, and parvovirus infections in swine fetuses have led to some general understanding. The source of the virus is most often from maternal infection with viremia and transplacental passage.

In the second half of pregnancy, the course of most fetal infections is influenced by the developing fetal immune response. For example, infections with porcine parvovirus after days 65–70 of gestation result not in fetal death but in fetal antibody production and recovery. When bovine virus diarrhea virus infects the fetus in the first half of gestation, immune tolerance with an inapparent, lifelong carrier state may develop; infection late in gestation usually leads to an effective immune response. When viral replication in the fetus is rapid, as in alphaherpesvirus infections of horse, cow, pig, and dog, fetal death and abortion can occur even during the last trimester, because the immune response is inadequate.

Germ-line transmission of the endogenous retroviruses, as integrated proviral DNA, occurs in many species of animals.

VIRUS SHEDDING

The shedding of infectious virions maintains infection in populations of animals (see Chapter 15, on epidemiology), and usually occurs via

one of the body openings or surfaces involved in the entry of viruses. With localized infections the same openings are involved in both entry and exit (see Fig. 7-1); in generalized infections a greater variety of modes of shedding is recognized (see Fig. 7-3).

Skin

Although skin lesions are produced in several generalized diseases, only a few viruses are shed from skin lesions in a way that leads to transmission. Virions are not excreted from the skin lesions associated with most picornavirus, togavirus, or flavivirus infections. Foot-and-mouth disease, vesicular stomatitis, poxvirus, and some herpesvirus infections, on the other hand, produce vesicular or pustular lesions from which virus is readily shed. Even here, however, virus shed in saliva and aerosols is often more important, as far as transmission is concerned, than that shed via the skin lesions.

The skin is an important source of virus in diseases in which transmission is by direct contact (rubbing) via small abrasions, e.g., papillomatosis. Several poxviruses may be spread from animal to human, and sometimes from human to animal, by contact with skin lesions, e.g., the viruses of cowpox, vaccinia, orf, and pseudocowpox. Localization of virus in the feather follicles is important in providing a mechanism for the shedding of Marek's disease virus from infected chickens.

Respiratory Tract

Many different viruses that cause either localized disease of the respiratory tract or generalized infections are shed in fluid expelled from the respiratory tract. Large droplets fall rapidly to the ground or may contaminate various fomites. Smaller droplets, with their relatively large surface-to-volume ratio, evaporate rapidly and are reduced to small droplet nuclei which remain airborne. Respiratory viruses, like infectious bovine rhinotracheitis virus, paramyxoviruses, some coronaviruses, and bovine respiratory syncytial virus, are excreted in both nasal and oral secretions. Shedding may continue to occur during convalescence or recurrently after that time, especially with herpesviruses.

Digestive Tract

Enteric viruses are shed in the feces and are in general more resistant to inactivation by environmental conditions than are the respiratory viruses, especially when suspended in water, e.g., in dams and troughs contaminated with feces. Thus, unlike the respiratory viruses, which

TABLE 7-5
Some Viruses Excreted in the Semen of Domestic Animals

Family	Virus	Animal	Comments
Herpesviridae	Infectious bovine rhinotracheitis virus	Cattle	Virus persists in ganglia, reinfecting genital tract with excretion in semen intermittently
Retroviridae	Mammary tumor virus	Mouse	Virus transmitted via sperm in some strains of mice
	Bovine leukemia virus	Cattle	Virus persists in lymphoid tissues and leukocytes, with excretion in semen continuously
Reoviridae	Bluetongue viruses	Cattle	Virus infects leukocytes and is excreted in semen continuously or intermittently as long as bull is viremic
Picornaviridae	Foot-and-mouth disease virus	Cattle	Virus infects genital tract with excretion in semen

usually spread directly from infected to susceptible animals, enteric viruses can persist for some time outside the body, especially as some, like rotaviruses and enteroviruses, are relatively heat stable.

Genital Tract

There may be transfer of virus from male to female and vice versa during coitus, and several viruses that cause important diseases of cattle and sheep are excreted in the semen (Table 7-5). Artificial insemination and embryo transfer are being promoted to avoid the introduction of sexually transmitted viruses into herds, as well as to avoid the risk of introduction of exotic viruses into disease-free countries. Knowledge of the transmission characteristics of viruses in semen and ova is extremely important in this regard.

Other Routes of Shedding

Urinary Tract. Urine, like feces, tends to contaminate food supplies and housing. A number of viruses, e.g., rinderpest, infectious canine hepatitis, and foot-and-mouth disease viruses, replicate in tubular epithelial cells in the kidney and are shed in the urine. Viruria is lifelong in

arenavirus infections of rodents and constitutes the principal mode of contamination of the environment by these viruses.

Milk. Several kinds of viruses are excreted in milk, which may serve as a route of transmission of caprine arthritis–encephalitis virus, mouse mammary tumor virus, and some of the tick-borne flaviviruses. Foot-and-mouth disease virus can be spread by feeding calves with infected milk.

Blood and Internal Organs. Blood is important as the source from which arthropods acquire arboviruses, and blood may also be the route of transfer of viruses to the ovum or fetus. Equine infectious anemia virus and bovine leukemia virus are often transmitted by needles and other equipment contaminated with blood. Carnivores and omnivores may be infected by consuming virus-containing meat; e.g., hog cholera, African swine fever, and vesicular exanthema of swine viruses are often transmitted to swine that eat garbage containing contaminated pork scraps.

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CHAPTER 8

Determinants of Host Resistance

Although some viruses can infect and cause disease in many species (i.e., they have a wide host range), many are host specific. Furthermore, within a susceptible host species there is often a striking difference between individual animals in their levels of resistance. What are the determinants of host susceptibility or resistance? Clearly, they are multifactorial. Within susceptible species, resistance varies not only with the genetic constitution of the host, but also with age, nutritional status, stress, and many other factors. Together, these genetic and physiological factors determine what is called the "nonspecific" or "innate" resistance of the host, in contrast to the immunologically specific resistance that results from the operation of the immune response, which is described in Chapter 9.

HOST SUSCEPTIBILITY AND VIRAL VIRULENCE

Susceptibility to infection, or its reciprocal, resistance, can be measured by determining the dose of virus required to cause infection or death in 50% of the test group: the 50% infectious dose (ID_{50}) or the 50% lethal dose (LD_{50}) (see Chapter 3). Different strains of inbred mice may vary many thousandfold in their susceptibility or resistance to a given

virus. The severity of an infection depends on the interplay between the virulence of the virus and the resistance of the host. One can regard an acute infection as a race between the ability of the virus to replicate, spread in the body, and cause disease, and the ability of the host to restrict and control these events. A highly virulent strain of virus is less lethal for a highly resistant animal than for a susceptible animal; conversely, a relatively avirulent strain of virus may be lethal for an unusually susceptible animal.

The variability in the response of individual animals to infection with a given virus is regularly observed during epizootics; for example, during an outbreak of Venezuelan equine encephalitis, one horse may die, another may merely develop a febrile disease, and a third may have a completely subclinical infection, the only evidence of which is a sharp rise in antibody and lifelong immunity to reinfection. The dose of infecting virus may be influential, but this is by no means the only factor. Both genetic and physiological factors can influence the outcome of exposure to a virus.

GENETIC DETERMINANTS

Genetic differences in susceptibility are most obvious when different animal species are compared. Common viral infections often tend to be less pathogenic in their natural host species than in certain exotic or introduced species. For instance, foot-and-mouth disease virus causes a severe disease in European cattle, but none in the African buffalo. Donkeys are more resistant to African horse sickness virus than are horses or mules, while zebras are refractory.

Accurate genetic data on resistance to infection is almost unobtainable in many species, because genetic, physiological, and environmental differences are generally confounded. Using inbred strains of mice, however, it has been possible to study the genetics of resistance to viral infection in some detail. For example, susceptibility to certain flaviviruses and to mouse hepatitis virus (a coronavirus) is under the control of a single gene which determines the capacity of macrophages to support the growth of virus.

Cellular Receptors

In a few instances it has been shown that the susceptibility of animals is dependent on the presence of the appropriate cellular receptor for the particular virus on cells of key target organs. The susceptibility of differ-

ent strains of chickens to Rous sarcoma virus is attributable to a single gene that codes for a cellular receptor; susceptibility is dominant.

Human polioviruses provide an example of the importance of cellular receptors at the species level. These viruses ordinarily infect only primates; mice and other nonprimates are not susceptible because their cells lack appropriate receptors. However, poliovirus RNA, when introduced into mouse cells *in vivo* or in culture, can undergo a single cycle of replication. Since progeny virions from such an artificial infection face mouse cells lacking receptors, they are unable to initiate a second cycle of replication.

Immune Response Genes

Immunological responsiveness to particular antigens differs greatly from one strain of mouse to another, being under the control of specific *immune response (Ir) genes*. There are many of these genes, most of them situated in the region known as the major histocompatibility complex (MHC) (see Chapter 9). Most other genetic determinants of virus susceptibility are not directly related to the immune response and map outside the MHC locus. Individuals with a genetically determined poor immune response to neutralizing epitopes on the surface proteins of a given virus would presumably have difficulty in controlling infection with that particular virus. In the mouse at least, absence of a specific response is generally recessive. Susceptibility of mice to infection with cytomegaloviruses, retroviruses, and lymphocytic choriomeningitis virus has been shown to be linked to particular MHC genotypes. Some breeds of domestic animals (e.g., sheep) are so inbred that particular viral susceptibility and resistance patterns have been found to be associated with specific immune responsiveness patterns.

PHYSIOLOGICAL FACTORS

Malnutrition

Malnutrition can interfere with any of the mechanisms that act as barriers to the replication or progress of viruses through the body. It has been repeatedly demonstrated that severe nutritional deficiencies will interfere with the generation of antibody and cell-mediated immune responses, with the activity of phagocytes, and with the integrity of skin and mucous membranes. However, often it is impossible to disentangle adverse nutritional effects from other factors such as poor husbandry.

Moreover, just as malnutrition can exacerbate viral infections, so viral infections can exacerbate malnutrition, thus creating a vicious cycle.

Age

The high susceptibility of newborn animals to many viral infections is of considerable importance in livestock husbandry. It can also be exploited for the laboratory diagnosis of viral diseases. Before cell culture techniques became available, foot-and-mouth disease virus isolation, titration, and neutralizing antibody assays were carried out in suckling mice. Infant mice are still useful for the isolation of togaviruses, flaviviruses, bunyaviruses, and rhabdoviruses.

In laboratory animals the first few weeks of life are a period of very rapid physiological change. For example, during this time mice pass from a stage of immunological nonreactivity (to many antigens) to immunological maturity. This change profoundly affects their reaction to viruses like lymphocytic choriomeningitis virus, which induces a persistent tolerated infection when inoculated into newborn mice, but an immune response in mice infected when over a week old. Most domestic animals are reasonably mature immunologically at the time of birth, but still very susceptible to infection with those viruses against which their dam has no antibody. If the umbrella of maternal antibody usually provided in mammals through colostrum or transplacental transfer is missing, the newborn animal is particularly vulnerable to infections with viruses such as canine distemper virus, canine parvovirus, hog cholera virus, bovine virus diarrhea virus, enteropathogenic coronaviruses, rotaviruses, and various herpesviruses during the first few weeks of life.

In humans, there are viruses that tend to produce more severe disease in adults than in children. For example, varicella virus, usually the cause of an uncomplicated disease in children, may produce severe pneumonia in adults; and mumps in adults may be complicated by orchitis. There are few parallels in domestic animals, but one example is bovine virus diarrhea virus, which generally infects calves subclinically, whereas older animals have a higher probability of developing clinical disease (see Chapter 25).

Hormones, Pregnancy, and Stress

There are few striking differences in the susceptibility of males and females to viral infections (except in the obvious instances of viruses with a predilection for tissues such as testes, ovaries, or mammary glands). Pregnancy significantly increases the likelihood of severe disease follow-

ing infection with certain viruses, e.g., Rift Valley fever virus in sheep. Herpesvirus infections are often reactivated during pregnancy, contaminating the birth canal and leading to infection of the newborn.

The therapeutic use of corticosteroids exacerbates many viral infections; e.g., infections with infectious bovine rhinotracheitis, pseudorabies, or equine herpesvirus 1 viruses are often more severe in domestic animals receiving corticosteroids. The precise mechanism is not understood, but corticosteroids reduce inflammatory and immune responses and depress interferon synthesis. It is also clear that adequate levels of these hormones are vital for the maintenance of normal resistance to infection. The stress of overcrowding and long-distance transport is believed to contribute to shipping fever in cattle via adrenocortical immunosuppression (see Chapter 10).

Fever. Almost all viral infections in domestic animals are accompanied by fever. The principal mediator of the febrile response appears to be the macrophage product, interleukin-1 (previously known as endogenous pyrogen). Interleukin-1 is induced by immunological mechanisms, e.g., generalized antigen–antibody and cell-mediated immune reactions. It is found in inflammatory exudates and acts on the temperature-regulating center in the anterior hypothalamus. Interferons are also pyrogenic when present in sufficiently high concentration; their antiviral and immunomodulatory functions are discussed below.

Fever profoundly disturbs body functions. The increased metabolic rate, by increasing the metabolic activity of phagocytic cells and the rate at which inflammatory responses are induced, might be expected to exert antiviral effects. *In vitro* experiments have shown that antibody production and T-cell proliferation induced by interleukin-1 are greatly increased when cells are cultured at 39°C rather than at 37°C. Furthermore, when fever was prevented in animals experimentally infected with vaccinia virus or influenza virus, the ensuing disease was more severe and very much more virus was excreted.

Lwoff suggested many years ago that fever constitutes a natural defense against viruses, and that virulent strains of virus have evolved with the ability to replicate in the host at temperatures achieved during fever (indeed, latent infections with some herpesviruses are actually reactivated by fever, hence the synonym "fever blisters" for recurrent herpes simplex in humans). It was subsequently suggested that temperature-sensitive (*ts*) viral mutants might therefore be expected to be less virulent, and this correlation has now been observed with *ts* mutants of many viruses, some of which are being used as vaccines (see Chapter 14).

INTERFERONS

Interferons are proteins that are induced in virus-infected cells and interfere with the replication of viruses. Their properties and mode of action were described in Chapter 6; here we consider their role in the animal.

Production and Distribution *in Vivo*

It is difficult to determine which cell types, or even which tissues and organs, are responsible for most interferon production *in vivo,* but, extrapolating from findings with cultured cells, one can probably assume that most cells in the body are capable of producing interferons in response to viral infection. Certainly, interferons can be found in the mucus bathing epithelial surfaces such as the respiratory tract, and interferon is produced by most or all cells of mesenchymal origin. Lymphocytes, especially T cells, NK cells, and K cells, as well as macrophages, produce large amounts of interferons α and γ, and probably comprise the principal source of circulating interferon in viral infections characterized by a viremic stage.

Role in Recovery from Viral Infections

There are data supporting a central role for interferons in the recovery of animals and humans following at least some viral infections. The most telling evidence that interferon can indeed be instrumental in deciding the outcome of a natural viral infection is that mice infected with any of several nonlethal viruses, or with sublethal doses of more virulent viruses, die if antiinterferon serum is administered. In general, however, we know very little about the relative importance of the various interferons. While it is widely postulated that interferons constitute the first line of defense in the process of recovery from viral infections, it would be naive to believe that they are the only, or even the most important factor. If this were so, one might expect that a systemic infection with any virus, or indeed, immunization with a live vaccine, might protect an animal, for a period at least, against challenge with an unrelated virus. While some experimental data suggest that this may occur, the phenomenon cannot be generally demonstrated. Evidence is somewhat stronger that infection of the upper respiratory tract with one virus will provide temporary local protection against others. Perhaps this distinction provides the clue; the direct antiviral effect of interferons is limited in both time and space. Their main antiviral role may be to protect cells in the immediate vicinity of the initial focus of infection.

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CHAPTER 9

The Immune Response to Viral Infections

Vertebrates differ from other organisms in having evolved the capacity to respond in a highly specific way to foreign macromolecules by means of what is called the immune system. Foreign macromolecules, including viral components, that activate this system are called *antigens;* immunoglobulins which recognize these antigens specifically are called *antibodies.* There is a parallel cellular system in which antigenic stimulation leads to the selection and activation of specific lymphocytes; this is called *cell-mediated immunity.*

The immune response, if rapid in onset, may terminate some viral infections before much damage has been done. Consequently, many viral infections are mild or even *subclinical*—they produce few or no signs of disease. However, for a variety of reasons the immune system does not always function effectively. Some viruses are almost always lethal for particular hosts, others establish persistent infections (see Chapter 11), and sometimes the immune response is actually harmful, causing tissue damage in vital organs (see Chapter 10). This chapter deals with the role of the immune response in recovery from viral infection and resistance to reinfection.

CELLULAR AND HUMORAL COMPONENTS OF THE IMMUNE SYSTEM

Classically, the immune response was considered to have two arms: (1) the antibody response, referred to as *humoral immunity*, and (2) a T lymphocyte-mediated response, known as *cell-mediated immunity*. Today, the distinction is blurred by the realization that cells, including but not exclusively T lymphocytes, are involved in the generation of both arms of the immune response. Indeed, a cardinal feature of the immune response is the interdependence of its parts, all acting cooperatively. This interdependence operates via soluble mediators and by direct contact between subsets of cells, in an intricate and precisely controlled network.

Seven principal kinds of lymphocytes (four types of T cells, B cells, K cells, NK cells), as well as macrophages (including dendritic cells), each interact with virions, viral antigens, or virus-infected cells. Lymphocytes, with their specific surface receptors, are the key to immunological specificity. Any given T or B lymphocyte possesses receptors with specificity for a particular *epitope (antigenic determinant)*. When antigen binds to their antigen receptors, both T and B lymphocytes respond by differentiating into lymphoblasts, which divide to form an expanded clone of cells (clonal expansion). Eventually some of these cells revert to small lymphocytes, which are long-lived and are involved with *immunological memory*. B lymphoblasts (plasma cells) secrete specific antibody, whereas T lymphoblasts secrete antigen-specific and nonspecific soluble factors known as *lymphokines*. Furthermore, unlike B cells, most T cells cannot bind soluble antigen; they recognize a foreign antigen only when it is presented in association with "self" MHC (major histocompatibility) antigen on the surface of another cell (Fig. 9-1).

Our knowledge of cellular immunology, in particular, is heavily dependent on studies in mice, while among larger mammals more detailed and extensive work has been carried out in humans than in most domestic animals. Hence principles described in this chapter often draw on experiments with mice and observations in humans; where investigated, counterparts have been found in domestic animal species.

T Lymphocytes

T lymphocytes, so named because of their dependence on the thymus for their maturation from pluripotent hematopoietic stem cells, are distinguishable from B lymphocytes (a term derived from the bursa in birds or its equivalent, the bone marrow, in mammals) not only by their

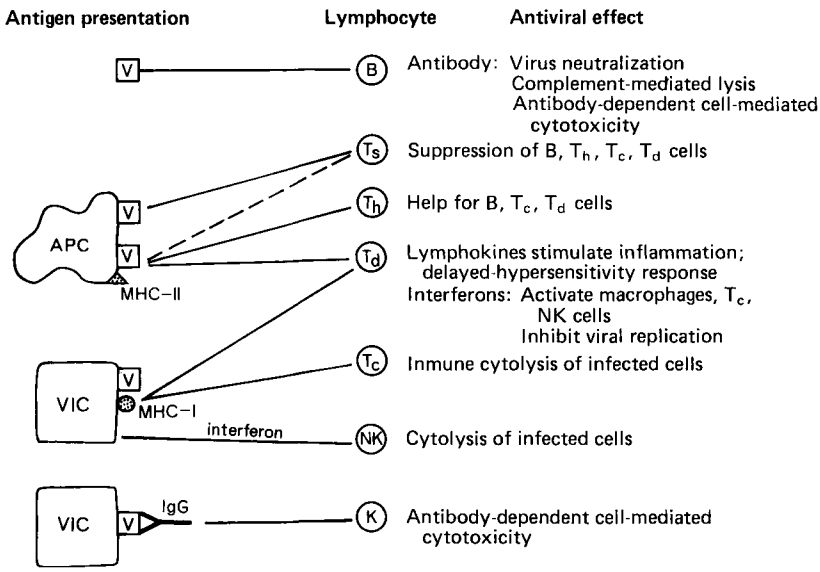

FIG. 9-1. *Cells involved in antiviral immune responses. V, Virus or viral antigen; APC, antigen-presenting cell (macrophage or dendritic cell); VIC, virus-infected cell. MHC-I, MHC-II, MHC antigens of class I and II, respectively. T_s, Suppressor; T_h, helper; T_d, delayed hypersensitivity; T_c, cytotoxic T lymphocytes. NK, Natural killer cell. K, Killer cell. (Modified from C. A. Mims and D. O. White, "Viral Pathogenesis and Immunology." Blackwell Scientific Publications, Oxford, 1984.)*

different antigen receptors, but also by other surface markers and by functional differences. Functionally, T lymphocytes are classified into four subsets. *Helper* (T_h) and *suppressor* (T_s) lymphocytes are regulator cells; *cytotoxic* (T_c) and *delayed hypersensitivity* (T_d) lymphocytes are effector cells (Table 9-1). Recent evidence with T cell clones indicates that a single cell type can discharge more than one of these functions and secrete a range of different lymphokines, but that it may recognize a particular epitope only in association with only a particular class of MHC antigen on the surface of the cell (see Fig. 9-1).

Helper T (T_h) cells recognize viral antigen in association with class II MHC glycoprotein, usually on the surface of a macrophage, dendritic cell, or B cell. They secrete both antigen-specific and nonspecific substances known as helper factors. Various subsets of T_h cells activate B cells, T_d cells, and T_c cells, respectively.

Cytotoxic T (T_c) cells generally recognize viral antigen in association

TABLE 9-1
Subsets of T Lymphocytes

Subset	MHC restriction	Function
Helper (T_h)	Class II	Help for B, T_c, or T_d lymphocytes
Suppressor (T_s)	Nil?, Class II?	Suppression of B, T_h, T_c, or T_d lymphocytes
Cytotoxic (T_c)	Class I, usually	Cytolysis of virus-infected cells
Delayed hypersensitivity (T_d)	Class I or II	Delayed hypersensitivity

with class I MHC glycoprotein; they lyse cells that have viral antigen on their surface.

Delayed hypersensitivity T (T_d) cells recognize antigen in association with either class I or class II MHC glycoprotein. T_d cells secrete a variety of lymphokines which attract and activate macrophages and other T cells, so greatly augmenting the immune response.

Suppressor T (T_s) cells are the least well-characterized class of T cells. They can probably bind viral antigen directly, after it has been processed by an antigen-presenting cell; sometimes they recognize viral antigen only in association with class II MHC glycoprotein. They secrete suppressor factors which regulate the immune response by suppressing the activity of B, T_d, T_h, or T_c cells, normally after they have fulfilled their function and are no longer needed.

The effector response is generally transient; e.g., T_c and T_d activities usually peak about 1 week after the onset of a viral infection, and disappear by 2 to 3 weeks. This may be attributable to the destruction of infected cells and removal of the antigenic stimulus. It is also probable that such immunoregulation is a function of T_s cells, which are first demonstrable at about the time other cell types decline in number. Memory T_c, T_d, and T_h cells persist for years.

T cells act principally via the lymphokines which they secrete following antigen binding. Some of these are antigen-specific factors, others nonspecific (in their action, though specific in their induction). Among many T cell-derived nonspecific lymphokines that have been described are (1) interferons α and γ, which are important immunomodulators as well as antiviral agents, (2) interleukin-2, a mitogen that promotes the clonal expansion of antigen-activated T lymphocytes, and (3) B cell growth factor, which amplifies clones of B cells following their activation.

B Lymphocytes

Some of the pluripotent hematopoietic stem cells originating from fetal liver and later from bone marrow differentiate into immature B cells, which are characterized by the presence on their surface of specific antigen-binding receptors, receptors for complement (C3), and receptors for the Fc portion of immunoglobulin. On encounter with antigen, the particular clones of B cells bearing receptors complementary to any of the several epitopes on that antigen, bind it and, after receiving the appropriate antigen-specific and nonspecific signals from T_h cells, respond by division and differentiation into antibody-secreting plasma cells. B cells may also process antigen and present peptides, in association with class II MHC antigen, to T cells, which respond by secreting helper factors.

Each clone of plasma cells secretes antibody of only a single specificity and avidity, corresponding to the particular receptors it expresses. Early in the immune response, when large amounts of antigen are present, there is an opportunity for antigen-reactive B cells to be triggered even if their receptors fit the epitope with relatively poor *affinity;* the result is the production of antibody which binds the antigen with low *avidity.* Later on, when only small amounts of antigen remain, B cells with receptors that bind the antigen with high affinity are selected, hence the avidity of the antibody secreted increases correspondingly. On reexposure to the same antigen, even years later, memory cells respond promptly with renewed multiplication and the production of larger amounts of specific antibody, mainly of the IgG class, after a delay of only a day or two.

Natural Killer (NK) Cells

These are immunologically nonspecific lymphocytes that have the capacity to kill virus-infected cells and tumor cells. The basis of their selectivity for virus-infected cells is not known. They do not specifically recognize viral and/or MHC antigens, nor do they have Fc receptors. They may be an important early defense mechanism, since their activity is greatly enhanced within 1 or 2 days of infection with viruses. Virus-induced activation is mediated by interferon.

K Cells

These are non-B, non-T lymphocytes that have receptors for the Fc of IgG molecules and bind only to target cells to which IgG antibodies are attached. They play a role in *antibody-dependent cell-mediated cytotoxicity.*

Macrophages

Cells of the macrophage–monocyte lineage are a diverse group of cells, and macrophages in different parts of the body, or even different subpopulations at one site, may have quite distinct antiviral properties. In Chapter 7 we discussed the important role played by macrophages in disposing of viruses (see Fig. 7-4). They also play a central role in the immune response to viruses, notably in antigen presentation (see Fig. 9-1); only viral antigen presented in association with the appropriate MHC antigen (generally class II) on the surface of a fixed tissue macrophage, *dendritic cell,* or Langerhans cell is recognized by T_d and T_h cells. As a class these cells are called *antigen-presenting cells.* Unlike typical macrophages, dendritic cells are nonphagocytic and lack Fc and C3 receptors; in other respects, including morphology, they resemble macrophages and express large amounts of class II MHC antigen. Indeed, the central requirement of an antigen-presenting cell is that it must carry class II glycoprotein.

Macrophages secrete soluble factors (*monokines*), one of which, *interleukin-1,* serves as an essential signal for the initiation and amplification of all T cell-dependent immune responses and the inflammatory response. Interferon α (see Chapter 6) can be regarded as another monokine, in that macrophages are one of the major (but not the only) source of this important class of antiviral and immunomodulating substances.

Macrophages play a role in antibody-dependent cell-mediated cytotoxicity, since their Fc receptors bind IgG nonspecifically. Under some circumstances macrophages may destroy virus-infected cells directly.

Antibodies

The end result of the activation of B cells is the production of antibodies, which react specifically with the epitope that stimulated their production. They fall into four main classes: IgG, IgM, IgA, and IgE. All immunoglobulins of a particular class have a similar structure, but differ widely in the amino acid sequence comprising the antigen-binding sites, reflecting the great variety of epitopes that have stimulated their production.

The basic structure of the commonest immunoglobulin found in serum, IgG, is shown in Fig. 9-2. Each molecule consists of two "heavy" and two "light" chains, and each chain consists of a "constant" and a "variable" domain. The chains are held together by disulfide bonds. Papain cleavage separates the molecules into two identical *Fab fragments,* which contain the antigen-combining sites, and an *Fc fragment,* which

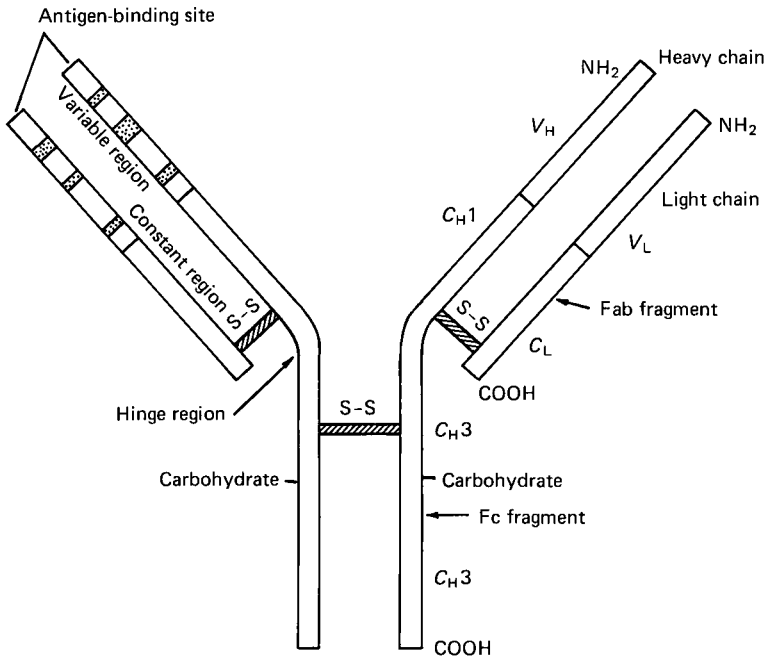

FIG. 9-2. *Schematic diagram of IgG molecule indicating Fab and Fc fragments, the location of the constant (C_L, C_H1, C_H2, C_H3) and variable (V_L, V_H) domains on the light and heavy chains of the Fab fragments, and the location of the antigen-binding sites on the Fab fragments.*

carries the sites for various effector functions such as complement fixation, attachment to phagocytic cells, and placental transfer.

The biological activity of an antibody molecule centers on its ability to bind specifically to a particular epitope. The binding site is located at the amino-terminal end of the molecule and is composed of certain hypervariable segments within the "variable" domains of both light and heavy chains. Of the approximately 220 amino acids of the variable domain of a heavy chain–light chain pair, between 15 and 30 appear to make up a binding site. Antibody specificity is a function of both the amino acid sequence at these sites and their three-dimensional configuration.

The major class of antibody in the blood is immunoglobulin G (IgG), which occurs in the mouse as IgG_1, IgG_2, IgG_3, and IgG_4 subclasses. Following systemic viral infections, IgG continues to be synthesized for many years and is the principal mediator of protection against reinfection. The subclasses of IgG differ in the "constant" region of their heavy

TABLE 9-2
Concentrations of Immunoglobulin Classes IgG, IgA, and IgM in Colostrum and Milk of Some Mammalian Species

	Immunoglobulin concentration (g/liter)					
	Colostrum			Milk		
Species	IgG	IgA	IgM	IgG	IgA	IgM
Human	0.3	**120**[a]	1.2	0.1	**1.5**	0.01
Cattle	**36–77**	4–5	3.2–4.9	**1.0–1.8**	0.2	0.04
Swine	**62**	10	3.2	1.4	**3.0**	1.9
Horse	**80**	9	4	0.35	**0.8**	0.04
Dog	2.0	**13.5**	0.3	0.01	**3.6**	0.06

[a]Bold type indicates major components.

chains and consequently in biological properties such as complement fixation, binding to phagocytes, and passage into colostrum and milk. In cattle and sheep IgG_1 is the major immunoglobulin in the colostrum (Table 9-2) and plays a major role in protecting newborn animals against infections.

IgM is a particularly avid class of antibody, being a pentamer of five IgG equivalents, with 10 Fab fragments and therefore 10 binding sites. It is formed early in the immune response, and is later replaced by IgG. Hence, specific antibodies of the IgM class are diagnostic of recent or chronic infection. IgM antibodies are also the first to be found in the fetus as it develops immunological competence in the second half of pregnancy. Since IgM does not cross the placenta from dam to fetus in any species, the presence of IgM antibodies against a particular virus in a newborn animal suggests intrauterine infection.

Secretory IgA is the principal immunoglobulin on mucosal surfaces, and in some species of animals, in milk and colostrum (Table 9-2). IgA antibodies are important in resistance to infection of the mucosal surfaces, such as the respiratory, intestinal, and urogenital tracts, and IgA antibody responses are much more effectively elicited by oral or respiratory than by systemic administration of antigen, a matter of importance in the design and route of delivery of certain vaccines (see Chapter 14).

Antibodies directed against certain epitopes on a particular protein on the surface of virions neutralize infectivity, by mechanisms discussed below. Antibodies also act as opsonins, facilitating the uptake and de-

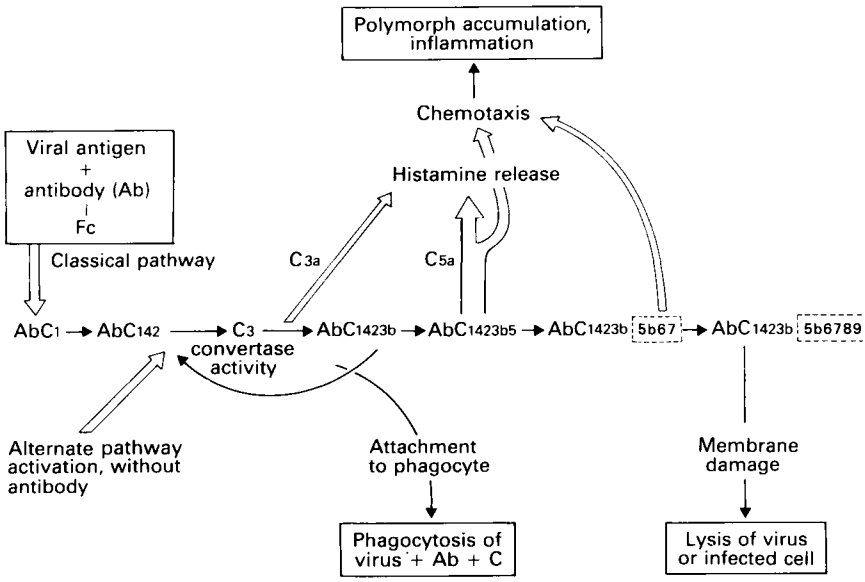

FIG. 9-3. *Diagram to show the complement activation sequence by classical and alternate pathways and the antiviral action of complement. The numbers of the complement components are not sequential because they were assigned before the sequence of action was elucidated. (From C. A. Mims and D. O. White, "Viral Pathogenesis and Immunology." Blackwell Scientific Publications, Oxford, 1984.)*

struction of virions by macrophages. In addition, antibody may attach to viral antigens on the surface of infected cells, leading to their destruction following activation of the classical or alternate complement pathways, or by arming and activating Fc receptor-bearing cells such as K cells, polymorphonuclear leukocytes, and macrophages (antibody-dependent cell-mediated cytotoxicity).

Complement

The *complement system* consists of a series of serum components which can be activated to "complement" the immune response (Fig. 9-3). As well as the "classical" complement activation pathway, which is dependent on the presence of an antigen–antibody complex, there is also an "alternate" antibody-independent pathway, which is not. Both are important in viral infections.

Activation of complement by the classical pathway may lead to the destruction of virions or virus-infected cells, as well as to inflammation.

Virions are destroyed as a result of opsonization, enhancement of neutralization, or lysis of the viral envelope. Antibody–complement mediated lysis of infected cells is discussed below. Complement activation following interaction of antibody with viral antigens in tissues leads to inflammation and the accumulation of leukocytes. Activation of complement via the alternate pathway appears to occur mainly after infections with enveloped viruses that mature by budding through the plasma membrane, and since it does not require antibody, can occur immediately after viral invasion.

IMMUNE RESPONSES TO VIRAL INFECTION

The major features of the immune response to a typical acute viral infection are illustrated in Fig. 9-4. The large boxes highlight three crucially important phenomena which contribute to recovery from infection: (1) destruction of infected cells, (2) production of interferon, and (3) neutralization of viral infectivity. The flowchart illustrates, in a simplified fashion, the interactions of the various cell types that participate in these events. The role of interferon in minimizing the harmful effects of viral infection is described in Chapter 8. The destruction of virus-infected cells by immunological mechanisms and the neutralization of viral infectivity are discussed below. First, however, let us briefly summarize the sequence of events that characterizes the immune response to a typical virus infection, moving from left to right across Fig. 9-4.

Shortly after infection, virus particles are phagocytosed by macrophages. Except in the case of certain viruses that are capable of growing in macrophages, the engulfed virions are destroyed. Their proteins are cleaved into shorter peptides which are presented on the surface of the macrophage (or Langerhans cell in the skin, or dendritic cell in T cell areas of lymphoid tissue) in association with class II MHC antigen (see Fig. 9-1). This combination is recognized by corresponding T_d and T_h lymphocytes.

T_d lymphocytes respond by clonal proliferation and release of lymphokines, which attract blood monocytes to the site and induce them to proliferate and to differentiate into activated macrophages, the basis of the inflammatory response. T_h lymphocytes respond by secreting helper factors which assist the appropriate clones of B cells, following binding of viral antigen, to divide and differentiate into plasma cells. T_c cells are activated following recognition of viral antigens in association with MHC class I glycoprotein on the surface of infected cells. The T_c response usually peaks at about 1 week after infection, compared with the

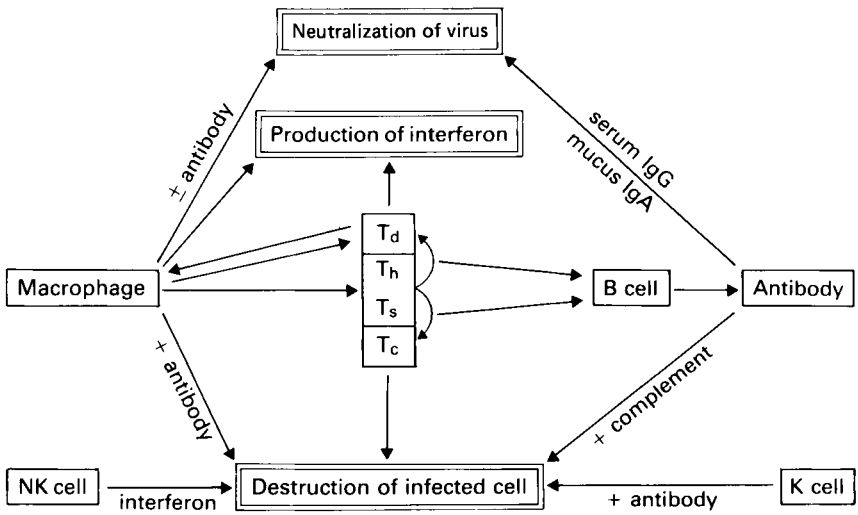

FIG. 9-4. *Immune responses to viral infection. For explanation see text. (From C. A. Mims and D. O. White, "Viral Pathogenesis and Immunology." Blackwell Scientific Publications, Oxford, 1984.)*

antibody response which peaks later (2–3 weeks). Natural killer (NK) cell activity is maximal by 2 days, and interferon activity peaks in concert with the peak of the infecting virus.

Antibody synthesis takes place principally in the spleen, lymph nodes, gut-associated lymphoid tissues, and bronchus-associated lymphoid tissues. The spleen and lymph nodes receive viral antigens via the blood or lymphatics and synthesize antibodies mainly restricted to the IgM class early in the response and IgG classes subsequently. On the other hand, the submucosal lymphoid tissues of the respiratory and digestive tracts, such as the tonsils and Peyer's patches, receive antigens directly from overlying epithelial cells, and make antibodies mainly of the IgA class.

Immune Cytolysis of Virus-Infected Cells

Destruction of infected cells is an essential feature of recovery from viral infections, and results from the complex orchestration of at least five different processes: cytotoxic T cells, antibody–complement mediated cytotoxicity, antibody-dependent cell-mediated cytotoxicity, NK cells, and macrophages acting in the presence of antibody. Because some viral proteins appear in the plasma membrane before any virions

have been produced, lysis of the cell at this stage, or shortly thereafter, brings viral replication to a halt before significant numbers of progeny virions are released.

The discovery that T_c cells recognize viral antigen only in association with "self" class I (or more rarely, class II) MHC antigen (Fig. 9-1) has had wide-ranging implications for immunology. One can visualize the role of T_c cells as one of immunological surveillance—constantly browsing over the surface of cells searching for "altered self," i.e., foreign epitopes. As many of these T_c clones recognize epitopes on antigens that are conserved in different viral serotypes, memory T lymphocytes primed by an earlier infection may be reactivated by subsequent exposure to a different serotype of the same virus genus or family.

Antibody–complement mediated cytotoxicity is readily demonstrable *in vitro* even at very low concentrations of antibody. The alternate complement activation pathway (Fig. 9-3) appears to be particularly important in this phenomenon. Antibody-dependent cell-mediated cytotoxicity does not involve complement or immunologically specific lymphocytes but is mediated by certain types of leukocyte that carry Fc receptors, namely K cells, macrophages, and polymorphonuclear leukocytes. NK cells, on the other hand, are activated by interferon, or directly by viral glycoproteins. They demonstrate almost no immunological specificity as we generally understand it, but preferentially lyse virus-infected cells. Finally, in the presence of antibody, macrophages can phagocytose and digest virus-infected cells.

Neutralization of Viral Infectivity

While specific antibody of any class can bind to any accessible epitope on a surface protein of a virion, only those antibodies that bind with reasonably high avidity to particular epitopes on a particular protein of the outer capsid or envelope of the virion are capable of neutralizing viral infectivity. The key protein is usually the one by which the virion attaches to receptors on the host cell.

Neutralization is not simply a matter of coating the virion with antibody, nor indeed of blocking attachment to the host cell. Except in the presence of such high concentrations of antibody that most or all accessible antigenic sites on the surface of the virion are saturated, neutralized virions generally attach satisfactorily to susceptible cells. The neutralizing block occurs at some point following adsorption and entry. One hypothesis is that, whereas the virion is normally uncoated intracellularly in a controlled way that preserves its infectivity, a virion–antibody complex tends to be destroyed by cellular enzymes, presum-

ably in the phagolysosome. Neutralizing antibody to picornaviruses appears to distort the capsid, producing conformational changes sufficient to interfere with the normal process of infection.

Our understanding of the antigenicity and immunogenicity of viral proteins has been revolutionized within the last few years by the application of the techniques of modern molecular biology and immunochemistry. Mapping of epitopes on the hemagglutinin (HA) protein of influenza virus has been particularly instructive, and recently atomic resolution structural studies of certain picornaviruses have revealed that the neutralizing epitopes cluster in a similar way in three or four areas on the surface of the capsomers.

Mapping of Epitopes on the Hemagglutinin of Influenza Virus. Epitopes on the HA molecules from a number of influenza viruses that have arisen sequentially in nature by antigenic drift have been mapped. Minor antigenic changes characteristic of drift are a consequence of the gradual accumulation of point mutations in the HA gene, expressing themselves as single amino acid substitutions in the primary sequence of the protein. Analysis of the serological reactivity patterns of a set of anti-HA monoclonal antibodies provided an indication of the diversity of distinguishable epitopes on the HA molecules of different strains of influenza virus. When monoclonal antibodies were used to select viral mutants *in vitro*, the mutants were generally found to contain only a single amino acid substitution, this being sufficient to prevent that particular monoclonal antibody from binding. Other monoclonal antibodies, recognizing topographically distinct epitopes, could still bind and neutralize the mutant, as could polyclonal antibody against the parent strain. The three-dimensional model of the HA protein, derived by a combination of X-ray crystallography and nucleic acid sequencing (Fig. 9-5), showed that the positions of the particular substituted amino acids are located on certain prominent regions on the surface of the exposed "head" of the HA molecule, as it occurs in the peplomers on the surface of the virion. Most of the epitopes occur in a large area extending from the "tip" and "interface" of the HA molecule to a conspicuous "loop" a little further down. All these epitopes are in the immediate vicinity of the receptor-binding site (the "cleft" just to the left of the "tip" in Fig. 9-5), so it seems plausible that antibodies binding to any of these epitopes may neutralize the infectivity of the virion (and inhibit hemagglutination) by steric hindrance of adsorption of the virion to cells. The other major antigenic site, the "hinge," is closer to the site of cleavage of the HA monomer into its two component polypeptides HA1 and HA2, which is considered to be vitally involved in the fusion of

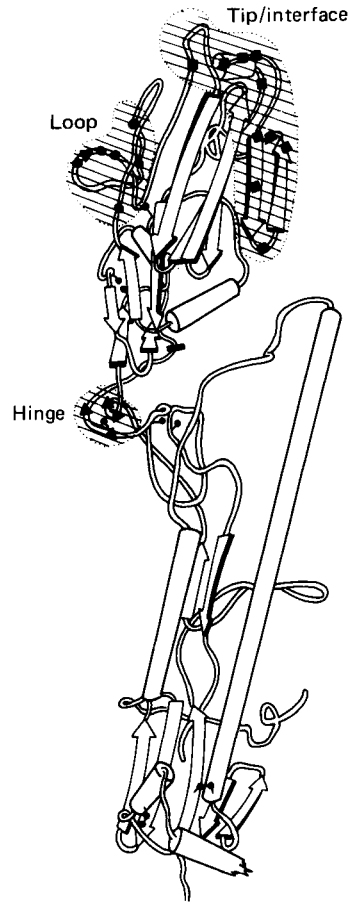

FIG. 9-5. *Three-dimensional model of the monomeric HA molecule of the trimeric HA peplomer of influenza virus. The dots indicate the positions of those amino acids that are substituted in different strains of influenza virus arising within the H3 subtype either by natural antigenic drift or by* in vitro *selection in the presence of monoclonal antibodies. The epitopes identified by these substitutions tend to cluster into four major antigenic domains on the exposed (distal) head of the molecule, designated "tip," "loop," "interface," and "hinge." [Modified from D. C. Wiley, I. A. Wilson, and J. J. Skehel,* Nature (London) **289,** *373 (1981).]*

the infecting virus with lysosomal membrane at pH 5 and thus in uncoating (see Chapter 4); it is possible that neutralizing antibodies binding to this region of the molecule may block the entry and/or uncoating of the virion.

RECOVERY FROM VIRAL INFECTION

Cell-mediated immunity, antibody, complement, phagocytes, and interferons are all involved in the response to viral infections and are together responsible for recovery. These components, illustrated diagrammatically in Fig. 9-4, operate in concert, and to some extent any attempt to assess their relative importance in particular viral infections is an artificial exercise. The fact that a particular cell, substance, or phenomenon may be unequivocally demonstrated to produce antiviral effects in culture does not prove its importance *in vivo*. The reductionist approach simply allows us to analyze a phenomenon in isolation, using relatively defined reagents *in vitro*. Having alerted ourselves in this way to the potential importance of the mechanism in question, we must return to the living animal to establish its biological significance. Yet even this is not sufficient. For example, the fact that one can save the life of an infected experimental animal by adoptive transfer of primed T_c cells is dramatic indeed, but does not establish the *relative* importance of this cell in recovery under natural circumstances. The dosage, timing, or state of activation of the artificially transferred cells may be quite out of proportion to those in the natural setting, or the cell may be acting via a quite different, perhaps unknown, property or secreted product from that identified *in vitro*. Nevertheless, much has been learned by such approaches.

The Role of T Lymphocytes

T cell depletion by neonatal thymectomy or antilymphocyte serum treatment increases susceptibility of experimental animals to most viral infections; for example, T cell-depleted mice infected with ectromelia virus fail to show the usual inflammatory mononuclear cell infiltration in the liver, but develop extensive liver necrosis and die, in spite of the production of antiviral antibodies and interferon. Viral titers in liver and spleen of infected mice can be greatly reduced by transfer of immune T cells taken from recovered donors; this process is class I MHC restricted, implicating T_c cells, and is lifesaving.

Another experimental approach is to use various drugs and antisera, or to ablate completely all immune potential, then add back one or more of the separate components of the immune system. For example, adult mice may be thymectomized to eliminate the source of T cells, X-irradiated to eliminate other lymphoid cells, and then reconstituted with bone marrow cells to restore hematopoiesis. The separate components of the immune response can then be restored one at a time to these animals and the effect on resistance to viral infections studied. For example,

virus-primed T_c lymphocytes of defined function and specificity, cloned in culture then transferred to infected mice, have been shown to save the lives of mice infected with lymphocytic choriomeningitis virus, influenza virus, and several other viruses.

The approach least subject to laboratory artifact is simple clinical observation of the occurrence and progress of viral infections in animals or children suffering from primary immunodeficiencies. There are many such diseases, but few of them manifest as a "pure" T or B cell deficit. Athymic ("nude") mice which are congenitally deficient in T cells are highly susceptible to many viral infections. One of the most severe primary immunodeficiencies known in domestic animals occurs in certain families of Arabian horses: a primary severe combined immunodeficiency, in which there is a total or near total absence of both B and T lymphocytes. Characteristic findings are a lymphopenia and a hypogammaglobulinemia, which render foals unusually susceptible to infections, especially with equine adenoviruses. There are also several types of B lymphocyte deficiency which predispose newborns of various species to very severe infections. Among these are a primary agammaglobulinemia of thoroughbred horses, a selective deficiency in foals of IgM-producing B cells, a deficiency of IgG_2-synthesizing cells in some breeds of cattle, and a dysgammaglobulinemia in certain lines of white leghorn chickens. Furthermore, there are conditions characterized by a T cell deficiency due to thymic hypoplasia. Of different origin and significance, but of great practical importance, are secondary agammaglobulinemias and hypogammaglobulinemias in foals, piglets, lambs, and especially calves, associated with the failure of antibody transfer via colostrum.

The data obtained from these clinical conditions and experimental approaches indicates a key role for T lymphocytes in recovery from generalized viral infections. Lymphocytes and macrophages normally predominate in the cellular infiltration of virus-infected tissues; in contrast with bacterial infections, polymorphonuclear leukocytes are not at all plentiful. Animals or humans with severe T cell deficiencies due to thymic aplasia, lymphoreticular neoplasms, or chemical immunosuppression show increased susceptibility to herpesviruses and to certain other viral infections. Perhaps the most informative example is that of measles in infants with thymic aplasia. In these infections there is no sign of the usual rash but an uncontrolled and progressive growth of virus in the respiratory tract, leading to fatal pneumonia. This reveals two aspects of the role of T cells. Evidently, in the normal child, the T cell-mediated immune response controls infection in the lung and also plays a vital role in the development of the characteristic skin rash.

The Role of NK Cells

The role of NK cells in recovery is not yet certain. "Beige" mice, which have little or no NK cell activity, or normal mice depleted of NK cells by treatment with NK-specific antiserum, show increased replication of some viruses but not others. Athymic mice have normal numbers of NK cells but usually die if infected with viruses that produce generalized viral infections.

The Role of Antibody

Viruses producing generalized disease characterized by a viremia in which virions circulate free in the plasma, rather than in leukocytes, appear to be controlled principally by circulating antibody. Because no instances of severe pure B cell deficiency have been identified in animals, we need to turn to human examples to help elucidate the situation. Unlike those with a T cell deficit, children with severe primary agammaglobulinemia recover normally from measles or varicella, but are about 10,000 times more likely than normal individuals to develop paralytic disease after vaccination with live attenuated poliovirus vaccine. They have normal cell-mediated immune and interferon responses, normal phagocytic cells, and complement, but cannot produce antibody, which is essential if poliovirus spread to the central nervous system via the bloodstream is to be prevented.

While there is reasonably good evidence that antibody plays a key role in recovery from picornavirus, togavirus, flavivirus, and parvovirus infections, it does not necessarily follow that the antibody is acting solely by neutralizing virions. Indeed it has been shown that certain non-neutralizing monoclonal antibodies can save the lives of mice, presumably by antibody-dependent cell-mediated cytotoxicity or antibody–complement mediated lysis of infected cells, or by opsonization of virions for macrophages.

PASSIVE IMMUNITY

There is abundant evidence for the efficacy of antibody in preventing infection. For example, *passive immunization* (injection of antibodies) temporarily protects against infection with canine distemper, feline panleukopenia, hog cholera, and many other viral infections (see Chapter 14). Furthermore, maternal antibody transferred from dam to fetus or newborn protects the newborn for the first few months of life against most of the infections that the dam has experienced. This is known as *maternal immunity* or *natural passive immunity.*

Natural Passive Immunity

Natural passive immunity is important for two major reasons: (1) it is essential for the protection of young animals, during the first weeks or months of life, from the myriad of microorganisms, including viruses, that are present in the environment into which animals are born; (2) maternally derived antibody interferes with active immunization of the newborn and must therefore be taken into account when designing vaccination schedules (see Chapter 14).

Transfer of Maternal Antibodies. Maternal antibodies may be transmitted in the egg yolk in birds, or across the placenta or via colostrum and/or milk in mammals. Different species of mammals differ strikingly in the predominant route of transfer of maternal antibodies, depending on the structure of the placenta of the species (Table 9-3). In those species in which the maternal and fetal circulations are separated by relatively few (one to three) placental layers, antibody of the IgG (but not IgM) class is able to cross the placenta, and maternal immunity is transmitted mainly by this route. However, the placenta of most domestic animals is more complex (five to six layers) and acts as a barrier even to IgG; in these species maternal immunity is transmitted to the newborn via colostrum, and to a much lesser extent, via milk.

Different species differ strikingly in regard to the particular class or subclass of immunoglobulin that is preferentially transferred to the newborn in colostrum (Table 9-2), but in most domestic animals it is mainly IgG. In cattle there is a selective transfer of IgG_1 from the serum across the alveolar epithelium of the mammary gland during the last few weeks

TABLE 9-3
Transfer of Natural Passive Immunity in Mammals

Species	Type of placentation	Number of placental layers		Prenatal transfer (via placenta)	Postnatal transfer (via gut)	Translocation cutoff time (days)
		Maternal	Fetal			
Cow Swine Horse	Epitheliochorial	3	3	0	+++	2
Sheep Goat	Syndesmochorial	2 or 3	3	0	+++	2
Dog Cat	Endotheliochorial	1	2 or 3	±	+++	2
Mouse Rat	Hemochorial	0	3	++	+	16–20

of pregnancy, such that the level of IgG_1 in colostrum may reach 40 g/liter compared with about 13 g/liter in serum.

The selective transfer of IgG from the maternal circulation across the mammary alveolar epithelium is a function of the Fc fragment of the molecule. The very large amounts of IgG present in colostrum are ingested and *translocated* in large intracytoplasmic vesicles by specialized cells present in the small intestine, to reach the circulation of the newborn in an undegraded form. Small amounts of other antibodies (IgM, IgA) present in colostrum or milk may in some species also be translocated across the gut, but quickly disappear from the circulation of the young animal. The period after birth during which antibody, ingested as colostrum, is translocated is sharply defined and very brief (about 48 hours) in most domestic animals (see Table 9-3). The mechanism of *translocation cutoff* is not known.

In birds there is a selective transfer of IgG from the maternal circulation; the level of IgG in chicken egg yolk is 25 g/liter compared with 6 g/liter in the maternal circulation. A laying hen produces about 100 g of IgG per year for transfer to yolk, which is about as much as she synthesizes for her own needs. IgG enters the vitelline circulation and hence that of the chick from day 12 of incubation. Some IgG is also transferred to the amniotic fluid and swallowed by the chick. Close to the time of hatching, the yolk sac with the remaining maternal immunoglobulin is completely taken into the abdominal cavity and incorporated into the wall of the small intestine of the chick.

Maternal antibody in the bloodstream of the newborn mammal or newly hatched chick is destroyed quite rapidly, with first-order kinetics. The half-life, which is somewhat longer than in adult animals, ranges from about 21 days in the cow and horse, through 8–9 days in the dog and cat, to only 2 days in the mouse. Of course, the newborn animal will be protected against infection with any particular virus only if the dam's IgG contains specific antibodies, and protection may last much longer than one IgG half-life if the titer against that virus is high.

Although the levels of IgA transferred via colostrum to the gut of the newborn animal are considerably lower than those of IgG, it helps to protect the neonate against enteric viruses against which the dam has developed immunity. Moreover, there is evidence that after translocation cutoff immunoglobulins present in ordinary milk, principally IgA but also IgG and IgM in certain animals, may continue to provide some protective immunity against gut infections. Often the newborn encounters viruses while still partially protected. Under these circumstances the virus replicates, but only to a limited extent, stimulating an immune response without causing significant disease. The newborn thus acquires active immunity while partially protected by maternal immunity.

Failure of Maternal Antibody Transfer. The failure or partial failure of maternal antibody transfer is the most common immunodeficiency disease of domestic animals. For example, between 10 and 40% of dairy calves and up to 20% of foals fail to receive adequate levels of maternal antibody. Mortality during the neonatal and early adolescent period, particularly from respiratory and enteric diseases, is higher than at any other time of life and there is a strong correlation with failure of antibody transfer. Among the reasons for failure are human intervention by imposition of unnatural conditions on parturition and early suckling, birth of weak or deformed animals, delay to first suckle, death of the dam, poor colostrum production, low antibody levels in maternal serum and thus in colostrum, poor maternal instinct particularly in primiparous dams, premature lactation, too many in the litter, and bullying of the weak in the litter by the strong. Of these, the most critical factors are the amount of colostrum available and the delay between birth and first suckling.

The transfer of maternal antibody to the newborn and its persistence are of paramount importance for the control of infectious diseases of domestic animals, and maternal immunization to protect the newborn is an important strategy in veterinary medicine (see Chapter 14).

IMMUNITY TO REINFECTION

Whereas a large number of interacting phenomena contribute to recovery from viral infection, the mechanism of acquired immunity to reinfection with the same agent appears to be much simpler. The first line of defense is antibody, which, if acquired by active infection with a virus that causes systemic infections, continues to be synthesized and protects against reinfection for many years. If the antibody defenses are inadequate, the mechanisms that contribute to recovery are called into play again, the principal differences on this occasion being that (1) the dose of infecting virus is reduced by antibody, and (2) primed memory T and B lymphocytes generate a more rapid (*secondary* or *anamnestic*) response.

As a general rule the secretory IgA antibody response is short-lived compared to the serum IgG response. Accordingly, resistance to reinfection with respiratory viruses and some enteric viruses tends to be of limited duration. Reinfection with the same serotype of parainfluenza virus or with respiratory syncytial virus is not uncommon. In addition, repeated attacks of respiratory disease due to rhinoviruses and influenza viruses result from infections with antigenically distinct strains of virus.

The immune response to the first infection with a virus can have a dominating influence on subsequent immune responses to antigenically related viruses. The second virus often induces a response that is directed mainly against the antigens of the original viral strain. The best-studied example of this is in influenza; the antibody response of humans to sequential infections with different strains of influenza A virus is largely directed to antigens characterizing the particular strain of virus with which that individual was first infected. This phenomenon has been called "original antigenic sin" and is seen not only in influenza but also with enteroviruses, reoviruses, paramyxoviruses, and togaviruses. The immunological basis of original antigenic sin remains unexplained, but it has important implications for interpretation of seroepidemiological data, for understanding immunopathological phenomena, and particularly for the development of efficacious vaccination strategies.

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CHAPTER 10

Mechanisms of Disease Production: Acute Infections

In the previous four chapters we have analyzed various aspects of viral infections of animals, exploring how viruses affect cells, how infection of animals occurs, how viruses spread to various parts of the body, and how the host animal responds to infection by immunological and other mechanisms. The clinical end product of these interactions, namely disease, is the subject of this chapter and Chapters 11 (persistent infections) and 12 (virus-induced tumors). The first part of this chapter covers the ways in which viral replication damages tissues and organs and the ways in which the body's own responses may cause damage. The second part of the chapter comprises examples illustrating the pathogenesis of four basic kinds of acute viral infection: respiratory, intestinal, generalized, and neurological.

DIRECT DAMAGE AT THE CELLULAR LEVEL

The mechanisms by which viruses damage cells have been discussed at the cellular and subcellular levels in Chapter 6. Here we apply these

concepts at the level of tissues and organs. The severity of disease in the animal is not necessarily correlated with the degree of cytotoxicity of the virus *in vitro*. Many viruses that are cytocidal in cultured cells are harmless *in vivo* (e.g., many enteroviruses), whereas some that are noncytocidal *in vitro* cause a lethal disease *in vivo* (e.g., rabies virus). A great deal of cell and tissue damage can occur without producing signs of disease, e.g., a large number of intestinal or liver cells can be destroyed without significant clinical signs. Even when damage to cells impairs the function of an organ or tissue, this may be of minor importance, e.g., in muscle or subcutaneous tissue, but of great importance in the heart or the brain. Likewise, edema of a tissue may be unimportant in most sites in the body but may have serious consequences in the brain, because of the resulting increase in intracranial pressure, or in the lung, where it may interfere with gaseous exchange, or in the heart, where it may interfere with conduction.

Sometimes the whole pathological process in an animal may be explained by the direct damage to cells caused by a highly cytocidal virus. Mice infected intravenously with a large dose of Rift Valley fever virus, for example, develop overwhelming hepatic necrosis within 4 hours of injection, because the virions pass quickly through the Kupffer cells to infect the hepatic cells, which are rapidly lysed. In this experimental model, the defense mechanisms of the host, both nonspecific and specific, are quite unable to cope with the rapid lethal damage to a vital organ.

Epithelial Damage Predisposing to Secondary Bacterial Infection

As well as having direct adverse effects, viral infections of the respiratory or digestive tracts often predispose animals to secondary bacterial infections. Viral infection increases the susceptibility of the respiratory tract to bacteria that are normal commensals in the nose and throat. For example, in cattle, parainfluenza 3 virus or other viruses may destroy ciliated epithelia and cause exudation, allowing *Pasteurella haemolytica* and other bacteria to invade the lungs and cause secondary bacterial pneumonia, known in many countries as "shipping fever." Rhinoviruses and respiratory syncytial viruses damage the mucosa in the nasopharynx and sinuses, predisposing to bacterial superinfection which commonly leads to purulent rhinitis, pharyngitis, and sinusitis. Similarly, in the intestinal tract, rotavirus and coronavirus infections may lead to an increase in susceptibility to enteropathogenic *E. coli*, and the synergistic effect leads to severe diarrhea.

Veterinary virologists are particularly conscious of the potentiating effect on viral diseases of coinfection with parasites. Animals are almost universally infected with protozoa and helminths, and a high parasitic load generally lowers their resistance to viruses and bacteria.

DAMAGE TO THE IMMUNE SYSTEM

Since the immune system plays a key role in protection against infections, viral damage to its components can exacerbate the severity of disease or predispose to superinfection with other viral or nonviral agents. Both specific acquired immunodeficiency and generalized immunosuppression can occur in viral infections.

Infection of the bursa of Fabricius in chickens (the site of B cell differentiation) with infectious bursal disease virus (a birnavirus) leads to atrophy of the bursa and a severe deficiency of B lymphocytes, with an increase in susceptibility to Marek's disease, Newcastle disease, infectious bronchitis, and infectious laryngotracheitis viruses. B and T cell deficiencies are produced in feline leukemia, thereby allowing intercurrent opportunistic infections to cause death.

Recently, a comparable example of great public health significance has emerged in humans. The agent that causes acquired immunodeficiency syndrome (AIDS) in humans, now designated human immunodeficiency virus (HIV), is a retrovirus, of the subfamily *Lentivirinae*. The virus destroys helper T (T_h) cells, causing profound immunosuppression, which, after a prolonged clinical course, leads to death from opportunistic infections. This tropism for T cells is shared by other lentiviruses; e.g., visna–maedi virus of sheep, as well as by simian T-lymphotropic virus-III (STLV-III), which causes a similar disease in some species of monkeys.

Infections with certain other viruses (e.g., hog cholera, bovine virus diarrhea, canine distemper viruses, feline and canine parvoviruses) may temporarily suppress humoral and/or cell-mediated immune responses, but the results are not usually so catastrophic. The immune response to unrelated antigens is reduced or abrogated in such animals, and the situation is thus distinct from suppression of the immune response to the specific virus in question, which is called immune tolerance. The mechanisms involved in such general immunosuppression are not fully understood, but may result from the replication of virus in lymphocytes and/or macrophages, or from abnormal induction of suppressor T cells. Many viruses are capable of replication in macrophages (see Chapter 11), and several have been shown to grow in T cells, especially activated

T cells. Some herpesviruses replicate nonproductively in B cells, transforming them and altering their function.

While viral infections can induce immunosuppression, conversely immunosuppression allows enhanced viral replication. When the immune system is suppressed by endogenous or exogenous factors, latent herpesvirus, adenovirus, or papovavirus infections can be reactivated. Such situations are frequently encountered following the use of cytotoxic drugs or irradiation for organ transplantation in humans. No doubt immunosuppression, usually of unknown origin, is often responsible, at least in part, for reactivation of herpesviruses in animals (see Chapters 11 and 19).

IMMUNOPATHOLOGICAL EFFECTS AND MECHANISMS

The immune response to viral infection may itself frequently contribute to the pathology of the disease. Inflammation with accompanying cellular infiltration is a regular feature of viral infection. Such common signs as erythema, edema, and enlargement of lymph nodes have an immunological basis. But there are viral diseases in which the cardinal manifestations are caused by the body's immune response. In the extreme case, the disease may be prevented by suppressing the immune response.

Immunopathological (hypersensitivity) reactions are traditionally classified into types 1, 2, 3, and 4. Although advances in cellular immunology have now blurred some of the distinctions, the classification is still convenient (Table 10-1). For most viral infections it is not known whether immunopathological effects make a significant contribution to disease, and if so, which of the four classical "hypersensitivity reactions" is implicated. Nevertheless, it is instructive to speculate about the possible involvement of different kinds of hypersensitivity reactions in viral diseases.

Hypersensitivity Reactions—Type 1

These are anaphylactic reactions, which depend on the interaction of antigens with IgE antibodies on the surface of mast cells, resulting in the release of histamine and heparin and the activation of serotonin and plasma kinins. Except for its possible contribution to some types of rash and possibly in some acute respiratory infections, anaphylaxis is probably not important in viral immunopathology, but is responsible for some adverse reactions to viral vaccines.

TABLE 10-1

Basic Types of Hypersensitivity Reactions[a]

Item	Hypersensitivity type			
	1	2	3	4
Designation	Anaphylactic	Cytotoxic	Immune complex	Delayed—cell mediated
Time course				
Initiation	Minutes	Minutes	3–6 hours	18–24 hours
Persistence	Minutes	Dependent on antigen and antibody	Dependent on antigen and antibody	Weeks
Transfer with	IgE	IgM, IgG	IgG	T lymphocytes
Complement required	No	Usually	Yes	No
Histamine dependent	Yes	No	No	No
Histology	Edema, congestion, eosinophils	Cell destruction, phagocytosis	Necrosis, neutrophils, later plasma cells	Lymphocytes, macrophages, necrosis
Viral immunopathological effects	Minor ?some rashes	Minor ?some rashes	Major acute: fever chronic: immune complex disease	Major in brain, lung

[a]As categorized by Gell and Coombs.

Hypersensitivity Reactions—Type 2

These cytolytic reactions occur when antibody, having combined with viral antigen on the cell surface, activates the complement system, leading to cell lysis. Alternatively, antibodies can sensitize virus-infected cells to destruction by K cells, polymorphonuclear leukocytes, or macrophages, via antibody-dependent cell-mediated cytotoxicity. While it has been clearly demonstrated that virus-infected cells are readily lysed by all of these mechanisms *in vitro*, their significance in viral diseases *in vivo* is unclear, although there is some evidence that they may be operative in certain herpesvirus infections.

Hypersensitivity Reactions—Type 3

Antigen–antibody complexes cause inflammation and cell damage by a variety of mechanisms. If the reaction occurs in extravascular tissues there is edema, inflammation, and infiltration of polymorphonuclear leukocytes, which may later be replaced by mononuclear cells. This is a common cause of mild inflammatory reactions. These "immune complex" reactions constitute the classical Arthus response, and are of major importance, especially in persistent viral infections. If they occur in the blood, they produce circulating immune complexes, which are found in many viral infections, both acute and persistent. The fate of the immune complexes depends on the ratio of antibody to antigen. If there is a large excess of antibody, each antigen molecule is covered with antibody and removed by macrophages, which have receptors for the Fc fragment of the antibody molecule. If the amounts of antigen and antibody are about equal, lattice structures which develop into large aggregates are formed and removed rapidly by the reticuloendothelial system.

However, in some persistent infections viral antigens or virions themselves are continuously released into the blood, but the antibody response is weak and antibodies are of low avidity. Complexes continue to be deposited in glomeruli over periods of weeks, months, or even years, leading to impairment of glomerular filtration and eventually to chronic glomerulonephritis (see Chapter 11). A classic example is lymphocytic choriomeningitis infection in mice infected *in utero* or as neonates. Viral antigens are present in the blood and small amounts of nonneutralizing antibody are formed, giving rise to immune complexes which are progressively deposited upon renal glomerular membranes. Depending on the strain of mouse, the end result may be glomerulonephritis, uremia, and death. Circulating immune complexes may also be deposited in the walls of the small blood vessels in skin, joints, and choroid plexus, where they attract macrophages and activate complement. Prodromal

rashes, seen commonly in exanthems in humans but rarely in domestic animals, are probably caused in this way.

In addition to these local effects, antigen–antibody complexes generate systemic reactions, such as the fever which marks the end of the incubation period in generalized viral infections. Fever is mediated by interleukin-1, which is liberated from macrophages and polymorphonuclear leukocytes.

Rarely, systemic immune complex reactions may activate the enzymes of the blood coagulation cascade, leading to histamine release and increased vascular permeability. Fibrin is deposited in the kidneys, lungs, adrenals, and pituitary gland, causing multiple thromboses with infarcts and scattered hemorrhages—a condition known as disseminated intravascular coagulation. This is seen in hemorrhagic fevers in humans, many of which are zoonoses caused by arenaviruses, bunyaviruses, filoviruses, or flaviviruses. Kittens infected with feline infectious peritonitis virus, a coronavirus, also display this phenomenon, and it probably occurs in other severe systemic diseases.

Hypersensitivity Reactions—Type 4

Unlike all the previous types, these "delayed hypersensitivity" reactions are mediated by cells rather than antibody. They are T lymphocyte-mediated immune reactions, involving inflammation, lymphocytic infiltration, macrophage accumulation, and activation by lymphokines secreted by T_d cells. Once again, the classic model is lymphocytic choriomeningitis virus infection, this time primary infection of adult mice. After intracerebral inoculation this noncytocidal virus replicates harmlessly in the meninges, ependyma, and choroid plexus epithelium until about the seventh day, when a T_c lymphocyte-mediated immune response occurs, causing severe meningitis, cerebral edema, and death. Elsewhere than in the central nervous system, T_c cells help to control infection; within the rigid confines of the skull these changes are fatal. The death of mice infected in this way can be prevented by chemical immunosuppression, by X irradiation, or by antilymphocyte serum. Type 4 hypersensitivity reactions may also contribute to consolidation of the lung, probably mediated by T_d cells, in various severe lower respiratory tract diseases.

Although occasionally the cause of immunopathology, cell-mediated immune responses are generally an important component of the process of recovery from viral infections (see Chapter 9), as becomes evident if they are abrogated by cytotoxic drugs, or are absent, as in some immunodeficiency diseases.

OTHER PHYSIOLOGICAL DISTURBANCES

Some pathological changes found in viral infections cannot be attributed to direct cell destruction by the virus, nor to inflammation, nor to immunopathology. Perhaps the most important of these effects relate to alterations in the function of various endocrine glands, notably the adrenals, in response to the "stress" of the infectious disease. Sometimes endocrine epithelial cells affected by noncytocidal viruses are not killed, but their secretory functions are damaged; e.g., infection of mice with encephalomyocarditis virus (a picornavirus) may lead to diabetes because of the action of the virus on the β cells of the islets of Langerhans. Lymphocytic choriomeningitis virus, by infecting somatotrophic cells of the anterior lobe of the pituitary gland of mice, may reduce the output of growth hormone, leading to dwarfism. These examples come from well-studied experimental animal models; similar changes probably occur in natural infections, but they have not yet been documented.

Most viral diseases are accompanied by a number of vague general clinical signs, such as fever, malaise, anorexia, and lassitude. Little is known about the causes of these signs, which collectively can significantly reduce the animal's performance and impede recovery. As discussed in Chapter 8, fever can be attributed to interleukin-1 and possibly to interferons. These and other soluble mediators produced by leukocytes, or released from virus-infected cells, may be responsible for the other clinical signs also.

REPRESENTATIVE DISEASE MODELS

We are now in a position to examine in some detail the pathogenesis of selected examples of each of the four main categories of acute infections: respiratory, intestinal, generalized, and those affecting the central nervous system. We begin with two prototypes of viral respiratory diseases: influenza, about which a great deal is known from experimental studies, and the complex respiratory infections of cattle known as shipping fever, as an example of synergistic virus–bacteria infection.

Influenza

The sequence of events in respiratory infections is rather similar no matter what kind of virus is involved. Acute respiratory infections are exemplified by influenza in horses and swine. Virus particles in aerosolized droplets or on fomites are inhaled and alight on the film of

mucus that covers the epithelium of the upper respiratory tract. Droplets of different sizes alight at different levels of the respiratory tree, and infection may accordingly be initiated at particular levels, but in general the upper respiratory tract—i.e., the nasal passages, the pharynx, and the trachea—are the sites of initial infection. Immediately upon alighting, the virus is met by host defense mechanisms; if the animal has previously been infected with the same or a very similar strain of influenza virus, antibody (mainly IgA) present in the mucus may neutralize the virus. Mucus also contains glycoproteins similar to the receptor molecules on respiratory epithelial cells; these glycoproteins may combine with virions and prevent them from attaching to epithelial cells. In turn, the viral neuraminidase may destroy enough glycoprotein to allow virions to attach to and infect an epithelial cell.

If these host defenses fail, the virus moves deeper into the airways, where it faces another physiological barrier mechanism. This is the cleansing action of beating cilia; inhaled particles, including virions, are normally carried via the mucous flow generated by cilial beating action to the pharynx, where they are swallowed. However, it has been shown with influenza viruses and other respiratory viruses that initial invasion and destruction of just a few epithelial cells can initiate a lesion which can progressively damage the mucus protective layer and lay bare more and more epithelial cells.

Infection of epithelial cells of the respiratory tract follows attachment of the virus via its hemagglutinin to the specific receptor, *N*-acetylneuraminic acid, the terminal sugar of the oligosaccharide side-chains of common cellular glycoproteins and glycolipids. Once attached, the virus is taken up by endocytosis and, in the acid conditions of the phagolysosome, the viral envelope fuses with the phagolysosome membrane, releasing the viral nucleocapsid into the cytoplasm of the epithelial cell. Viral replication progresses and large numbers of progeny virions are budded from the plasma membrane into the lumen of the airway. Early in infection, cilial beating helps to move released progeny virus along the airway, thereby spreading the infection. As secretions become more profuse and viscous, the cilial beating becomes less effective, and later cilial beating is stopped as epithelial cells are destroyed.

In studies in experimental animals the spread of the infection via contiguous expansion from initial foci often does not stop until virtually every columnar epithelial cell at that airway level is infected. The result is complete denuding of large areas of epithelial surface (Plate 10-1) and the accumulation of large amounts of transudates, exudates, inflammatory infiltrates, and necrotic epithelial debris in the airways. The consequent respiratory distress is made worse by forced movement of

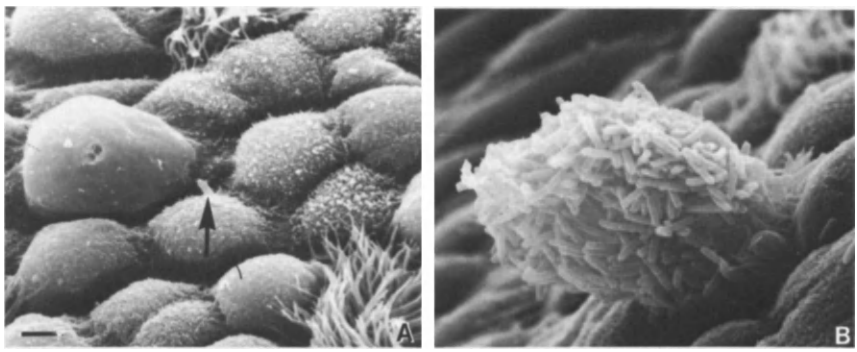

PLATE 10-1. *Scanning electron micrographs showing the adherence of* Pseudomonas aeruginosa *to the mouse trachea (bar = 2 μm). (A) Normal mouse trachea, showing a single bacterium (arrow) on a serous cell. (B) Microcolony adhering to desquamating cells in an influenza virus-infected trachea. [From R. Ramphal* et al., Infect. Immun. **27,** *614 (1980); courtesy Dr. P. A. Small, Jr.]*

animals and by coughing and sneezing. Where infection of the epithelium of the nasal passages, trachea, and bronchi proceeds to a fatal outcome, there is usually one or more of three complications: (1) bacterial superinfection, nurtured by the accumulation of fluid and necrotic debris, "growth medium," in the airways, (2) infection and destruction of the lung parenchyma, the alveolar epithelium, and/or (3) blockage of airways that are so small in diameter that mucus plugs cannot be opened by forced air movements. Blockage of the airways is of most significance in the newborn. In all of these complications there is hypoxia, and a pathophysiological cascade that leads to acidosis and uncontrollable fluid exudation into airways.

Degeneration of respiratory tract epithelial surfaces during influenza infection is extremely rapid, but so is regeneration. In studies of influenza in ferrets, for example, it has been shown that the development of a complete new columnar epithelial surface via hyperplasia of remaining transitional cells may be complete in a few days. The transitional epithelium and the newly differentiated columnar epithelium that arises from it are resistant to infection, probably by virtue of interferon production and a lack of virus receptors. Other host defenses, including antibody and cell-mediated immune mechanisms, also play a part in terminating the infection.

Influenza viruses, like most other respiratory viruses, have evolved a rapid transmission cycle, so as to outrace the host immune response. Influenza viruses, particularly human influenza viruses, have also

evolved systems of genetic drift and shift (see Chapter 5) to circumvent host immunity, but they also depend on management programs that favor continual exposure of new susceptible horses and swine, as well as poultry—hence our need for vaccine.

Whereas influenza virus infection in mammals, as described, is generally restricted to the cells of the respiratory tract, in birds it is primarily an inapparent infection of the digestive tract; viremia and spread to other organs, leading to severe disease, occur with virulent strains of avian influenza virus.

Viral–Bacterial Synergism in Respiratory Infections (Shipping Fever)

In cattle there is a seasonal incidence of bronchopneumonia that corresponds with the extra stress of harsh climatic conditions and husbandry practices in fall and early winter, as well as the extra activity of respiratory viruses and mycoplasma at this time of year. This syndrome of bronchopneumonia, often extending to a true fibrinous pleuropneumonia, is called shipping fever in many countries. In the United States and Canada the syndrome represents the most economically important health problem in cattle, especially feedlot cattle. The syndrome, despite having diverse initial causes, has a common final pathway and etiology, the terminal manifestations being caused by overwhelming infection by *Pasteurella haemolytica* and to a lesser extent *P. multocida.*

Respiratory virus infections, augmented by the pathophysiological effects of stress, alter the susceptibility of cattle to *Pasteurella* species that are normally present in the upper respiratory tract by a number of independent and interdependent mechanisms. The influence of respiratory epithelial tissue damage and fluid exudation into airways, as described above for influenza, is a major factor favoring bacterial growth following all respiratory virus infections. Viral infections can also alter bovine host defense mechanisms in other ways: (1) they can be directly immunosuppressive or can damage reticuloendothelial (macrophage and neutrophil) function in the lungs and airways, (2) they may induce such exuberant inflammatory response that the delicate epithelial surfaces of the alveoli are destroyed and the structure of the lungs collapsed and consolidated, or (3) they can alter the surface properties of respiratory epithelial cells so as to favor bacterial adherence, and the growth of bacterial microcolonies. These microcolonies in turn resist phagocytosis and the effects of antibodies and antibiotics, and can more readily enter the lower respiratory tract. Another effect of viral damage to epithelial cells is the release of iron, which enhances bacterial growth and colo-

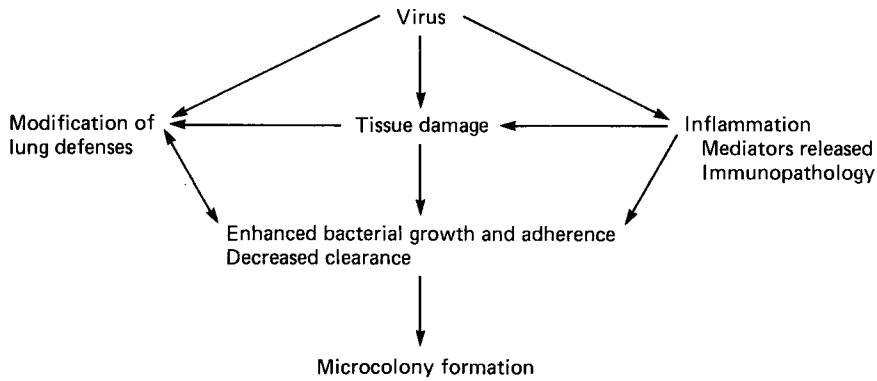

FIG. 10-1. *Interactions between events associated with viral and bacterial infections in the development of respiratory disease ("shipping fever") in cattle. [From L. A. Babiuk,* In *"Applied Virology" (E. Kurstak, ed.), p. 431. Academic Press, Orlando, 1984.]*

nization. The complex of interactions between various factors during the development of respiratory disease is represented diagrammatically in Fig. 10-1.

Control of the viral infections that initiate shipping fever appears to be more important than control of the terminal bacterial pneumonia. The *Pasteurella* species involved are always present in the environment and in the upper respiratory tract of cattle, and it is not likely that they could be eliminated. Likewise, these bacterial species have proved most difficult to affect with vaccines. The speed with which antibiotic therapy is initiated does influence the eventual outcome, but in many cases irreparable damage has already been done by the time of diagnosis. If microcolonies have been established and have progressed to abscess formation, there is added difficulty in delivering effective antibiotic levels so as to reach the bacteria. Even when the animal does not die, it generally does not grow well, and it is prone to further debilitating diseases. Viral vaccine programs, aimed at preventing this pathogenetic pattern, must be measured and justified indirectly, by the effect they have on pneumonia prevalence. Producers and veterinarians must therefore collaborate in developing overall strategies for dealing with this syndrome.

Pathogenesis of Viral Diarrheas

Diarrhea in animals is often multifactorial, and interactions of infectious agents with immunological, environmental, and nutritional factors can often exacerbate the disease. The principal causes of viral diarrhea in

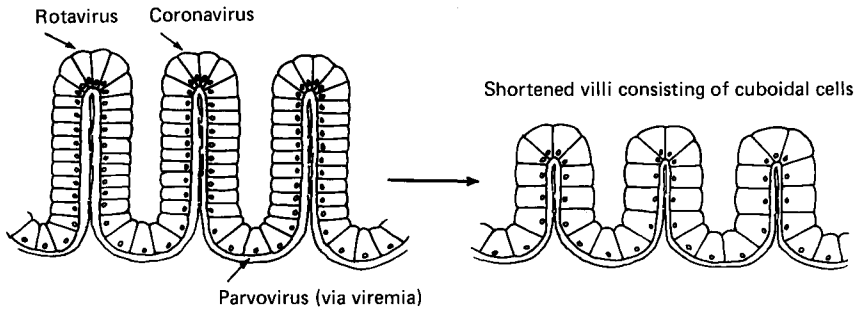

FIG. 10-2. *Preferred locations of replication of enteropathogenic viruses in intestinal villi and the effects on the villi: coronaviruses and rotaviruses in cells at the tips, parvoviruses in the multiplying cells in the crypts. [Based on L. A. Babiuk,* In *"Applied Virology" (E. Kurstak, ed.), p. 349. Academic Press, Orlando, 1984.]*

domestic animals are rotaviruses and coronaviruses; other viruses involved are caliciviruses, astroviruses, and parvoviruses. Infection occurs by ingestion of virus, except with parvoviruses, in which infection of the intestinal tract is part of a systemic infection. The incubation period is very short.

Different viruses characteristically infect different parts of the villi of the intestinal tract, with rotaviruses and coronaviruses infecting cells at the tip and parvoviruses cells in the crypts (Fig. 10-2). All cause marked shortening and occasional fusion of adjacent villi (Plate 10-2), so that the absorptive surface of the intestine is reduced, resulting in fluid accumulation and diarrhea. Infection generally begins in the proximal part of the small intestine and spreads progressively to the jejunum and ileum and sometimes to the colon. The extent of such spread depends on the initial dose, the virulence of the virus, and the host's immunological status. In the presence of maternal antibody, infection can occur, but the degree of replication is limited and diarrhea is mild or does not occur.

With rotaviruses and coronaviruses, which infect cells at the tips of the villi, as infection progresses the absorptive cells are replaced by immature cuboidal epithelial cells whose absorptive capacity and enzymatic activity is greatly reduced. These cells are relatively resistant to viral infection, so that the disease is often self-limiting if dehydration is not so severe as to be lethal. The rate of recovery is rapid, since the crypt cells are not damaged. In contrast, recovery is slow after infections with parvoviruses, which infect cells of the crypts.

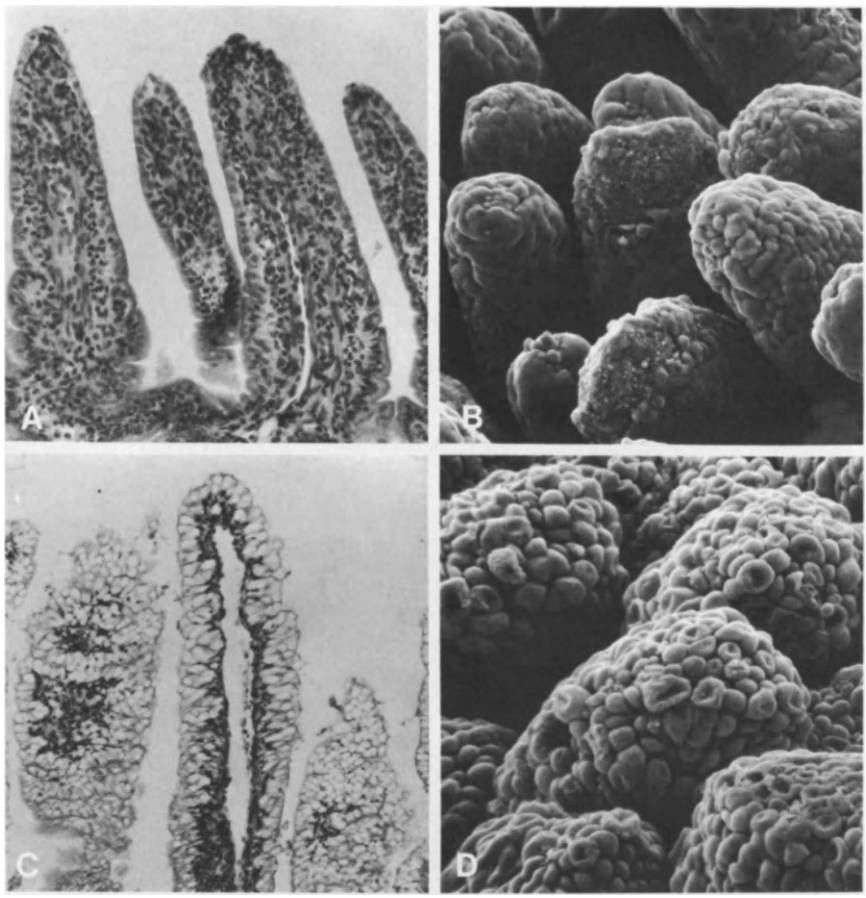

PLATE 10-2. *Scanning electron and light micrographs of intestinal tissues from a gnotobiotic calf sacrificed 0.5 hour after onset of rotavirus diarrhea. (A) Proximal small intestine with shortened villi and a denuded villus tip (second from right) (H and E, ×112). (B) Appearance of same level of intestine as in (A) depicting denuded villi by scanning electron microscopy (×170). (C) Distal small intestine with normal vacuolated epithelial cells and normal villi (H and E, ×70). (D) Same area as in (C) seen by scanning electron microscopy. Epithelial cells appear round and protruding (×200). [From C. A. Mebus* et al., Vet. Pathol. **14,** *273 (1977); and A. Z. Kapikian and R. M. Chanock,* In *"Virology" (B. N. Fields* et al., *eds.), Raven Press, New York; Courtesy A. Z. Kapikian.]

The mechanism of fluid loss in viral infections is different from that in bacterial infections, but the net loss may be of the same magnitude. In viral infections fluid loss is mainly a loss of extracellular fluid due to impaired absorption, and osmotic loss due primarily to the presence of undigested lactose in the lumen (in sucking animals), rather than active secretion. As virus destroys the absorptive cells there is a loss of those enzymes responsible for the digestion of disaccharides, and the loss of differentiated cells diminishes glucose, sodium carrier, and Na^+,K^+-ATPase activities. This leads to a loss of sodium, potassium, chloride, bicarbonate, and water, and the development of acidosis. Another cause of acidosis is the result of increased microbial activity associated with the fermentation of undigested milk. Acidosis can create a K^+ ion exchange across the cellular membrane, affecting cellular functions that maintain the normal potassium concentration. Hypoglycemia due to decreased intestinal absorption, inhibited glyconeogenesis, and increased glycolysis follow, completing a complex of pathophysiological changes that if not promptly corrected results in death of the animal.

Effective management of viral diarrheas in young animals requires prompt action to prevent continued loss of fluids and electrolytes. This is most readily achieved on the farm by removal of milk from the diet. This reduces the amount of undigested lactose in the lumen of the intestine, and therefore reduces fluid loss and acidosis. Therapy also includes administration of balanced electrolyte solutions orally or parenterally. The use of intravenous fluid replacement and careful monitoring of dehydration status could save a large percentage of the most severely affected animals, but in many settings this is impractical.

In many cases of diarrhea in animals more than one virus is active; if two viruses have different sites of replication their combined effect may be severe. Furthermore, many bacterial infections, e.g., with enterotoxic *E. coli*, are more severe if combined with a viral infection.

Susceptibility decreases rapidly with increasing age; the viral diarrheas are essentially diseases of the first few weeks of life. To prevent infection of newborn animals, antibody must be present continuously in the lumen of the gut. This does not continue for more than about 7 days unless the dam is hyperimmunized against the common etiological agents. Since local immunization of the newborn is often not practical, this strategy of prevention should be actively promoted (see Chapter 14).

Pathogenesis of a Generalized Infection, Canine Distemper

Canine distemper is caused by a virus of the genus *Morbillivirus* (family *Paramyxoviridae*), which is closely related to the viruses of measles and

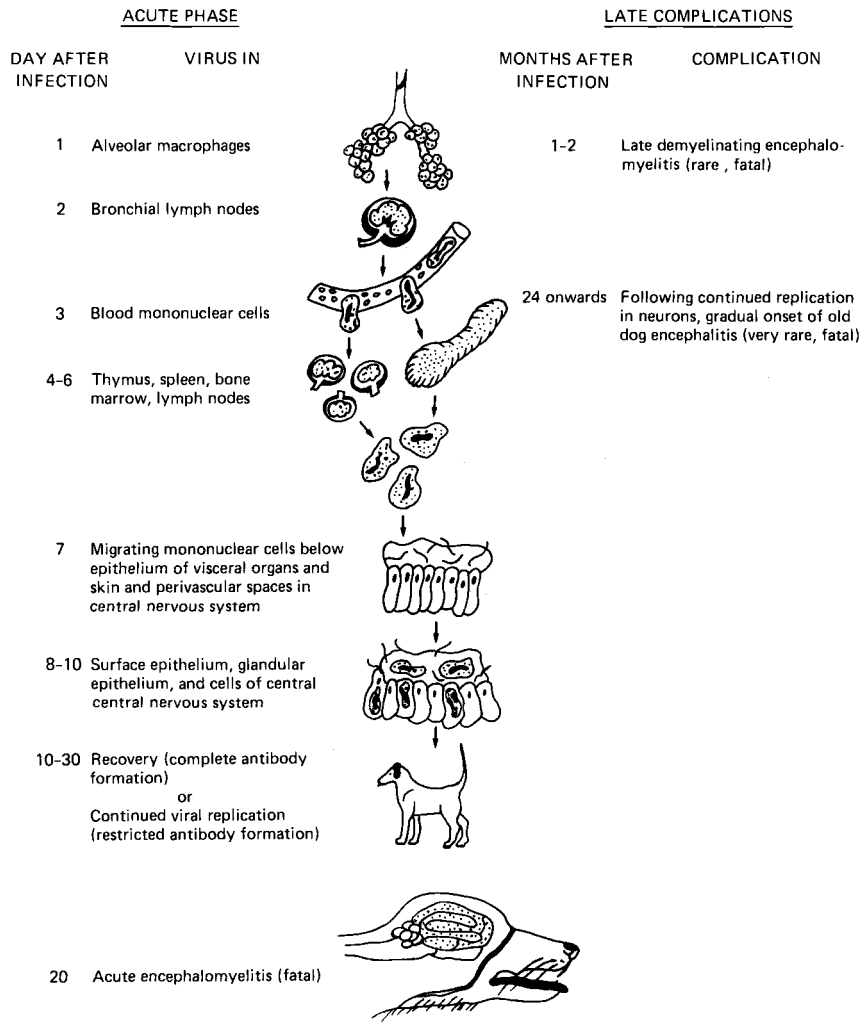

FIG. 10-3. *Diagram illustrating the pathogenesis of canine distemper. (Based on the work of Dr. M. J. G. Appel.)*

rinderpest. It usually causes an acute, self-limited systemic disease, but in some dogs virus invades the central nervous system and causes encephalitis. In a minority of dogs the virus persists in the brain and may cause the late neurological disease, old dog encephalitis (Fig. 10-3).

Infection occurs via virus inhalation into the respiratory tract. Studies of the course of infection by fluorescent-antibody staining of tissues and

organs have revealed that following initial infection of the respiratory epithelium and alveolar macrophages, virus is transferred within 2 days into mononuclear cells in the bronchial lymph nodes and tonsils. During the first week, before the onset of symptoms, cell-associated virus spreads through the bloodstream to the bone marrow, spleen, thymus, cervical and mesenteric lymph nodes, and macrophages in the lamina propria of the stomach and small intestine.

The rate of spread and distribution of virus after the eighth to ninth day varies, and appears to depend on the rate of development of neutralizing antibody, although the role of cell-mediated immunity has not been adequately studied. No antibody is found on the seventh day, but in some dogs the titer reaches 1 : 100 or higher on the eighth or ninth day. In such animals there is no further spread of virus, and virus disappears rapidly from the lymphatic tissues; the infection remains subclinical. If measurable antibody is not present on the ninth day or a titer of 1 : 100 has not been attained by the fourteenth day, virus spreads throughout the body. As well as continued infection of mononuclear cells in the lymphatic system, extensive infection of the epithelium in the intestinal, respiratory, and urogenital tracts, skin, and exocrine and endocrine glands occurs. Infection of the gastrointestinal tract causes vomiting and diarrhea, infection of the respiratory tract causes bronchitis and sometimes pneumonia, and infection of the skin is associated with dermatitis.

The brain is sometimes infected, usually when infection in visceral organs is subsiding. The virus appears first in meningeal macrophages and mononuclear cells in perivascular adventitia and later in ependymal cells, glial cells, and neurons. The infection of neurons is associated with behavioral changes, local myoclony, tonic–clonic spasms, and paresis, which often persist after recovery. Forty to sixty days after apparent recovery, some dogs suffer from postinfection encephalitis, with characteristic demyelination, often leading to death. In these dogs, high titers of neutralizing antibody occur in both blood and cerebrospinal fluid. In addition, very occasionally dogs that have recovered from distemper suffer from encephalitis years later—"old dog encephalitis." Like subacute sclerosing panencephalitis in humans who have recovered from measles, this appears to be due to the very slow replication and spread of distemper virus in the brain. This complication, like acute canine distemper itself, has become more rare as distemper vaccination has become more general.

The course of acute canine distemper is affected by the extent of secondary bacterial infection, but this factor does not affect the central nervous system diseases. Recovery from canine distemper is followed by prolonged immunity, probably lifelong.

Pathogenesis of a Neurological Disease, Rabies

The pathogenesis of rabies is remarkable in that invasion, spread to the central nervous system, and the development of signs occur with mimimal immunological responses, yet even after infection has occurred, early administration of antibody and/or vaccine can often prevent disease.

Infection by the bite of a rabid animal usually results in deposition of rabies-infected saliva deep in the striated muscles, but rabies can occur, albeit with less certainty, after superficial abrasion of the skin. Initially virus replicates in the muscle cells or cells of the subepithelial tissues until it has reached a sufficient concentration to reach motor or sensory nerve endings in the muscle or skin. Here it appears to bind specifically to the acetylcholine receptor or other receptors and enters nerve endings. This begins the second phase of infection, in which neuronal infection and centripetal passive movement of viral nucleic acid within axons leads to involvement of the central nervous system. The incubation period—i.e., the time between the infective bite and the development of signs of central nervous system involvement—is usually between 14 and 90 days, but may occasionally be much longer, possibly because virus remains sequestered in striated muscle cells before entering peripheral nerves and ascending to the brain.

Although rabies proteins are highly antigenic, neither humoral nor cell-mediated responses occur during the stage of movement of virus from the site of the bite to the central nervous system, probably because very little antigen is delivered to the immune system; most is sequestered in muscle cells or within nerve axons. However, this early stage of infection is accessible to antibody and the classical Pasteurian postinfection vaccination (especially with the additional administration of hyperimmune immunoglobulin). Immunological intervention is effective during the long incubation period because of a delay between the initial viral replication in muscle cells and the entry of virus into the protected environment of the nervous system.

Movement along the nerves eventually delivers virus to the central nervous system, usually the spinal cord initially. An ascending wave of neuronal infection and neuronal dysfunction then occurs. Virus reaches the limbic system, where it replicates extensively, and the release of cortical control of behavior leads to "furious" rabies. Spread within the central nervous system continues, and when replication occurs in the neocortex the clinical picture changes to "dumb" rabies. Depression, coma, and death from respiratory arrest follow.

However, before this—and, indeed, coincidentally with its replication in the limbic system that leads to fury—virus moves centrifugally from

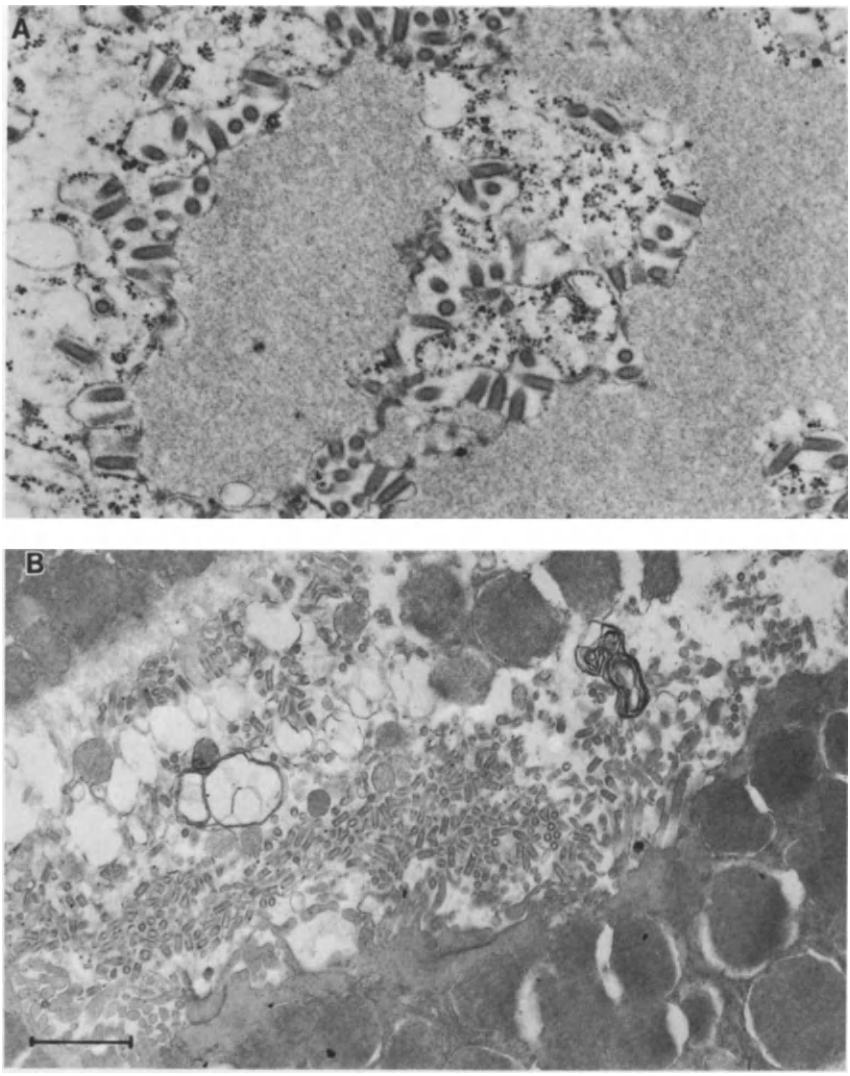

PLATE 10-3. *Electron micrographs of rabies virus infection in the brain (A) and salivary gland (B) (bar = 500 nm). In both organs the infection is noncytopathic, but in the brain nearly all virus is formed by budding upon internal membranes of neurons and so is trapped, while in the salivary gland nearly all virus is formed by budding upon the apical plasma membrane of mucous epithelial cells, where it is free to enter the salivary duct. Some reservoir host species can have* 10^6 ID_{50} *of rabies virus per milliliter of saliva at the time of peak transmissibility. (A) Street rabies virus from a dog in the cytoplasm of a neuron of a mouse 10 days after infection. Bullet-shaped virions are budding upon internal cellular membranes; the granular material is excess viral nucleocapsids forming an inclusion body which by light microscopy is seen as a Negri body. (B) Street rabies virus in the saliva of a fox. Bullet-shaped virions, having budded from mucous epithelial cells, are accumulating in the salivary duct where they are free to be transmitted in saliva injected during a bite.*

the central nervous system down peripheral nerves to a variety of organs: adrenal cortex, pancreas, and, most importantly, the salivary glands. In the nervous system most virus is assembled upon cytoplasmic membranes within cells; the cells are not lysed, so that little viral antigen is released to stimulate host defense mechanisms. In the salivary gland, however, virions bud almost exclusively from plasma membranes at the luminal surface of mucous cells and are released in high concentrations into the saliva (Plate 10-3). Thus at the time when viral replication within the central nervous system causes the infected animal to become furious and bite indiscriminately, the saliva is highly infectious.

On histopathological examination there is little evidence of brain damage, yet electron microscopic or fluorescent-antibody studies show that almost all neurons are infected. There is minimal cellular destruction to match the extensive neurological dysfunction seen in this disease.

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CHAPTER 11

Mechanisms of Persistent Infections

The features of the pathogenesis of viral infections described in Chapter 7—namely, the establishment of infection, followed by spread of the virus through the animal's body, either locally or generally—are found in all viral infections. With many viruses, especially those that produce infections localized in the skin, the respiratory tract, or the intestinal tract, the sequel to the establishment and spread of the virus is an acute disease, which results either in death, or recovery with complete elimination of the virus from the body. The pathogenesis of several such acute infections is described in Chapter 10. However, even in some of these acute infections, such as canine distemper, in a minority of cases virus is not eliminated, but may persist for months or years, causing late pathological manifestations. Viruses of some families, notably the herpesviruses, have long been known to cause infections that usually persist for the life of the animal, although episodes of clinical disease might occur infrequently and at long intervals. In addition, viruses have been found to be responsible for many chronic diseases, in which virus persists for months or for life and causes continuing, often subtle, pathological effects. These *persistent viral infections* are important for four reasons:

1. They are often of epidemiological importance, as a source of infection of other animals, and provide a mechanism for the maintenance of the virus in nature.
2. They may be reactivated and cause acute episodes of disease.
3. They may lead to immunopathological disease.
4. They may lead to neoplasia.

Persistent infections of one type or another are produced by viruses of all families; indeed in veterinary medicine acute self-limiting infections seem to be the exception rather than the rule, apart from the viral diarrheas and most viral respiratory infections. As in cultured cells (see Chapter 6), such infections may be characterized by the continuous or intermittent production of infectious virus or by the persistence of the viral genome either as provirus or as an episome. Some viruses, characteristically the alphaherpesviruses, produce cytocidal infections in most cultured cell systems, but persistent as well as cytocidal infections *in vivo*.

CATEGORIES OF PERSISTENT INFECTIONS

For convenience, persistent infections may be subdivided into three categories:

1. *Latent infections:* virus is *latent*, i.e., generally not demonstrable, except when reactivated to replicate, such episodes being sometimes associated with recurrence of disease (see Table 11-1).
2. *Chronic infections:* virus is continuously demonstrable and often shed; disease may be absent, or chronic, or may develop later, often with an immunopathological basis (see Table 11-2).
3. *Slow infections:* virus gradually increases during a very long preclinical phase, leading to slowly progressive lethal disease (see Table 11-3).

The key distinctions between these three groups of persistent infections are illustrated diagrammatically in Fig. 11-1. It may be noted that these three categories are defined in terms of the extent of viral replication in the body during the long period of persistence; presence or absence of virus shedding and of disease are secondary issues as far as these definitions are concerned. In chronic infections, virus is produced continuously and may be shed in body secretions, or may always be present in the blood, or may be sequestered in cells of an internal organ, e.g., the brain. Some persistent infections in all three categories are associated with disease, some are not, but the "carrier" status of all such

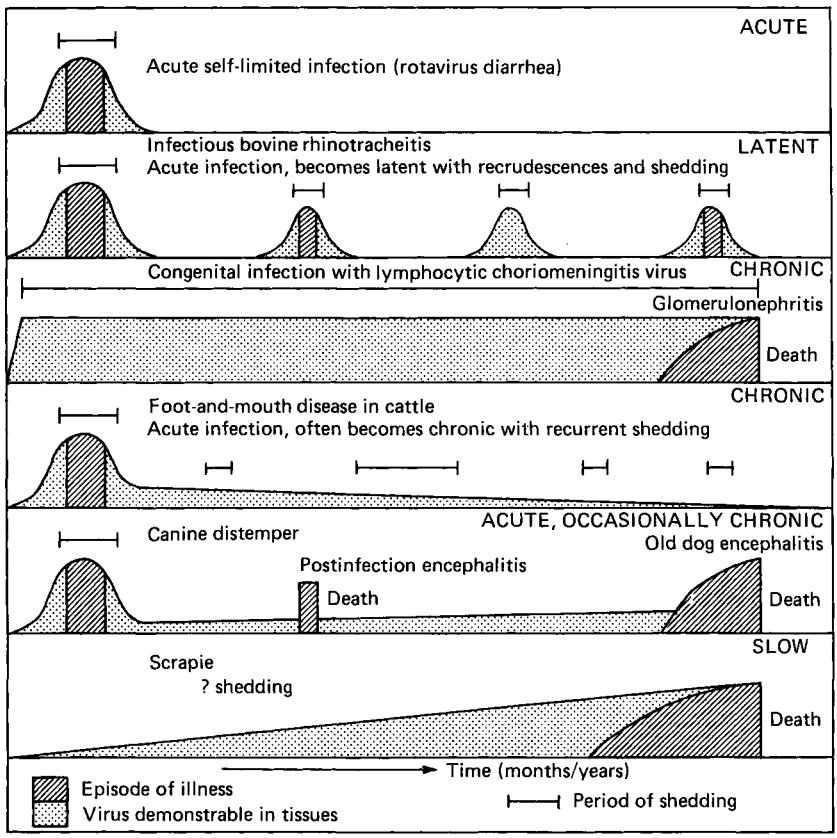

FIG. 11-1. *Diagram depicting the presence and shedding of virus and the occurrence of clinical signs in acute self-limited infections and various kinds of persistent infection, as exemplified by the diseases indicated. The time scale is notional and the duration of various events approximate.*

animals makes them a source of infection for others. While there are some persistent infections that possess features of more than one of these categories, it is useful to retain the terms so as to focus attention on the vital question: namely, what is the virus doing during its lifelong sojourn in the body?

LATENT INFECTIONS

Latency following recovery from a primary acute infection is a general feature of infections with herpesviruses (Table 11-1). The mechanisms of

TABLE 11-1

Examples of Latent Infections: Persistent Infections with Viral Latency and Recrudescence, with or without Recurrent Clinical Episodes

Virus	Disease	Sites of infection		Virus shedding	Neutralizing antibodies
		Between disease episodes	During disease episodes		
Bovine herpesvirus 1	Infectious bovine rhinotracheitis	As episomal DNA, in cerebral or dorsal root ganglion neurons	Respiratory and genital mucosa	During disease episodes in saliva and genital secretions	+
Cytomegalovirus (several host-specific viruses)	Usually subclinical	As episomal DNA, in salivary gland and bladder epithelial cells and leukocytes	Various epithelial cells	Sporadically throughout life in saliva and urine	+

latency and reactivation are assumed to be comparable for all herpesviruses, although they have been described in greatest detail in herpes simplex, varicella-zoster, and EB virus infection in humans, and to a lesser extent in pseudorabies in swine and infectious bovine rhinotracheitis. During a primary infectious bovine rhinotracheitis virus infection, virions move to the cranial or spinal ganglia along the axons of sensory nerves. Virus persists in ganglion neurons as episomal viral DNA, some of which may be transcribed to mRNA, but little or none of which is translated except when reactivation and the production of infectious virus occurs. In recurrent infectious bovine rhinotracheitis infections, virus moves down the sensory nerves again until it reaches the nasal mucous membranes or the skin, where further replication occurs in epithelial cells, with virus shedding. There is debate as to whether intermittent reactivation of latent virus is associated with the development of lesions or clinical disease, but reactivation and shedding of virus is the mechanism whereby virus is maintained from generation to generation in the bovine population.

Betaherpesviruses (cytomegaloviruses) establish latent infections, probably in salivary gland and bladder epithelium as well as in monocytes and/or lymphocytes. Virus is shed, intermittently or continuously, particularly into the oropharynx, from which it may be transmitted via saliva, and into the urine, from which it may be transmitted directly.

CHRONIC INFECTIONS

A large number and variety of viral infections fall into this category of persistent infections (Table 11-2), which is marked by continuous virus production. There may be no disease (e.g., chronic foot-and-mouth disease infections), chronic disease (e.g., African swine fever), or disease occurring only as a late complication (e.g., old dog encephalitis after canine distemper). Virus may be shed for years (e.g., foot-and-mouth disease in cattle), or not at all (e.g., old dog encephalitis). Some of the listed infections are described more fully in the appropriate chapters of Part II. Here we present three illustrative examples: lymphocytic choriomeningitis in congenitally infected mice, chronic foot-and-mouth disease infection in cattle, and old dog encephalitis.

Lymphocytic Choriomeningitis

Lymphocytic choriomeningitis, caused by an arenavirus, is the classical persistent viral infection, consideration of which was important for the formulation by Burnet of the concept of *immunological tolerance*. Lym-

TABLE 11-2

Examples of Chronic Infections: Persistent Infections with Virus Demonstrable for Long Periods, with or without Disease

Virus (genus)	Host	Sites of persistent infection	Virus shedding	Antibodies	Chronic disease
African swine fever virus	Swine	Hematopoietic system	Variable	+ (Nonneutralizing)	Intermittent fever
Foot-and-mouth disease virus (*Aphthovirus*)	Cattle, buffalo	Soft palate and pharynx	Intermittent and prolonged	+	No signs
Canine distemper virus (*Morbillivirus*)	Dog	Brain	Nil	++In serum +In cerebrospinal fluid	Rarely: old dog encephalitis
Lymphocytic choriomeningitis virus (*Arenavirus*)	Mouse	Widespread	Continuous	+ (Nonneutralizing)	Sometimes late glomerulonephritis
Aleutian disease virus (*Parvovirus*)	Mink	Macrophages	Continuous	++(Nonneutralizing)	Late: vasculitis, glomerulonephritis, fatal
Hog cholera virus (*Pestivirus*)	Swine (congenital infection)	Widespread	Continuous	+(Nonneutralizing)	Systemic, progressive, fatal

phocytic choriomeningitis virus is transmitted horizontally and *in utero*, such that every mouse in a colony may become infected. Such infected mice are normal at birth and appear normal for most of their lives, although careful study of the physiological activity of some endocrine glands shows that their specialized functions may be impaired. Infected mice have persistent viremia and viruria; almost every cell in the mouse is infected and remains so throughout life. Circulating free antibody cannot be detected, but immunological tolerance is not complete; some antibody is formed and circulates as virion–IgG–complement complexes, which are infectious. However, there is no cell-mediated immune response to the virus. Late in life inbred mice (but not wild house mice) may exhibit "late disease" due to the deposition of viral antigen–antibody complexes in the renal glomeruli. Other members of the family *Arenaviridae* cause similar persistent infections of wild rodents in Africa and South America and produce severe disease, with hemorrhagic signs, when humans are accidentally infected (Lassa fever, Bolivian and Argentine hemorrhagic fevers).

Foot-and-Mouth Disease

Although convalescence after foot-and-mouth disease in cattle is often protracted, it used to be thought that recovery was complete, with elimination of the virus. However, it is now known that foot-and-mouth disease viruses can cause a persistent infection of the pharynx of cattle, sheep, goats, and other ruminants. Not all infected animals become carriers, nor is there any correlation between antibody levels and the carrier state. Cattle vaccinated with inactivated vaccine may become carriers if subsequently infected.

The recovery of virus from pharyngeal fluids is often intermittent, possibly because of variability in sampling technique, but isolations have been made from cattle and buffalo for up to 2 years after infection. The mechanism of persistence is unknown and its epidemiological significance is difficult to assess. Pharyngeal fluids may contain large amounts of virus, which may be aerosolized by cattle when they cough, but attempts to demonstrate transmission from carrier to susceptible cattle have given equivocal results, although transfer of infection from persistently infected African buffalo to cattle is known to occur.

Old Dog Encephalitis

Canine distemper is an acute systemic infection in which the majority of dogs recover completely within a month of the onset of signs (see Chapter 10). It has now been recognized that in a minority of cases dogs

TABLE 11-3

Slow Infections: Long Preclinical Phase, Slowly Progressive Fatal Disease

Group	Virus or agent	Host	Sites of infection	Antibodies	Disease
Lentivirinae	Visna–maedi virus	Sheep	Macrophages, brain and lung	+ (Nonneutralizing)	Slowly progressive pneumonia or encephalitis
	Ovine progressive pneumonia virus				
	Caprine arthritis–encephalitis virus	Goat	Macrophages, brain and joints	+ (Nonneutralizing)	Arthritis, encephalitis
	HIV	Human	Helper T lymphocytes	+ (Nonneutralizing)	Acquired immune deficiency syndrome (AIDS)
Subacute spongiform encephalopathy	Scrapie agent	Sheep	Central nervous system and lymphoid tissue	–	Slowly progressive encephalopathy
	Mink encephalopathy agent	Mink			
	Kuru agent	Human			
	Creutzfeldt–Jacob agent	Human			
	Wasting disease agent	Deer, elk			

that have recovered from canine distemper continue to harbor the virus in brain cells, where it replicates slowly and eventually produces old dog encephalitis. The situation is analogous to that of subacute sclerosing panencephalitis in the corresponding human infection, measles.

In subacute sclerosing panencephalitis, at the time of death, certain nerve cells contain large masses of viral nucleocapsids, but virions are not made, apparently because a mutant virus has been selected *in vivo* which is defective in the production of matrix protein and possibly other envelope components. Nevertheless, the complete viral genome must be present, as measles virus can be isolated *in vitro* by cocultivation of brain cells with permissive cells. The situation is similar in old dog encephalitis, but virus can be readily cultivated from the brains of affected dogs.

SLOW INFECTIONS

The term "slow infections" was originally used to describe slowly progressive retrovial diseases found in sheep in Iceland. The term is now used to categorize several such infections that have a very long preclinical phase (incubation period) and then cause a slowly progressive, invariably lethal disease. They are persistent infections in that virus can be recovered from infected animals during the preclinical phase and also after clinical signs have appeared. The group (Table 11-3) includes the lentiviruses and the unclassified agents that cause the subacute spongiform encephalopathies.

Lentiviral Diseases: Visna/Maedi and AIDS

Visna/maedi virus belongs to the family *Retroviridae*, subfamily *Lentivirinae*. It and related viruses cause chronic demyelinating disease of the central nervous system and chronic pneumonia in sheep and chronic arthritis and encephalitis in goats. It produces persistent productive infection, mainly in circulating leukocytes, and may also exist as integrated DNA provirus. A feature of visna–maedi, and of another persistent lentivirus infection, equine infectious anemia, is the occurrence of antigenic drift in surface proteins during the progress of infection in a single animal.

Human immunodeficiency virus (HIV), the etiological agent of AIDS in humans, is also a lentivirus. It replicates in and destroys helper T cells, and it is demonstrable in the blood of patients for life. Within 5 years of being infected, at least 10% of those infected develop the acquired immune deficiency syndrome and eventually die from oppor-

tunistic infections that result from the immunosuppressive effects of the virus. The antibody that is made is nonneutralizing, and infected individuals can transmit virus to contacts by sexual intercourse, blood transfusion, or intravenous drug abuse practices.

Subacute Spongiform Viral Encephalopathies

This term is used as a generic name for five diseases that have strikingly similar clinicopathological features and causative agents, namely, scrapie of sheep and goats, mink encephalopathy, wasting disease of deer and elk, and kuru and Creutzfeldt–Jakob disease in humans. The basic lesion is a progressive vacuolation in neurons, and to a lesser extent, in astrocytes and oligodendrocytes, an extensive astroglial hypertrophy and proliferation, and finally a spongiform change in the gray matter.

Scrapie. Scrapie is an infection of sheep, usually transmitted from ewe to lamb. Infection was widely disseminated in Britain by the inoculation of sheep with louping ill vaccine that was contaminated with the scrapie agent. The preclinical phase (incubation period) is very long, up to 3 years, and once signs have appeared the disease progresses slowly but inevitably to paralysis and death.

Research was limited until it was discovered that mice and hamsters could be infected, with incubation periods of less than a year. Experimental studies in mice reveal that scrapie behaves as a typical infectious disease, and filtration shows that the causative agent is the size of a very small virus. Unusual features are the apparent absence of an immune response and the lack of effect of either interferon or measures that augment or depress the immune system. Tests on the inactivation of infectivity by a variety of physical and chemical treatments suggest that the scrapie agent has a higher degree of resistance than conventional viruses. These unusual biological and physicochemical properties appear to be shared by the agents of the other four subacute spongiform encephalopathies.

PATHOGENESIS OF PERSISTENT INFECTIONS

The term "persistent infections" embraces such a wide variety of different conditions that it is not surprising to find that there are several mechanisms whereby such viruses bypass the host defenses that eliminate virus in acute infections. They include factors related primarily to

the virus on the one hand, or to the host defenses on the other, although the two kinds of factors interact in some instances.

Unique Properties of the Virus

Nonimmunogenic Agents. The uncharacterized agents that cause the subacute spongiform encephalopathies seem to be completely nonimmunogenic; they do not induce interferon, nor are they susceptible to its action. If with further study this turns out to be true, there may be no mechanism whereby the host can control the replication and pathological effects of these agents.

Integrated Genomes. Retroviruses whose proviral DNA is integrated are maintained indefinitely, from one generation to the next, as part of the genome of the host. Apart from the possibility that such proviral DNA may be implicated in tumorigenesis (see Chapter 12), it does not produce disease. Persistent provirus is not involved in lentiviral infections, but viral replication causes systemic disease, or lesions in the lungs, brain, lymphoid tissues, and joints.

Growth in Protected Sites. During their latent phase, most alphaherpesviruses avoid immune elimination by remaining within cells of the nervous system, as DNA in ganglion cells during the intervals between disease episodes, and within axons prior to acute recurrent episodes of disease. Betaherpesviruses (e.g., cytomegalovirus) and gammaherpesviruses (e.g., Marek's disease virus) avoid immune elimination by persisting in lymphocytes.

Certain other viruses grow in epithelial cells on luminal surfaces (e.g., kidney tubules, salivary gland, or mammary gland) and are shed more or less continuously in the corresponding secretions. Most such viruses are not acutely cytopathogenic, and, perhaps because they are released on the luminal borders of cells, they do not provoke immune or inflammatory reactions. Secretory IgA, which is present in the secretions at such sites, does not activate complement, hence complement-mediated cytolysis does not occur.

Antigenic Variation. Visna–maedi and equine infectious anemia viruses are retroviruses which avoid the host's immune response by antigenic drift. During the persistent infection, a succession of antigenic variants develops within the infected animal, enabling each successive variant to evade the immune response. In equine infectious anemia clinical signs occur in cycles, each cycle being initiated by a new antigenic variant of the virus. Persistence of influenza virus, not in individual animals but in populations, occurs by a similar mechanism, oper-

ating over a longer time span and cumulatively in a succession of animal hosts.

Modification of Host Defense Mechanisms

Modification of the immune response is achieved in a variety of ways, some of which are also seen in nonpersistent viral infections (Table 11-4). They fall into several broad categories: ineffective antibodies, dis-

TABLE 11-4
Ineffective Immune Responses in Persistent Viral Infections[a]

Phenomenon	Mechanism	Examples[b]
No antibody	Nonimmunogenic agent	Scrapie
Nonneutralizing antibody	Small amounts, or low affinity, or reacting with irrelevant epitopes	Aleutian disease virus African swine fever virus Lymphocytic choriomeningitis virus
Enhancing antibody	Antibody attached to virus enhances infection of macrophages	Cytomegaloviruses Lactic dehydrogenase virus Dengue viruses
Disturbance of lymphocyte functions	Infection of lymphocytes	Infectious bursal disease virus Cytomegaloviruses HIV
Disturbance of macrophage functions	Infection of macrophages	Lactic dehydrogenase virus African swine fever virus
Antigen-specific suppression	Induction of T_s cells or clonal deletion of T cells	Lymphocytic choriomeningitis virus Herpesviruses
Avoidance of immune lysis	1. Little or no antigen on cell membrane	Herpes simplex virus in ganglion cell Marek's disease virus in T cell
	2. Loss of viral antigen by "stripping"/endocytosis	Canine distemper virus in neurons
	3. Antigen on inaccessible membrane	Cytomegaloviruses, rabies virus
	4. Fc receptor induced on infected cell; IgG binds nonspecifically	Alphaherpesviruses Cytomegaloviruses
Antigenic variation	Antigenic drift within host	Visna–maedi virus Equine infectious anemia virus

[a]Some of these mechanisms are also operative in nonpersistent infections.

[b]Speculative only; in several of these instances an association exists but no cause-and-effect relationship has been demonstrated between the immunological phenomenon and the persistent infection listed.

turbance of function of cells of the immune system, avoidance of immune lysis of infected cells, and antigenic variation of the virus. Persistent infections often become reactivated when another disease, form of treatment, or physiological condition interferes in some way with an immune response which was formerly operating effectively.

Defective Antibody Response. Viruses that cause persistent plasma-associated viremia not only replicate in lymphoid tissue and macrophages but also characteristically induce production of nonneutralizing antibodies. These antibodies combine with viral antigens and virions in the serum to form immune complexes which may produce "immune complex disease," usually by deposition in the renal glomeruli. Such antibodies may also block immune cytolysis of virus-infected target cells by T cells.

Many persistent infections are associated with a very weak antibody response, and such antibodies as are produced are nonneutralizing. The severe specific hyporeactivity found in congenital infections with bovine virus diarrhea, hog cholera, and lymphocytic choriomeningitis viruses, and in some retrovirus infections is called *immunological tolerance.* Tolerance to particular viral antigens is genetically determined.

Defective Cell-Mediated Immunity. Persistent infections may be caused by partial suppression of the host's cell-mediated immune response, as a result of any one or a combination of several factors: immunosuppression by the causative virus, immunological tolerance, the presence of "blocking" antibodies or virus–antibody complexes, failure of immune lymphocytes to reach target cells, increase in the ratio of T_s to T_h cells, inadequate expression of viral antigens on the surface of the target cell, or stripping/endocytosis of surface antigens. These factors are probably important in persistent infections caused by lentiviruses and herpesviruses.

Growth in Macrophages. In many chronic infections the virus appears to grow mainly in reticuloendothelial tissue, especially in macrophages. This may have two effects relevant to persistence: (1) impairment of the humoral and cell-mediated immune response, and (2) impairment of the phagocytic and cytotoxic activities of the reticuloendothelial system. Furthermore, some persistent viruses replicate in lymphocytes; e.g., in AIDS in human beings, helper T cells are virtually eliminated from the body.

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CHAPTER 12

Mechanisms of Viral Tumorigenesis

It may be useful at the outset of this chapter to define certain commonly used terms. A *benign tumor* is a lump produced by abnormal cell proliferation which remains localized and does not invade adjacent tissue. A *malignant tumor,* in contrast, is usually locally *invasive* and may also be *metastatic,* i.e., spread by lymphatic and blood vessels to other parts of the body. Such malignant tumors are often referred to as *cancers.* Malignant tumors of epithelial cell origin are known as *carcinomas,* those arising from cells of mesenchymal origin as *sarcomas,* and those from lymphocytes as *lymphomas* (if solid tumors) or *leukemia* (if circulating cells are involved). The process of development of tumors is termed *tumorigenesis,* synonyms for which are *oncogenesis* and *carcinogenesis.* The capacity to study tumorigenesis at a molecular level was greatly facilitated when it became possible to induce essential genetic changes in cultured cells (*transformation,* see Chapter 6).

Since the discovery of the viral etiology of avian leukemia by Ellerman and Bang and of avian sarcoma by Rous in 1908 and 1911, respectively, there has been a steady stream of discoveries clearly incriminating vi-

TABLE 12-1
Viruses That Induce Malignant Tumors in Domestic Animals

Host	Viral family	Virus	Transformation of cultured cells	Tumor
Cattle	*Retroviridae*	Bovine leukemia virus	No	Leukemia
	Papovaviridae	Bovine papillomavirus 4	Yes	Carcinoma of intestine, bladder
Cat	*Retroviridae*	Feline leukemia virus	No	Leukemia
		Feline sarcoma virus	Yes	Sarcoma
Chicken	*Retroviridae*	Avian leukosis virus	No	Leukosis, osteopetrosis, nephroblastoma
		Rous sarcoma virus	Yes	Sarcoma
		Avian erythroblastosis virus	Yes	Erythroblastosis
		Avian myeloblastosis virus	Yes	Myeloblastosis
		Avian reticulo-endotheliosis	Yes	Reticulo-endotheliosis
	Herpesviridae	Marek's disease virus	Yes	Lymphoma

ruses in a variety of benign and malignant tumors of numerous species of mammals, birds, amphibia, and reptiles (Table 12-1). Many retroviruses and a few herpesviruses cause malignant tumors under natural conditions, while papillomaviruses of many species of animals produce benign tumors, which may become malignant in cattle, rabbits, and humans, at least. Hepadnaviruses are implicated in the production of primary hepatocellular carcinoma in humans, ducks, and woodchucks. Poxviruses of several genera produce fibromas or other benign tumors of the skin, but these never become malignant. Polyomaviruses and some adenoviruses are tumorigenic when inoculated into newborn rodents, but are not in nature.

Intensive studies over the past two decades, using polyomavirus and SV40 in baby rodents and retroviruses of chickens and mice, have revolutionized our understanding of the molecular mechanisms of viral tumorigenesis and of tumorigenesis in general. An important element in

our present understanding has come from the discovery of *oncogenes*, which derives from work with retroviruses.

TUMOR INDUCTION BY RETROVIRUSES

Viruses of the subfamily *Oncovirinae* of the family *Retroviridae* are the major cause of leukemias and lymphomas in many species of animals and birds, including cattle, cats, apes, mice, and chickens. Although the subfamily *Oncovirinae* is the only one of the three subfamilies of the family *Retroviridae* that includes tumorigenic viruses, workers in the field tend to use the family rather than the subfamily name (i.e., in the vernacular, "retroviruses" rather than "oncoviruses") when referring to these tumorigenic retroviruses, a practice that we will follow here, but not in Chapter 31, which deals with all three subfamilies.

Categories of Tumorigenic Retroviruses

Retroviruses of the subfamily *Oncovirinae* have been grouped into several genera on the basis of virion morphology, and members of the most abundant genus—those with a so-called C-type virion morphology—are further categorized according to their hosts (see Chapter 31). More important from the point of view of the mechanisms of viral tumorigenesis, however, are subdivisions of the C-type retroviruses according to other criteria, as outlined below.

Defective and Nondefective Retroviruses. The genome of a typical nondefective endogenous retrovirus (Fig. 12-1A) contains two identical copies of an RNA molecule, each of which has three genes, known as *gag* (encoding the four core proteins), *pol* (encoding the unique viral polymerase, reverse transcriptase), and *env* (encoding the two envelope glycoproteins). The rapidly tumorigenic exogenous retroviruses carry a fourth gene, *onc* (for *oncogene,* which is responsible for the malignant change in the infected cell). Because the oncogene has been incorporated into the viral RNA in place of part of one or more normal viral genes (Fig. 12-1C), the genome is *defective;* hence such viruses are dependent on nondefective *helper* retroviruses for their replication. Rous sarcoma virus, upon which many of the classical studies were made, is atypical in that its genome contains a viral oncogene (v-*src*) in addition to complete copies of all the retrovirus genes, *gag, pol,* and *env* (Fig. 12-1D).

Endogenous and Exogenous Retroviruses. As outlined in Chapter 31, a complete DNA copy of the genome (*provirus*) of one, or sometimes more

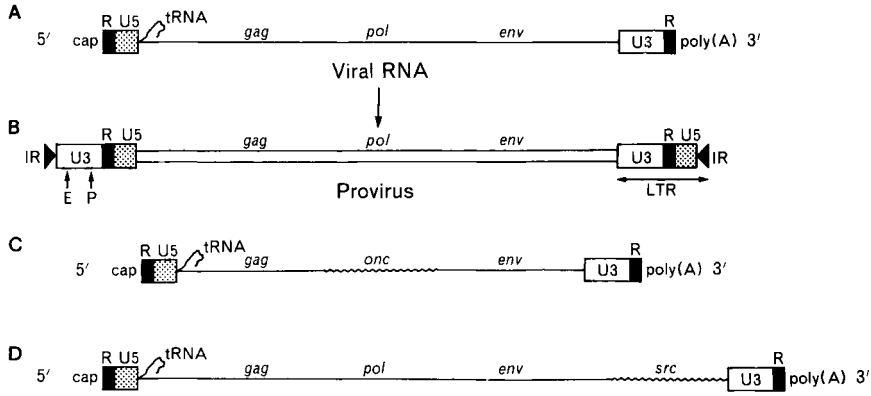

FIG. 12-1. *Structure of retrovirus genome and integrated provirus. (A) One of the two identical ssRNA molecules that comprise the diploid genome of a replication-competent slowly transforming retrovirus. The major coding regions,* gag, pol, *and* env, *encode the viral proteins. The 5′ terminus is capped and the 3′ terminus is polyadenylated. A short sequence (R) is repeated at both ends of the molecule, while unique sequences (U5 and U3) are located near the 5′ and 3′ termini, immediately proximal to R. A cellular tRNA molecule is attached in the vicinity of U5. (B) Provirus as integrated into cellular DNA. The genome is now dsDNA. It is flanked at each terminus by additional sequences known as the long terminal repeat (LTR). Each LTR comprises U3, R, and U5 plus short inverted repeat sequences (IR) at the distal end. U3 contains the promoter (P) and enhancer (E) sequences, as well as several other sequences with important functions. (C) One of the two identical ssRNA molecules of the genome of a defective rapidly transforming retrovirus. A v-*onc *gene has replaced all of the* pol *and part of the* gag *and* env *genes. (D) One of the two identical ssRNA molecules of the genome of a Rous sarcoma virus, a replication-competent rapidly transforming retrovirus. A v-*onc *gene (v-*src*) is present in addition to complete* gag, pol, *and* env *genes.*

than one of the many species of retroviruses may be transmitted in the germ-line DNA from mother to offspring (see Fig. 31-2) and may thus be perpetuated in the DNA of every cell of every individual of certain vertebrate species. Proviral genomes are under the control of cellular regulatory genes and are normally totally silent in feral animals. Such retroviruses are said to be *endogenous* (Table 12-2). However, proviruses can be activated (induced) by various factors such as irradiation, exposure to mutagenic or carcinogenic chemicals, and hormonal or immunological stimuli. Under these circumstances virions are synthesized. In contrast, other retroviruses behave as more typical infectious agents, spreading horizontally to contacts, and are said to be *exogenous*. Many exogenous retroviruses are recombinants that have arisen in the labora-

TABLE 12-2
Comparison of Endogenous and Exogenous Retroviruses

Parameter	Endogenous	Exogenous	
		Slowly transforming	Rapidly transforming
Transmission	Vertical (germ line)	Horizontal	Horizontal
Expression	Usually not, but inducible	Yes	Yes
Genome	Complete	Complete	Defective[a]
Replication	Productive	Productive	Requires helper[a]
Oncogene	No	No	Yes
Tumorigenicity	Nil, or rarely leukemia	Leukemia after long incubation period	Sarcoma, leukemia, or carcinoma—short incubation period
In vitro transformation	No	No	Yes

[a]Except for Rous sarcoma virus.

tory, or perhaps following fortuitous coinfection of an animal; they do not occur in an endogenous provirus form in nature.

The host species from which a particular retrovirus was originally recovered may not necessarily be its native host. Some endogenous retroviruses are *ecotropic* (i.e., replicate only in the host species from which they originate), while others are *amphotropic* (i.e., replicate in native and certain foreign hosts), and yet others are *xenotropic* (i.e., cannot replicate in the host species in whose genome they are carried as provirus, but can in certain other, not necessarily related, species).

Tumorigenicity. Most endogenous retroviruses never produce disease, cannot transform cultured cells, and contain no oncogene in their genome (see below). Most exogenous retroviruses, on the other hand, are tumorigenic; some characteristically induce leukemia or lymphoma, others sarcoma, yet others carcinoma—usually displaying a predilection for a particular type of target cell. Exogenous retroviruses can be further subdivided into rapidly tumorigenic and weakly tumorigenic viruses. The highly tumorigenic sarcoma viruses, like Rous sarcoma virus, are the most rapidly acting carcinogens known, causing death in as short a time as 2 weeks after infection in certain host species, and rapidly transforming cultured cells *in vitro*. These properties are attributable to a viral

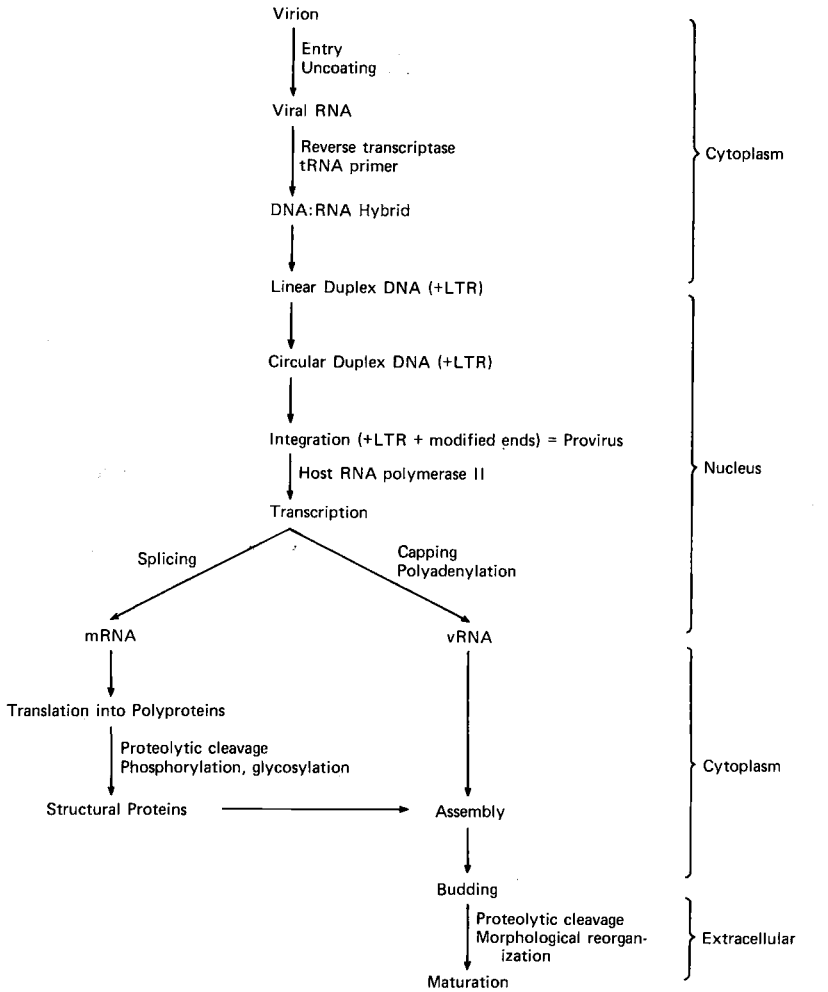

FIG. 12-2. *Steps in the replication of retroviruses.*

oncogene (v-*onc*, the general abbreviation for all viral oncogenes) which they carry in their genome. There are over 20 known viral oncogenes; most exogenous retroviruses carry only one particular type of v-*onc*, e.g., v-*src* in the case of Rous sarcoma virus. The weakly tumorigenic ("slowly transforming") viruses, on the other hand, contain no viral oncogene, but can induce B cell, T cell, or myeloid leukemia with low efficiency and after a much longer incubation period. For example, the avian leukosis viruses, which are endemic in many chicken flocks, pro-

duce lifelong viremia in some chickens, which usually causes no disease but in a small percentage of birds leads later in life to a wide variety of diseases involving the hematopoietic system or rarely solid tumors (sarcomas, carcinomas, and endotheliomas) (see Chapter 31).

Replication of Retroviruses

The complete replication cycle of retroviruses is described in Chapter 31, but certain aspects associated with the integration of the DNA copy of the RNA genome into the cellular DNA are described here, in order to explain viral tumorigenesis. The key steps in the integration and replication of the retroviral genome are depicted in Figs. 12-1A and B and 12-2. Following attachment, penetration, and uncoating to release the (+) sense diploid RNA genome, the reverse transcriptase makes a (−) sense DNA copy, using viral RNA as the template. The parental RNA molecule is then removed from the DNA–RNA hybrid by ribonuclease H, a second enzymatic activity carried by the reverse transcriptase molecule. By a process not yet fully elucidated, the nascent (−) sense DNA strand is converted into dsDNA, the termini of which are flanked by additional identical sequences some hundreds of nucleotides long, known as the *long terminal repeat (LTR).* The LTR comprises sequences derived from both ends of the viral RNA: U3 from the 3′ end, U5 from the 5′ end, and a short sequence, R, which is present at both ends of genomic RNA (Fig. 12-1). This linear dsDNA now migrates from the cytoplasm to the nucleus where it becomes circularized. Several copies of the circularized dsDNA are then integrated into host chromosomal DNA at random or quasi-random sites. During integration, short direct repeats of cellular DNA are synthesized and joined to each end of the provirus. The provirus is a complete and independent transcription unit, because the LTR contains a *promoter* which directs initiation of transcription, and an *enhancer* which enhances transcription, and may confer tissue specificity on the virus. The proviral DNA may simply remain integrated and be transmitted to that cell's progeny, or it may be transcribed into RNA, leading to the production of virions. Such productive infections are noncytocidal; the cell survives and divides.

ONCOGENES

The rapidly tumorigenic retroviruses carry a viral oncogene. Deletion of the oncogene removes the tumorigenicity of the virus. Furthermore, *transfection* of cultured cells with a DNA copy of the oncogene alone, linked to the viral LTR, which contains promoter and enhancer se-

quences, is all that is needed to induce cell transformation. For example, a DNA copy of the v-*src* oncogene of Rous sarcoma virus, in association with the appropriate LTR, transforms chicken fibroblasts into sarcoma cells. Moreover, cells transformed by a sarcoma virus containing a *ts* mutation in its v-*src* gene can be alternately transformed at the permissive temperature, returned to the normal phenotype at the nonpermissive temperature, then transformed again at the permissive temperature. These findings demonstrate that the protein product of an oncogene is required and sufficient for both the initiation and the maintenance of transformation.

Oncogenes are not necessary for viral replication; in most cases they render the virus defective because they displace parts of the viral structural genes. This observation provides a hint as to their real role and origin. In fact, they originate from *cellular oncogenes* (c-*onc*) (also known as *protooncogenes*), which are accidentally incorporated by recombination into the genome of a retrovirus. Over 20 distinct c-*onc* genes have been recognized. Unlike v-*onc* genes: (1) they are located at particular and stable positions on a chromosome of cells of every individual of the species, just like any other cellular gene, (2) they contain introns, (3) they segregate as classical Mendelian loci, (4) they are highly conserved through evolution (e.g., c-*src* genes from *Drosophila* and humans are 95% homologous), and (5) they are normally transcribed at low levels, but their expression may increase substantially in particular tissues and/or at particular stages of differentiation. This strongly suggests that c-*onc* genes are essential genes and that they may play key roles in the normal regulation, division, and differentiation of cells.

The 20 or more different retroviral v-*onc* genes closely resemble, but are not identical with, the corresponding c-*onc* genes, the principal differences resulting from (1) deletion in the v-*onc* genes of intron sequences and cellular transcriptional signals, (2) substitution of a small number of nucleotides by point mutation, and/or (3) deletion and/or rearrangement of some portion(s) of the coding sequence. The protein encoded by the v-*onc* gene usually has similar function, attributes, and intracellular location to the protein encoded by the corresponding c-*onc* gene from which the v-*onc* is presumed to be derived.

In 1982 it was found that the DNA of malignant cells from chemically induced experimental animal tumors or naturally occurring human carcinomas contains a gene which not only differs very slightly from a corresponding c-*onc* gene in nonmalignant cells, but also displays considerable homology with a v-*onc* gene from a known tumorigenic retrovirus, and can itself transform cultured cells. The DNA of human bladder carcinoma cells was shown to differ from that of normal bladder

epithelial cells by a single nucleotide substitution in a single c-*onc* gene. Transfection of NIH3T3 mouse fibroblasts in culture with this mutant gene transformed the cells into sarcoma cells. The c-*onc* gene involved (now known as c-*Ha-ras*) has a remarkable degree of homology with the v-*Ha-ras* oncogene of the Harvey murine sarcoma virus.

These findings indicate that c-*onc* genes can induce tumors as a consequence of mutation occurring either *in situ* or following recombination with the genome of a retrovirus which is subsequently reinserted into cellular DNA. There is evidence to suggest that tumors may also be produced as a result of unregulated expression of normal c-*onc* genes. Possible mechanisms whereby these events could occur are the subject of intensive research today, for, as J. M. Bishop has surmised, it is not unlikely that oncogenes may represent "the final common pathway to tumorigenesis . . . . An enemy has been found; it is part of us."

Proteins Encoded by Oncogenes

The 20 or more retroviral v-*onc* genes and their cellular counterparts have been grouped according to the nature and function of the protein for which they code (Table 12-3). One group, typified by v-*myc*, is a DNA-binding protein, which is thought to regulate transcription. By contrast, the proteins of the v-*ras* family are found in the plasma membrane and regulate adenylate cyclase. The v-*sis* gene-product is a growth

TABLE 12-3
Some Typical Retroviral Oncogenes and Their Products

Virus	Oncogene	Product	
		Function	Location
Rous sarcoma virus	v-*src*	Protein-tyrosine kinase	Plasma membrane
Avian erythroblastosis virus	v-*erbB*	Growth factor receptor[b]	Plasma membrane
Simian sarcoma virus	v-*sis*	Analog of platelet-derived growth factor	Cytoplasm
Murine sarcoma viruses	v-*ras*	Regulates adenylate cyclase	Plasma membrane
Avian myelocytomatosis virus	v-*myc*	? Regulates transcription (DNA binding)	Nucleus

[a]Usually; sometimes other cytoplasmic membranes.
[b]Homologous with the tyrosine kinase domain of the receptor for epidermal growth factor.

factor, and the *erbB* product, which is also located in the plasma membrane, resembles not a growth factor but the receptor for a growth factor. Specifically, this gene-product resembles that part of the receptor for epidermal growth factor which carries tyrosine-specific protein kinase enzyme activity. Approximately half of all known oncogenes, including v-*src*, the first to be described, encode proteins that either have protein-tyrosine kinase activity or display sequence homology with that enzyme.

The common thread linking these apparently disparate observations can be perceived by considering the structure of the receptor for epidermal growth factor which spans the plasma membrane of all cells. The external domain of this molecule constitutes the binding site for epidermal growth factor, and the domain which extends internally into the cytoplasm carries tyrosine kinase activity. When epidermal growth factor binds to the external receptor, a configurational change is transmitted to the internal domain and the tyrosine kinase is activated. Various cellular proteins in the vicinity are phosphorylated by the enzyme, abnormally, at tyrosine residues rather than at normal serine or threonine residues. Thus, the apparent paradox that oncogenes encode both growth factors and their receptors is resolved, and at the same time a possible mechanism for cellular deregulation and transformation is uncovered.

The phosphorylation of cellular proteins at an enhanced rate or at inappropriate times can have far-reaching effects, especially if the enzyme activation is permanent. For example, phosphorylation of proteins involved in the cellular cytoskeleton, situated just beneath the plasma membrane, might lead to perturbations in cellular structure and in the way a given cell interacts with its neighbors. In cell culture, normal cell division is inhibited by cell–cell contact, but continuous, unregulated cell division of transformed cells is said to represent "an escape from contact inhibition." Phosphorylation of cytoskeleton proteins may be involved in this *in vitro* analog of tumorigenesis in animals.

Postulated Mechanisms of Tumor Production by Oncogenes

Though still highly speculative, there is already evidence to support a number of alternative mechanisms of tumorigenesis involving oncogenes. It is perhaps most logical to classify them into those mediated by the products of v-*onc* genes or c-*onc* genes, although one must bear in mind that the v-*onc* genes of retroviruses were presumably derived from c-*onc* genes during recombination between proviral and cellular DNA.

Transduction of an Oncogene by a Retrovirus. This is the best documented of all the mechanisms. A c-*onc* gene acquired by recombination

becomes an integral part of a retrovirus genome, i.e., a v-*onc* gene. During subsequent integration of provirus into the cellular genome, there is a high rate of mutation, including point mutations, deletions, and various rearrangements, such that the v-*onc* gene generally differs significantly from its c-*onc* progenitor and may code for a somewhat different protein with a somewhat different function or substrate specificity. When later reinserted into the genome of another cell, the v-*onc* gene is controlled by powerful promoters and enhancers within the LTR. Thus, a v-*onc* gene may produce a tumor either because its protein product differs significantly from that encoded by the corresponding c-*onc* gene, or because it is expressed to an excessive level.

Activation of a Cellular Oncogene. There is evidence that c-*onc* genes may be responsible for some transformations. It is not difficult to imagine a tumor arising from overexpression of a c-*onc* gene, or inappropriate expression, e.g., in the wrong cell or at the wrong time. Such abnormal c-*onc* transcription may occur in a variety of ways: (1) insertional mutagenesis, (2) transposition, (3) gene amplification, and (4) mutation.

Insertional Mutagenesis. Integration of a provirus with its strong promoter and enhancer elements, upstream from a c-*onc* gene, may greatly amplify the expression of the latter. This is the likely mechanism whereby the weakly tumorigenic avian leukosis viruses, which lack a v-*onc* gene but have an LTR, produce tumors. When avian leukosis viruses cause malignancy, the viral genome is generally integrated at a particular location, immediately upstream from a c-*onc* gene. Integrated avian leukosis provirus increases the synthesis of the normal c-*myc* oncogene-product 30- to 100-fold. Experimentally, only the LTR need be integrated, and, furthermore, by such a mechanism, c-*myc* may be expressed in cells in which it is not normally expressed. In a more direct *in vitro* demonstration of the postulated mechanism, cells may be transformed by transfection with a particular v-*onc* gene, e.g., c-*ras*, linked to a retroviral LTR.

The inference from these examples is that a quantitative, rather than a qualitative difference in the c-*onc* gene-product can be tumorigenic in itself. In view of the crucial implications of this hypothesis, it is vital to ascertain whether absolutely normal (rather than premalignant) cells can be transformed to the fully malignant state *in vitro* and *in vivo* by such mechanisms (see below).

Transposition. Transposition of c-*onc* genes may result in enhanced expression by bringing them under the control of strong promoter and enhancer elements. For instance, the 8 : 14 chromosomal translocation that characterizes Burkitt's lymphoma (a tumor associated with EB herpesvirus infection, occurring in African children) brings the c-*myc* gene

into juxtaposition with immunoglobulin genes, thus perhaps under the control of the strong immunoglobulin promoter. It is possible that EB virus is responsible for this particular translocation and hence causes lymphoma. Retroviruses could do the same.

Gene Amplification. Amplification of oncogenes is a feature of many tumors of humans and animals; e.g., a 30-fold increase in the number of c-*ras* gene copies is found in one human cancer cell line, while the c-*myc* gene is amplified in several human tumors. The increase in gene copy number leads to a corresponding increase in the amount of the oncogene's product. Again, it is suggested that this "dosage effect" suffices to produce cancer.

Mutation. Mutation in a c-*onc* gene, e.g., c-*ras*, may alter the function of the protein for which it codes. Such mutations can occur either *in situ* as a result of chemical or physical mutagenesis, or in the course of recombination with retroviral DNA.

Cooperation between Oncogenes: Multistep Tumorigenesis

Tumors other than those induced by rapidly transforming retroviruses like Rous sarcoma virus do not generally arise as the result of a single event, but by a series of steps leading to progressively greater loss of regulation of cell division. Significantly, the genome of some retroviruses (e.g., avian erythroblastosis virus) carries two different oncogenes (and that of polyomavirus, three), while two or more distinct oncogenes are activated in certain human tumors (e.g., Burkitt's lymphoma). It has recently been shown that transfection of primary cultures of normal fibroblasts with two different oncogenes, one encoding a plasma membrane protein and one encoding a nuclear protein, regularly produces enhanced transformation.

Cotransfection of normal rat embryo fibroblasts with the mutant c-*ras* gene plus the polyomavirus large-T gene (Py-T, Table 12-5), or with c-*ras* plus the E1A early gene of tumorigenic adenoviruses, or with v-*ras* plus v-*myc*, converted them into tumor cells. It should be noted that, whereas v-*ras* and v-*myc* are typical v-*onc* genes, originally of c-*onc* origin, the other two had been assumed to be typical viral genes. However, E1A has now been shown to have partial sequence homology with v-*myc* and may therefore have been derived from c-*myc* at some point much earlier in evolution. Furthermore, it has been demonstrated that a chemical carcinogen can substitute for one of the two v-*onc* genes; following immortalization of cells *in vitro* by treatment with the carcinogen, transfection of a cloned oncogene converted the cloned continuous cell line to a tumor cell line.

Such experiments resurrect earlier unifying theories of cancer causation that viewed viruses as analogous to other mutagenic carcinogens, both being capable of initiating a chain of two or more events leading eventually to malignancy. If viruses or oncogenes are to be considered as cocarcinogens in a chain of genetic events culminating in a tumor, it will be necessary to determine whether their role is that of initiator or promoter, or both. The most plausible hypothesis may be that (1) a limited number of c-*onc* genes represent targets for carcinogens (chemicals, radiation, and tumor viruses), and (2) the full expression of malignancy may generally require the mutation or enhanced expression of more than one class of oncogene.

TUMOR INDUCTION BY HERPESVIRUSES

Herpesviruses of the subfamily *Gammaherpesvirinae* are the etiological agents of lymphomas or carcinomas in hosts ranging from amphibia, through birds, to primates including humans (Table 12-4).

Marek's disease virus (see Chapter 19) transforms T lymphocytes, causing them to proliferate to produce a lethal generalized lymphomatosis. The disease is contagious, being transmitted via virus shedding from cells of the feather follicles, and is a major problem for the poultry industry. It is preventable by vaccination with an attenuated live-virus vaccine derived from a related turkey herpesvirus.

Herpesvirus ateles and *H. saimiri* cause inapparent infections in their natural simian hosts, but when transmitted to other species of monkeys

TABLE 12-4
Tumorigenic Herpesviruses

Virus	Natural host	Natural tumor	Experimental tumor[a]
Marek's disease virus	Chicken	T cell lymphoma	Chicken
Herpesvirus ateles, Herpesvirus saimiri	Monkey	? Nil in natural hosts	Lymphomas, leukemias in other primate species
EB virus	Human	Burkitt's lymphoma Nasopharyngeal carcinoma	Monkeys
Herpesvirus papio	Baboon	Lymphoma	Primates
Herpesvirus sylvilagus	Rabbit	Lymphoma	Rabbit

[a]Produced by inoculation of the host species indicated.

induce fatal T cell lymphoma–lymphatic leukemia. Burkitt's lymphoma is a malignant B cell lymphoma found in children in East Africa, which is associated with EB virus infection.

Mechanism of Tumorigenesis by Herpesviruses

Oncogenes have yet to be recognized in herpesviruses, and the most important tumorigenic herpesvirus, Marek's disease virus, has been subjected to limited study at the molecular level. The mechanism by which herpesviruses produce malignancy has been best studied in Burkitt's lymphoma. The EB virus genome DNA is present in multiple copies in each cell of most African Burkitt's lymphomas. Though sometimes integrated into host chromosomes, it is generally in the form of closed circles of the complete viral DNA molecule, found free in the cytoplasm as autonomously replicating episomes. The cells express EB virus nuclear antigen detectable by immunofluorescence, but do not produce virus until induced to do so by cultivation *in vitro*.

The malignant cells also contain a characteristic chromosomal aberration, namely an 8 : 14 translocation. Recent work on retroviral oncogenes has given this karyological abnormality a genetic interpretation which may explain the mechanism of tumorigenesis by EB virus. The human c-*myc* oncogene, located on the distal segment of chromosome 8, is transposed to one of three chromosomes that contain genes for immunoglobulin—usually chromosome 14, sometimes 2 or 22—leading to its enhanced expression.

TUMOR INDUCTION BY PAPOVAVIRUSES AND ADENOVIRUSES

The family *Papovaviridae* contains two genera: *Papillomavirus* and *Polyomavirus*. Papillomaviruses are causes of naturally occurring carcinomas in cattle, rabbits, and humans. Polyomaviruses readily produce tumors when injected into infant rodents, but are now thought to have little or nothing to do with naturally occurring tumors. Likewise, certain adenoviruses, while highly tumorigenic when inoculated into suckling rodents, have never been implicated as a cause of malignancy in nature.

Transformation by Polyomaviruses and Adenoviruses

During the 1960s and 1970s two papovaviruses of the *Polyomavirus* genus, polyomavirus of mice and simian virus 40 (SV40), as well as certain human adenoviruses (types 12, 18, and 31), became the most

popular models for biochemical investigation of the mechanism of viral tumorigenesis. They attracted attention because they were found to induce malignant tumors following inoculation into baby hamsters and other rodents. Furthermore, although they replicate productively in and destroy cultured cells of their native host species, they transform cultured cells of certain other species. Though not necessarily capable of producing tumors when transplanted, these transformed cells display many of the properties of malignant cells (see Table 6-3 and Plate 6-3). As they can be readily cloned and grow to high density *in vitro,* they represent good experimental models for detailed analysis of the biochemical events in cell transformation. Much is known about the expression of the integrated viral genome in cells transformed by these viruses.

The polyomavirus- or adenovirus-transformed cell does not produce virus. Viral DNA is integrated at multiple sites on the cell's chromosomes. Most of the integrated viral genomes are complete in the case of the polyomaviruses, but defective in the case of the adenoviruses. Only certain early viral genes are transcribed, albeit at an unusually high rate. Their products, demonstrable by immunofluorescence, are known as *T (tumor) antigens* (Table 12-5). A great deal is now known about the role of these proteins in transformation. For example, the "middle-T" antigen

TABLE 12-5
Oncogenes of Adenoviruses and Papovaviruses and Their Products[a]

Virus	Oncogene	Product	
		Function	Location
Adenovirus	E1A	Regulates transcription	Nucleus, cytoplasm
	E1B	?	Nuclear membrane, plasma membrane, endoplasmic reticulum
Polyomavirus	Py-t	?	Cytoplasm
	Py-mT	Binds and regulates product of c-*src*	Plasma membrane
	Py-T	Initiates DNA synthesis and regulates transcription	Nucleus
SV40	SV-t	?	Cytoplasm
	SV-T	Initiates DNA synthesis, regulates transcription	Plasma membrane, nucleus

[a]Data from J. M. Bishop, *Cell* **42,** 23 (1985).

(Py-mT) of polyomavirus (like the product of the v-*ras* gene of retroviruses) seems to bring about the change in cell morphology and enables the cells to grow in suspension in semisolid agar medium as well as on solid substrates (*anchorage independence*), whereas the "large-T" antigen (Py-T), like the product of the v-*myc* gene of retroviruses, is responsible for the reduction in dependence of the cells on serum and enhances their life span in culture.

Virus can be "rescued" from polyomavirus-transformed cells, i.e., induced to replicate, by any of a number of manipulations, including irradiation, treatment with certain mutagenic chemicals, or cocultivation or fusion with certain types of permissive cell. This cannot be achieved with adenovirus-transformed cells, for the integrated adenoviral DNA contains substantial deletions.

It should be stressed that integration of viral DNA does not necessarily lead to transformation. As discussed in Chapter 11, persistent infections with some herpesviruses are characterized by integration of the viral genome without any indication of cellular transformation. Many or most episodes of integration of papovavirus or adenovirus DNA have no recognized biological consequence. Transformation by these viruses in the experimental systems is a rare event, requiring that the viral transforming genes be integrated in the location and orientation needed for their expression. Even then, many transformed cells revert (abortive transformation). Furthermore, cells displaying the characteristics of transformation (immortalization, anchorage independence, altered morphology and surface properties, growth to high density, etc., see Table 6-3) do not necessarily produce tumors. This needs to be demonstrated independently by transplantation of cells into athymic or syngeneic mice. As was discussed in the section on oncogenes, recent evidence suggests that certain ostensibly normal cell lines commonly used for *in vitro* transformation assays, such as 3T3 cells, are in fact "premalignant"; transformation of normal cells to the fully malignant state may require the cooperation of more than a single oncogene, e.g., polyoma large-T, adenovirus E1A, or retrovirus v-*myc*, together with at least one other.

Transformation by Papillomaviruses

Papillomaviruses produce papillomas (warts) on the skin and mucous membranes. These benign tumors are hyperplastic outgrowths which generally regress spontaneously. Occasionally, however, they may progress to malignancy. There is evidence that a cofactor may be required.

One of the most instructive models is the bovine papillomavirus, of

which six types are recognized (see Chapter 17). Different bovine papillomaviruses are associated with the development of tumors in different sites. In hot, sunny climates such as Australia and Texas, viral papillomas around the eye and on hairless or nonpigmented patches of skin may become malignant; the cofactor is postulated to be UV irradiation. In the Scottish Highlands, multiple benign papillomas are common, but only cattle consuming bracken fern will sometimes develop carcinoma of the alimentary tract or bladder. Mature virions are readily demonstrable in the papillomas but are absent from the carcinomas. However, *in situ* hybridization with a labeled bovine papillomavirus 4 DNA probe reveals that the cells of carcinomas contain the viral genome, not integrated but free, in the form of a closed circular molecule of DNA. Viral DNA is also found in distant metastatic tumors, ruling out the possibility that it represents contamination from papillomas. The fact that it is all episomal indicates that integration of viral DNA is not required for the induction of malignancy. Bovine papillomaviruses, as well as those from humans and other animals, will transform bovine or murine cells *in vitro* and induce fibromas in rodents. Examination of these transformed cells also reveals no virions but episomal viral DNA in essentially every cell. Moreover, the viral DNA, which is infectious, induces tumors in rodents and transforms cultured rodent cells. Indeed, transfection with a fragment of bovine papillomavirus 1 DNA representing 69% of the genome also transforms cultured cells. Only a small part of the corresponding papillomavirus genome is transcribed in bovine or rabbit carcinoma cells. Nevertheless, more of the viral DNA may be required for maintenance of the transformed state, as treatment with interferon has been reported to "cure" mouse cells transformed by bovine papillomavirus 1; revertants (abortively transformed cells) have lost their viral DNA.

TUMOR INDUCTION BY HEPADNAVIRUSES

Some members of the family *Hepadnaviridae* (see Chapter 34) are also strongly associated with naturally occurring carcinomas in their native hosts. Hepadnaviruses are characterized by a small circular DNA genome which commonly integrates into cellular DNA and persists indefinitely.

Hepatitis B, a very common infection of humans in some parts of the world (southern and eastern Asia and Africa), is the principal cause of primary hepatocellular carcinoma—the most common lethal human cancer in the world. The hepadnaviruses associated with hepatocellular

carcinoma in the woodchuck, *Marmota monax*, and in the Pekin duck provide models for the study of hepadnavirus tumorigenicity. Most wild woodchucks are chronic carriers of the virus and a significant number of them develop hepatomas. Viral DNA is found to be integrated into the chromosomes of the malignant liver cells, but the mechanism of tumorigenesis remains unknown.

TUMOR INDUCTION BY POXVIRUSES

Some poxviruses are regularly associated with the development of benign tumorlike lesions, which usually disappear within weeks but may persist for months. There is no evidence that they ever become malignant, nor is there evidence that poxvirus DNA is ever integrated into cellular DNA.

Poxviruses of several genera may induce these benign tumors. All leporipoxviruses produce localized fibromas in their natural hosts (leporids and squirrels), and if infection occurs in young animals these may persist, and continue to harbor large amounts of virus, for many months. Cutaneous infection of birds with avian poxviruses is usually associated with localized tumors, and infection of rhesus monkeys with Yaba monkey tumor poxvirus produces large benign tumors which are described as histiocytomas.

Little is known of the mechanism of development of these tumors, but it has recently been found that a very early viral protein produced in vaccinia virus-infected cells displays homology with epidermal growth factor, and is probably responsible for the epithelial hyperplasia often found in sites of infection with vaccinia virus. Possibly poxviruses that regularly produce fibromas, etc., produce more potent growth factors.

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CHAPTER 13

Laboratory Diagnosis of Viral Diseases

Tests for the specific diagnosis of a viral infection in an animal are of two general types: (1) those that demonstrate the presence of the virus, and (2) those that demonstrate the presence of specific viral antibody. The provision, by a single laboratory, of a comprehensive service for the diagnosis of viral infections of domestic animals is a formidable undertaking. There are about 200 individual viral species, in some 20 different viral families, that infect the eight major domestic animal species (cattle, sheep, goat, swine, horse, dog, cat, and chicken). If antigenic types within an individual viral species are considered, and the number of animal species is broadened to include turkey, duck, and zoo and laboratory animals, then the number of individual viruses exceeds 1000. It is therefore not surprising that few single laboratories could have available the necessary specific reagents, skills, and experience for the diagnosis of such a large number of infections.

One consequence of this great variety of viruses is that veterinary diagnostic laboratories tend to specialize, e.g., in diseases of food ani-

mals, or companion animals, or poultry, or in "exotic" viruses. Within these specialized laboratories there is considerable scope for the development of rapid diagnostic methods that short-circuit the need for the isolation of viruses, which is expensive, time-consuming, and rarely necessary.

RATIONALE FOR SPECIFIC DIAGNOSIS

Many viral diseases can be diagnosed clinically, others with the assistance of the pathologist; but there are several circumstances under which laboratory confirmation of the specific virus involved is desirable or, indeed, essential.

Exotic Diseases

The industrialized countries of Europe, North America, Australasia, and Japan are free of many devastating diseases of livestock that are still enzootic in other parts of the world, such as foot-and-mouth disease, African swine fever, rinderpest, and fowl plague. All industrialized countries maintain or share the use of specialized biocontainment laboratories (such as those at Plum Island in the United States and Pirbright in the United Kingdom) devoted to diagnosis and research on such "exotic" viruses. Clearly it is of the utmost importance that the clinical diagnosis of a suspected exotic virus should be confirmed quickly and accurately (see Chapter 16).

Zoonoses

Several animal viral diseases such as rabies, Rift Valley fever, and eastern, western, and Venezuelan encephalomyelitis are zoonotic and are of sufficient human public health significance to require the maintenance of specialized diagnostic laboratories. For example, confirmation of the diagnosis of rabies in a skunk that has bitten a child provides the basis for postexposure treatment of the human patient (see Chapter 30). Confirmation and early warning of an equine encephalomyelitis virus epizootic allows implementation of mosquito control and other measures such as restriction of the movement of horses.

Certification of Freedom from Specific Infections

For diseases in which there is lifelong infection, such as bovine and feline leukemia, equine infectious anemia, and herpesvirus infections, a negative test certificate is often required as a condition of sale, particu-

larly export sale, for exhibition at a state fair, or show, or for competition, as at race meetings.

Artificial Insemination, Embryo Transfer, and Blood Transfusion

Males used for semen collection and females used in embryo transfer programs, especially in cattle, and blood donors of all species, are usually screened for a range of viral infections to minimize the risk of transmission to recipient animals.

Test and Removal Programs

For retrovirus infections, Marek's disease, pseudorabies, and certain other diseases, it is possible to reduce substantially the incidence of disease or eradicate the causative virus from the herd or flock by test and removal programs. Laboratory diagnosis is essential for the effective implementation of such operations.

Veterinary Health Investigations

Provision of a sound veterinary service in any state or country depends on a knowledge of prevailing viral diseases; hence, epidemiological studies to determine the prevalence and distribution of particular viral infections are frequently undertaken, usually based on the detection of specific antibody.

Clinical Management Dependent on Precise Diagnosis

Many relatively nonspecific disease syndromes, such as respiratory disease (e.g., kennel cough and shipping fever), diarrhea, and some skin diseases, may be caused by a variety of agents, viral and bacterial. Proper management of individual cases or infected herds or flocks may require specific viral diagnosis.

METHODS OF VIRAL DIAGNOSIS

Specific diagnosis of the kind outlined above can be achieved by one of three methods: (1) isolation and characterization of the causative virus, (2) direct demonstration of virions, viral antigens, or viral nucleic acids in tissues, secretions, or excretions, and (3) detection and measurement of antibodies (Table 13-1). Each group of methods has its place. Viral isolation remains the benchmark against which other methods are

TABLE 13-1
Techniques for Identification of Viruses at Various Taxonomic Levels

Taxonomic level	Techniques of choice
Family (sometimes genus)	Cytopathology in cultured cells Electron microscopy Complement fixation
Species (type) Subtype	Neutralization Kinetic neutralization Monoclonal antibody serology
Variant	Nucleic acid hybridization Oligonucleotide fingerprints Restriction endonuclease fragment patterns
Mutant (including by point mutation)	Nucleic acid sequencing

measured, and is essential when decisions of major economic importance depend on the diagnosis, e.g., with suspected exotic diseases such as foot-and-mouth disease or fowl plague. On the other hand, the direct demonstration of virions or viral components may provide a much more rapid and cheaper method of specific diagnosis than viral isolation, particularly when large numbers of samples must be tested. Epidemiological surveys, eradication programs, and the provision of certificates of freedom from specific infections are often based on serological methods or rapid tests for viral antigen.

COLLECTION, PACKAGING, AND TRANSPORT OF SPECIMENS

It requires at least as much effort, and often more, to process a negative specimen as it does one from which virus is isolated. The chance of isolating a virus depends critically on the knowledge, care, and attention of the veterinarian who collects the specimen (see Plate 13-1). Obviously such a specimen must be taken from the right place and at the right time. The right time is always as soon as possible after the onset of clinical signs; virus is usually present in maximum amount at about this time and diminishes, sometimes quite rapidly, in the ensuing few days. Specimens taken as a last resort when days or weeks of empirically chosen antibiotic therapy have failed are almost invariably a waste of effort.

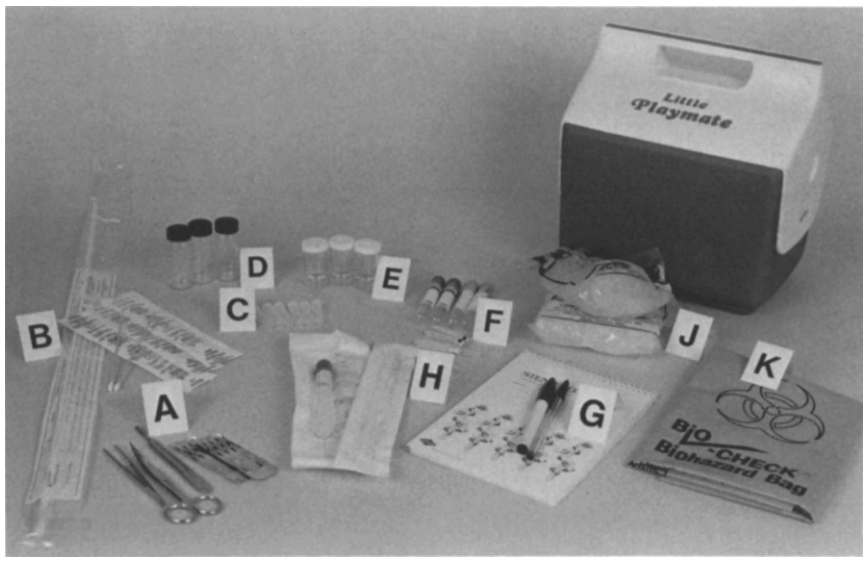

PLATE 13-1. *Equipment required for collection of virus samples. (A) Sterile forceps, scissors, and scalpels. (B) Selection of sterile swabs. (C) Vials for containing virus transport medium for collection of samples for virus isolation or identification. (D) Bottles for collection of feces, blood, and other samples that do not require virus transport medium. (E) Bottles containing formol saline or Bouin's fixative for tissues to be examined histologically. (F) Blood collection equipment—without additive for serum, with anticoagulant for virus isolation. (G) Notebook and equipment for labeling specimens. (H) Swabs and transport medium for bacteriological investigation. (J) Cool box. (K) Heavy-duty plastic bags for postmortem material.*

The site from which the specimen is collected will be influenced by the clinical signs and a knowledge of the pathogenesis of the suspected disease (Table 13-2). Having collected the appropriate specimen(s), it should be properly labeled and sent to the laboratory, with a history, including the provisional diagnosis. Where ambient temperatures are moderate and transit time to the laboratory is less than 1 day, ice or cold packs (<4°C) in a styrofoam box are frequently used. If the environmental temperature is high and transit times longer than a day, dry ice (−70°C) may be used, although wet ice with provision to replenish it in transit is better. If exotic or zoonotic viruses are suspected, the styrofoam boxes must be replaced by or enclosed within sturdier, double-walled containers with absorbent padding. Appropriate permits must be obtained for interstate and international transportation, and in

TABLE 13-2
Specimens Appropriate for Various Clinical Syndromes

Disease	Live animal	Postmortem
Respiratory and ocular disease	Nasal and conjunctival swabs, blood[a]	Tissues from affected system, lymph nodes
Skin disease; lesions of mucous membranes	Scrapings of lesion, swab affected area, blood	Tissue from affected system, lymph nodes
Gastroenteritis	Feces, blood	Tissues from affected system, lymph nodes, intestinal contents
Systemic disease	Blood, nasal and urogenital swabs; feces	Tissues from various organs
Disease of the central nervous system	Blood, cerebrospinal fluid (if feasible); feces, nasal and urogenital swabs	Tissues from affected system, lymph nodes
Disease of the urinary tract	Urogenital swab; urine, blood	Tissues from affected system, lymph nodes
Abortion	Blood from dam, vaginal mucus	Tissues from placenta and fetus; blood from fetal heart; intestinal contents

[a]Blood: refers to clotted sample for serology and sample with anticoagulant added for other tests. Large animals, 10–20 ml; small animals, 5–10 ml; others as appropriate. If possible remove clot before dispatch.

such circumstances the collection and transport arrangements need to be discussed with the laboratory and/or the appropriate government regulatory agency.

For particularly labile viruses such as respiratory syncytial virus, herpesviruses, or coronaviruses, it may be an advantage to take the cell culture to the animal.

VIRUS ISOLATION

Isolation and identification requires at least a week, usually longer, and it is expensive. However, it is probably the most sensitive available method, if properly collected material is used, and it provides material for further study.

Preparation of the Specimen for Inoculation

The sooner the specimen is processed and inoculated after arrival at the laboratory, the better. If delays of more than 1 day are anticipated,

the specimen may be frozen to −70°C. Swabs are processed by twirling them in the transport medium and expressing the fluid by pushing the swab firmly against the side of the container. Feces are dispersed on a vortex mixer. Tissue specimens are finely minced with scissors and homogenized in a glass or mechanical homogenizer.

Prior to inoculation, contaminating microorganisms are removed by filtering through membrane filters of average pore diameter 0.45 μm, although such filters allow the passage of mycoplasmas. Once virus is successfully isolated and grown to a high titer, the suspension can be refiltered through 0.22 μm filters to exclude mycoplasmas. Feces and tissue homogenates should be diluted at least 1 : 10 and centrifuged at 1000 *g* for 15 minutes to obtain a supernate that can be filtered. If the concentration of virus is suspected to be very low, high concentrations of antibiotics may be preferable to filtration. Whatever the origin of the specimen, some of the original sample and some of the filtrate should be retained at 4°C or frozen until the isolation attempt is finalized.

Virus can be grown from the suitably prepared specimen by inoculation into cell cultures, laboratory animals, or the species of host animal from which the specimen was obtained. By far the most widely used substrate is cultured cells.

Growth of Virus in Cultured Cells

Choice of Cultured Cells. The choice of the optimal cell culture for the primary isolation of a virus of unknown nature from clinical specimens is largely empirical. Primary or low-passage, homologous, monolayer cell cultures derived from fetal tissues probably provide the most sensitive substrate for isolation of the greatest variety of different viruses. Continuous cell lines derived from the homologous species are almost as good. Often the nature of the disease from which the material was obtained will suggest what species of virus may be found, and in such cases the optimum cell culture for that virus can be chosen, in parallel, perhaps, with a second type of culture with a wide spectrum. Cell lines offer some advantages and are available for most domestic animals except avian species (Table 13-3).

Monolayer cell cultures for virus isolation should be grown in sealed containers, such as plastic flasks or glass tubes with screw caps. Open-culture systems such as petri dishes or microtiter trays should not be used because of the risks of cross-contamination. For some viruses, rolling the cultures on a drum improves isolation rates.

Special types of cultures are utilized for particular viruses. For example, betaherpesviruses and gammaherpesviruses may be recovered from monolayer cultures of tissue taken directly from the diseased animal,

TABLE 13-3
Some Commonly Used Cell Lines

Animal species	Cell line	
	Origin	Acronym
Cattle	Fetal kidney	MDBK
	Embryonic trachea	EBTr
Sheep/goat	None	—
Swine	Kidney	PK
Horse	Skin	EDerm
Dog	Kidney	MDCK
Cat	Fetal kidney	CrFK
	Embryo	FEmb
Chicken	None	—

whereas inoculation of established monolayer cell cultures with cell-free material may be negative. For some coronaviruses and rhinoviruses that do not grow well in monlayer cultures, growth may occur in explant cultures (i.e., small cubes of tissue from the trachea or gut), probably because these do not dedifferentiate in culture (see Chapter 3).

Recognition of Viral Growth. Cultures are usually incubated at 37°C, despite the fact that the normal body temperatures of all domestic animal species are somewhat higher. Cultures are observed daily for cytopathic effects. The speed and nature of the cytopathic effect caused by different viruses varies considerably. Cytopathic effect must always be based on comparison with uninoculated cell cultures; this is particularly important for viruses requiring incubation periods of longer than a week. Where none or a doubtful cytopathic effect is observed, it is usual to make a second or even a third ("blind") passage.

When cytopathic effect is observed, there is a range of options:

1. The speed and appearance of the cytopathic effect, coupled with the case history, may immediately suggest the diagnosis.
2. After suitable manipulation, material from the cell culture may be examined by electron microscopy.
3. Infected monolayers on glass coverslips or special slide/culture chambers may be fixed and appropriately stained, and the cells examined for inclusion bodies, syncytia or other characteristic cell changes. Better, they may be stained with fluorescent antibody, which may provide an immediate definitive diagnosis. Where prior experience and knowledge suggest it, such slide cultures may be included at the time of primary inoculation, with a consequent saving in time.

Some viruses are relatively noncytopathogenic for cultured cells (see Chapter 6 and Table 3-1). Their growth in monolayer culture may sometimes be recognized by means of hemadsorption. Most viruses that hemagglutinate will also hemadsorb; the growth of paramyxoviruses, orthomyxoviruses, and, to a lesser extent, the flaviviruses and togaviruses, is routinely recognized in this way (see Plate 3-2).

Growth of Virus in Laboratory Animals

Nowadays laboratory animals play a minor role in the virus diagnostic laboratory. However, some virologists still regard intracerebral inoculation of baby mice as the method of choice for the isolation of rabies virus, flaviviruses, and togaviruses.

The developing chick embryo occupies a special place. Intraamniotic inoculation provides the most sensitive method for influenza viruses and for several avian viruses, and species diagnosis of orthopoxviruses can be made directly from the type of pock produced on the chorioallantoic membrane. In addition, chick embryos are extensively used as a source of primary monolayer cultures (fibroblasts, kidney cells) for the isolation of avian viruses.

Inoculation of the Natural Host Species

In veterinary medicine it is feasible to consider using the natural host species, especially susceptible young animals (e.g., calves, pigs, chicks), for the recovery of a virus from suspect material. Such animals, if free of antibodies, must be considered a highly sensitive substrate. However, their use would now be contemplated only for viruses not yet cultivable, or where cell culture procedures were negative in circumstances that strongly indicated a viral etiology, and/or where there might be serious repercussions if the diagnosis were missed.

Identification of Viral Isolates by Serology

A newly isolated virus can usually be provisionally allocated to a particular family, and sometimes to a genus or species, on the basis of the clinical findings, the host cell used for virus isolation, and the visible result of viral growth (cytopathic effect, hemadsorption, hemagglutination, electron microscopy of the cytopathogenic agent, etc.). Definitive identification, however, usually depends on serological procedures. By using the new isolate as antigen against known antisera, e.g., in a complement fixation test, the virus can often be placed into its correct family or genus. Having allocated it to a particular family (e.g., *Adenoviridae*), one can then go on to determine the species or serotype (e.g., canine

TABLE 13-4
Principal Serological Procedures Used in Diagnostic Virology

Technique	Principle
Virus neutralization	Antibody neutralizes infectivity of virion; inhibits cytopathology, reduces plaques, or protects animals
Hemagglutination inhibition	Antibody inhibits viral hemagglutination
Complement fixation	Antigen–antibody complex binds complement, which is thereafter unavailable for the lysis of hemolysis-sensitized sheep red blood cells
Immunoelectron microscopy	Antibody-aggregated virions are visible by electron microscopy
Immunofluorescence	Antibody labeled with fluorochrome binds to intracellular antigen; fluoresces by UV microscopy
Immunoperoxidase staining	Peroxidase-labeled antibody binds to intracellular antigen; colored precipitate forms on adding substrate
Enzyme-linked immunosorbent assay (ELISA)	Enzyme-labeled antibody (or antigen) binds to antigen (or antibody); substrate changes color
Radioimmunoassay	Radiolabeled antibody (or antigen) binds to antigen (or antibody), e.g., attached to solid phase
Immunodiffusion	Antibodies and soluble antigens produce visible lines of precipitate in a gel

adenovirus 1) by more discriminating serological procedures. This sequential approach is applicable only to families with a common family antigen.

The range of available serological techniques is now extremely wide (Table 13-4). Some are best suited to particular families of viruses. Each laboratory makes its own choice of favored procedures, based on considerations such as sensitivity, specificity, reproducibility, speed, convenience, and cost. Currently most serological procedures are carried out with "hyperimmune" sera comprising a polyclonal mixture of antibodies, sometimes after they have been absorbed to eliminate antibodies of certain specificities.

Monoclonal antibodies with defined specificity are now becoming available. These make it possible to proceed quickly to very specific diagnosis even to the level of subtypes, strains,or variants, e.g., rabies viruses from different geographical areas. Family-, genus-, and type-specific monoclonal antibodies are also being developed. As their properties are defined and they become commercially available, we can expect monoclonal antibodies to be widely used for all methods of serological identification.

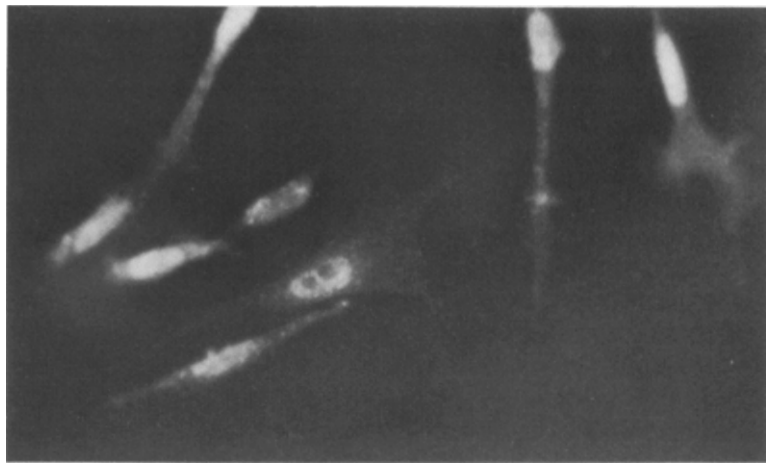

PLATE 13-2. *Immunofluorescence, used here for determining the site of assembly of components of influenza virus. Antibody against the nucleoprotein antigen shows nuclear accumulation at 4 hours after infection of chick cells. Procedure: guinea pig antiserum to nucleoprotein antigen is added to a monolayer of infected chick cells, then fluorescein-conjugated rabbit anti-guinea pig IgG. (Courtesy Dr. N. J. Dimmock.)*

Immunofluorescence. The simplest way of identifying a newly isolated virus is by fluorescent-antibody staining of the infected cell monolayer itself (Plate 13-2). This can provide definitive diagnosis within an hour or so of recognizing the earliest suggestion of cytopathic effect. Immunofluorescence is best suited to the identification of monotypic genera, or genera of which only a single species affects that particular species of animal, or to epidemic situations when a particular virus is suspected; otherwise, replicate cultures must be screened with a range of antisera. The advantages and disadvantages of monoclonal antibodies, in comparison with polyclonal or "absorbed" sera, discussed below in the context of radioimmunoassays, apply equally to other serological procedures, including immunofluorescence and neutralization.

Electron Microscopy and Immunoelectron Microscopy. These procedures (see Plate 13-5) are most useful in the rapid identification of cell culture virus isolates, as well as directly on specimens (see below). Electron microscopy allows identification only to the level of family, whereas immunoelectron microscopy using suitable specific antibody may permit finer distinctions to be made.

Complement Fixation. For the complement fixation test, the acute and convalescent sera are heated (56°C for 30 minutes) to inactivate complement, then serially diluted in a plastic tray. Two to four units of antigen (e.g., a crude preparation of live or inactivated virus) are then added to each serum dilution together with 2 units of complement, derived from a guinea pig. The reagents are allowed to interact at 4°C overnight, to allow the complement to become "fixed." Sheep erythrocytes, "sensitized" by the addition of rabbit antiserum to them ("hemolysin") are then added and the trays are incubated at 37°C for 45 minutes. In those cups where the complement has been fixed by the virus–antibody complex, the hemolysin fails to lyse the sheep erythrocytes; where complement is still present, they are lysed.

Crude cell culture supernatants often used for complement fixation tests contain not only mature virions but a range of soluble antigens, both structural and nonstructural. Since many of these are shared by many or all viruses within a particular genus or family, e.g., *Mastadenovirus*, they will cross-react with antibodies raised against any other member of the genus or family. This property makes complement fixation a useful method for preliminary screening of an isolate—to place it within the correct family or genus. Immune-adherence hemagglutination is basically a somewhat simplified version of the complement fixation test; currently it is applied more often to the detection of antibody than that of antigen.

Hemagglutination and Hemagglutination Inhibition. Virions of several viral families bind to red blood cells and cause hemagglutination. If antibody and virus are mixed prior to the addition of red blood cells, hemagglutination is inhibited (Table 3-4; Plate 13-3). The hemagglutination titer of certain viruses, e.g., canine distemper virus, may be increased by dissociation of the virions with detergents. Antisera may have to be pretreated to remove nonspecific inhibitors of hemagglutination (see Chapter 26).

The hemagglutination inhibition test is sensitive and, except in the case of the togaviruses, highly specific, since it measures antibodies binding to the surface protein most subject to antigenic change. Moreover, it is simple, inexpensive, and rapid, and is therefore the serological procedure of choice for identifying isolates of hemagglutinating viruses.

Virus Neutralization. The infectivity of a virus may be neutralized by specific antibody by a variety of mechanisms (see Chapter 9). Serum must first be "inactivated" by heating at 56°C for 30 minutes to remove nonspecific virus inhibitors. Serum–virus mixtures are inoculated into appropriate cell cultures, which are then incubated until the "virus

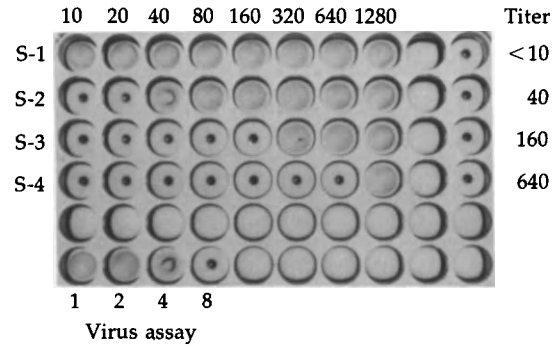

PLATE 13-3. *Hemagglutination inhibition test, used for titrating antibodies to the viral hemagglutinin. Titers are expressed as reciprocals of dilutions. In the example illustrated, a horse was immunized against the prevalent strain of influenza virus. Serum samples S-1, S-2, S-3, and S-4 were taken, respectively, before immunization, 1 week after the first injection, 4 weeks after the first injection, and 4 weeks after the second injection. The sera were treated with periodate and heated at 56°C for 30 minutes to inactivate nonspecific inhibitors of hemagglutination, then diluted in microtiter wells in twofold steps from 1/10 to 1/1280. Each well then received four hemagglutinating (HA) units of the relevant strain of influenza virus. After incubation at room temperature for 30 minutes, 0.05 ml of red blood cells was added to each well. Where enough antibody is present to coat the virions, hemagglutination has been inhibited; hence the erythrocytes settle to form a button on the bottom of the well. On the other hand, where insufficient antibody is present, erythrocytes are agglutinated by virus and form a mat. The virus assay (bottom line) indicates that the virus used gave partial agglutination (the end point) when diluted 1/4.* Interpretation: *The horse originally had no hemagglutinin-inhibiting antibodies against this particular strain of influenza virus. One injection of vaccine produced some antibody; the second injection provided a booster response. (Courtesy I. Jack.)*

only" controls develop cytopathic effects (Plate 13-4). Antibody, by neutralizing the infectivity of the virus, protects the cells against viral destruction.

In keeping with the general trend toward miniaturization, most neutralization tests are now conducted in disposable nontoxic sterile plastic trays with, say, 96 flat-bottomed wells in each of which a cell monolayer can be established. Virus–antiserum mixtures can be added to established monolayers, or, more usually, serum dilutions are made in the wells, a standard amount of virus is added, and the mixture incubated, after which cells are added. In the standard neutralization test the end point is read by cytopathic effect, the titer of the serum being defined as the highest dilution that inhibits the cytopathic effect. In a version of the neutralization test known as the *plaque reduction* assay, cell monolayers

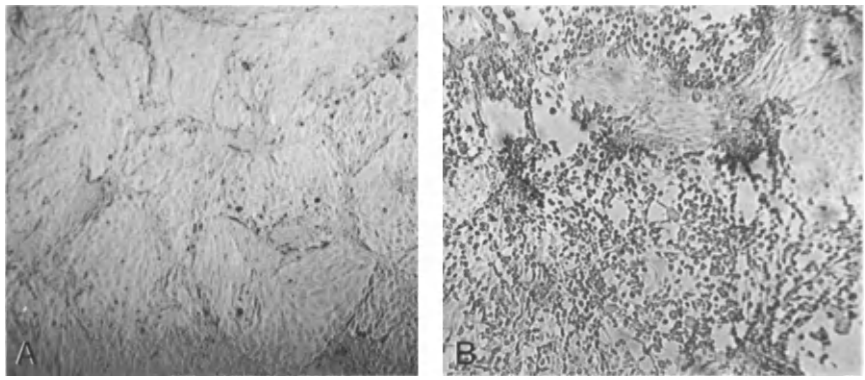

PLATE 13-4. *Virus neutralization test. A pig developed encephalitis during an epizootic of procine enterovirus 1 infection. An enterovirus was isolated from the feces. One hundred times the* $TCID_{50}$ *of this virus was incubated at 37°C for 60 minutes with a suitable dilution of "inactivated" (56°C. 30 minutes) anti-porcine enterovirus 1 serum (a reference serum raised in a rabbit). The mixture was inoculated onto a monolayer of swine kidney cells in wells of a microculture tray (A). Virus similarly incubated with normal rabbit serum was inoculated into well B. The cultures were incubated at 37°C for several days and inspected daily for cytopathic effect (unstained, ×23). (Courtesy I. Jack.)* Interpretation: *The infectivity of this virus isolate has been neutralized by anti-porcine enterovirus 1 serum (no cytopathic effect); the control culture (B) shows typical cytopathic effect.*

inoculated with virus–serum mixtures are overlaid with agar or methylcellulose and incubated until plaques develop (see Plate 3-3); the end point is usually taken to be the highest dilution of serum reducing the number of plaques by at least 50%.

If a newly isolated virus proves to be "untypeable," i.e., not neutralizable by antisera against any of the known serotypes, it may be a novel serotype, or it may indicate a mixed infection with two distinct viruses, or aggregation of virions in the specimen. Aggregates can be removed by vigorous agitation, filtration, or, in the case of some nonenveloped viruses, dispersed with sodium deoxycholate, prior to repeating the neutralization test.

Oligonucleotide and Restriction Endonuclease Fingerprinting

For most routine diagnostic purposes it is usually not necessary to "type" the isolate antigenically, even to the degree just described. Sometimes, however, important epidemiological information can be obtained by going even further, to identify differences between "variants" or

subtypes within a given serotype (see Table 13-1). Short of determining the complete nucleotide sequence of viral nucleic acid, the most useful methods of doing this are by oligonucleotide fingerprinting of viral RNA or the determination of restriction endonuclease fragment patterns of viral DNA. With RNA viruses, viral RNA is labeled with ^{32}P during replication of the virus in culture; the labeled RNA is phenol-extracted from purified virions, digested with ribonuclease T1, and the resulting oligonucleotide fragments separated by two-dimensional polyacrylamide gel electrophoresis, or by cellulose acetate electrophoresis followed by DEAE–cellulose chromatography. Autoradiography reveals a "fingerprint" unique to that particular viral strain. An example of the epidemiological use of this technique to trace the origin and spread of foot-and-mouth disease virus in Europe in 1981 is described in Chapter 23.

Similarly, viral DNA prepared from virions or infected cells can be cut with appropriately chosen restriction endonucleases and the fragments separated by agarose gel electrophoresis. When stained with ethidium bromide or silver, restriction endonuclease fragment patterns (also called fingerprints) are obtained. The method has found application with all dsDNA virus families, particularly in epidemiological studies, but also in understanding pathogenesis. Depending on the viral family, the resolution of these methods is such that different isolates of the same viral species may be distinguishable, unless they come from the same epizootic. Minor degrees of genetic drift, often not reflected in serological differences, can sometimes be detected in this way.

Interpretation

The isolation and identification of a particular virus from an animal with a given disease is not necessarily meaningful in itself. Fortuitous subclinical infection with a virus unrelated to the illness in question is not uncommon. Koch–Henle postulates (see Chapter 2) are as apposite here as in any other microbiological context, but are not always easy to fulfill. In attempting to interpret the significance of any virus isolation, one must be guided by the following considerations:

1. The site from which the virus was isolated is important; e.g., one would be quite confident about the etiological significance of equine herpesvirus 1 isolated from the tissues of a 9-month-old aborted equine fetus with typical gross and microscopic lesions, or of distemper virus isolated from the cerebrospinal fluid of a dog with encephalitis, because these sites are usually sterile, i.e., they have no normal bacterial or viral flora. On the other hand, recovery of an enterovirus from the feces, or a

herpesvirus from a nasal or throat swab may not necessarily be significant, because such viruses are often associated with inapparent infections at these sites.

2. Interpretation of the significance of the isolation in such instances will be facilitated by recovery of the same virus from several cases of the same illness during an epizootic.

3. Knowledge that the virus and the disease in question are often causally associated provides confidence that the isolate is significant.

Laboratory Safety

It is appropriate to conclude this section with some remarks about safety precautions in virus diagnostic laboratories in general and regulations about exotic viruses in particular. Diagnostic virology is one of the less hazardous human occupations, but over the years a number of deaths have been caused by laboratory-associated infections. Some of the commoner hazards are listed in Table 13-5. It is important to note that many of the procedures that may be dangerous for laboratory workers, particularly aerosolization, may also be sources of laboratory contamination—something that may give rise to mistaken diagnoses and sometimes a great deal of misdirected administrative action.

Precautions to avoid these hazards consist essentially of good laboratory technique, but special measures may be needed. Mouth-pipetting is banned. Gowns must be worn at all times, and gloves for anything

TABLE 13-5
Laboratory Hazards

Hazard	Cause
Aerosol	Homogenization (e.g., of tissue in blender)
	Centrifugation
	Ultrasonic vibration
	Broken glassware
	Pipetting
Ingestion	Mouth pipetting
	Eating or smoking in laboratory
	Inadequate washing/disinfection of hands
Skin penetration	Needle prick
	Hand cut by broken glassware
	Leaking container contaminating hands
	Pathologist handling infected organs
	Splash into eye
	Animal bite

TABLE 13-6
Summary of Recommended Biosafety Levels for Infectious Agents[a]

Biosafety level	Practices and techniques	Safety equipment	Facilities
1	Standard microbiological practices	None: primary containment provided by adherence to standard laboratory practices during open-bench operations	Basic
2	Level 1 practices, plus laboratory coats, decontamination of all infectious wastes, limited access, protective gloves and biohazard warning signs as indicated	Partial containment equipment (i.e., class I or II biological safety cabinets) used to conduct mechanical and manipulative procedures that have high aerosol potential that may increase the risk to personnel	Basic
3	Level 2 practices, plus special laboratory clothing, controlled access	Partial containment equipment used for all manipulations of infectious material	Containment
4	Level 3 practices, plus entrance through change room where street clothing is removed and laboratory clothing is put on, shower on exit, all wastes are decontaminated on exit from the facility	Maximum containment equipment (i.e., class III biological safety cabinet or partial containment equipment in combination with full-body, air-supplied, positive-pressure personnel suit) used for all procedures and activities	Maximum containment

[a]From Centers for Disease Control and National Institutes of Health, "Biosafety in Microbiological and Biomedical Laboratories." U.S. Government Printing Office, Washington, D.C., 1984.

dangerous. Various classes of safety cabinets are available for procedures of various degrees of hazard; their use is summarized in Table 13-6.

Besides personal hazard, exotic animal viruses pose special community risks such that major developed countries with large livestock indus-

TABLE 13-7
Animal Viruses the Importation of Which is Restricted[a]

Virus	Family	Virus	Family
African horse sickness virus	*Reoviridae*	Nairobi sheep disease virus	*Bunyaviridae*
African swine fever virus	Unclassified	Newcastle disease virus (velogenic strains)	*Paramyxoviridae*
Borna disease virus	Unclassified	Porcine polio-encephalomyelitis virus	*Picornaviridae*
Bovine ephemeral fever virus	*Rhabdoviridae*	Rift Valley fever virus	*Bunyaviridae*
Camelpox virus	*Poxviridae*	Rinderpest virus	*Paramyxoviridae*
Foot-and-mouth disease virus	*Picornaviridae*	Swine vesicular disease virus	*Picornaviridae*
Fowl plague virus	*Orthomyxoviridae*	Vesicular exanthema virus	*Caliciviridae*
Lumpyskin disease virus	*Poxviridae*	Wesselsbron disease virus	*Flaviviridae*

[a]For the United States. In other developed countries there are similar listings, some even longer; e.g., the Australian list includes, as well as these, bluetongue viruses, epidemic hemorrhagic disease of deer virus, hog cholera virus, malignant catarrhal fever virus, ovine progressive pneumonia virus, pseudorabies virus, rabies virus, the scrapie agent, and sheeppox virus, but it excludes bovine ephemeral fever virus, which is enzootic in Australia.

tries support special laboratories for their investigation. These are the so-called maximum containment laboratories, popularly designated by their location, e.g., Plum Island in the United States, Pirbright in the United Kingdom, and Geelong in Australia. "Restricted" animal viruses in the United States and Australia, the importation, possession, or use of which is prohibited or restricted by law or regulation, are listed in Table 13-7.

DIRECT IDENTIFICATION OF VIRUS, VIRAL ANTIGEN, OR VIRAL NUCLEIC ACID

We use the term direct identification, in contrast to virus isolation, to encompass a variety of methods that can be used to detect and often identify the etiological agent by the direct demonstration of virions or

viral constituents in the tissues, secretions, or excretions of infected animals. Although they do not provide the laboratory worker with a culture of the causative virus for further study, these direct methods have great advantages in terms of speed, cost, and the number of samples that can be examined. They can be subdivided into methods used to detect virions, viral antigens, or viral nucleic acids.

Direct Detection of Virions by Electron Microscopy

The introduction of negative staining procedures, together with a realization that in many clinical situations the concentration of virions frequently exceeds the critical lower limit of 10^6 per milliliter required for visualization in the electron microscope, has led to the use of this instrument for rapid viral diagnosis (Plate 13-5). The procedure is particularly suited to enteric infections, in which a crude fecal suspension can be clarified by low-speed centrifugation, followed by high-speed centrifugation to yield a pellet for negative staining. In addition to its value in the recognition of known viruses, this technique has led to the discovery of new viruses of etiological importance in diarrheal diseases which were, and in some cases remain, uncultivable (e.g., some adenoviruses, astroviruses, caliciviruses, coronaviruses, parvoviruses, and rotaviruses).

The procedure is also suited to viral infections of the skin and mucous membranes, the appropriate specimen being scabs, vesicular fluid, or scrapings made with a scalpel. Also, as described earlier, electron microscopy can be used for the rapid identification of viruses isolated in cell culture, allowing immediate and definitive diagnosis to the family or sometimes the genus or species level.

The sensitivity of electron microscopic methods can be enhanced by the use of immune serum, by a procedure known as immunoelectron microscopy. The sample, usually clarified by low-speed centrifugation, is mixed with antibody, and after overnight interaction, the immune complexes are pelleted by low-speed centrifugation and the pellet negatively stained. The antibody used may be serum from an old animal hyperimmune to a large number of viruses, or it may be type-specific polyclonal or monoclonal antibody, or such antibodies may be used sequentially. Solid-phase immunoelectron microscopy procedures have also been developed, in which virus-specific antibody is first bound to the plastic supporting film on the copper grid. Sensitivity is enhanced by a double-layering procedure, in which staphylococcal protein A (which binds the Fc moiety of IgG) is bound to the film, then virus-specific antibody, to which the sample is then added.

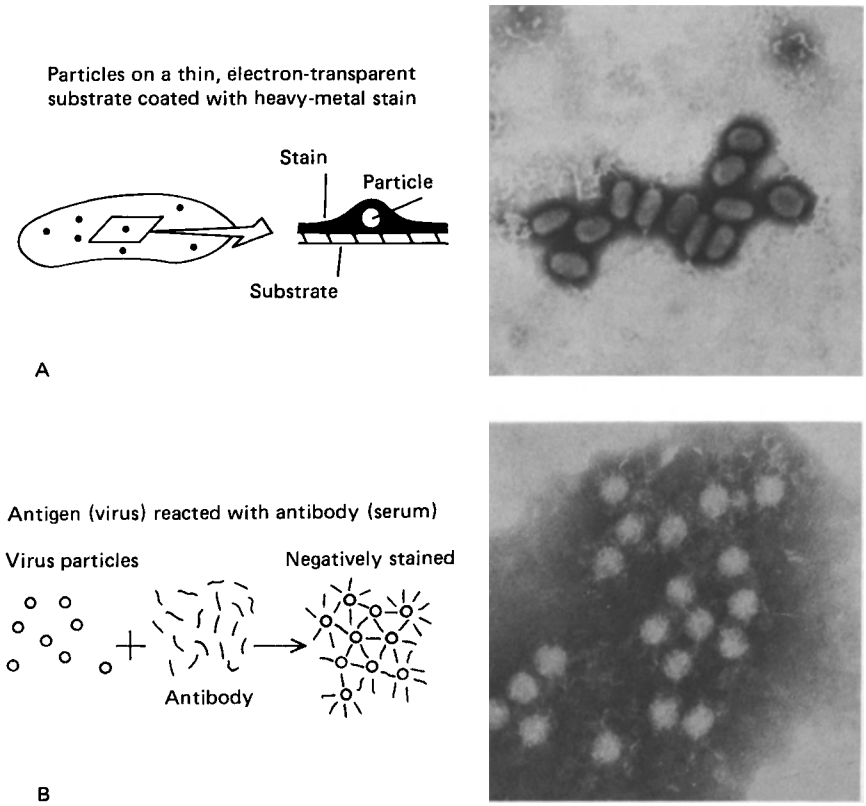

PLATE 13-5. *Negative staining for electron microscopy. (A) Direct staining: virions of bovine papular stomatitis virus. An electron-opaque stain (1% phosphotungstic acid, pH 7.3) was mixed with scrapings from the lesion and applied to plastic film supported by the copper electron microscope grid (×13,000). (B) Immunoelectron microscopy: an isolate of foot-and-mouth disease virus type O was incubated with homotypic antiserum and stained with phosphotungstic acid (×100,000). Note aggregation of virions by antibody. [From E. P. J. Gibbs* et al., Vet. Rec. **106,** *451 (1980).]*

Direct Detection of Viral Antigen(s)

These methods are based on direct interaction between virions or viral antigens, *in situ* in tissues or in excretions or secretions, and specific antibodies which are prelabeled in some way so as to permit the ready recognition of the interaction. The methods are specified by the method of labeling used: immunofluorescence, immunoperoxidase staining, radioimmunoassay, or enzyme-linked immunosorbent assay (ELISA). Vir-

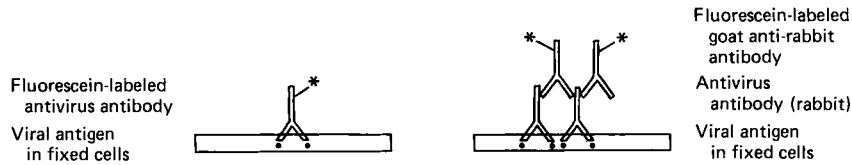

FIG. 13-1. *Immunofluorescence. Left, direct. Right, indirect.*

al antigens can also be detected by such time-honored serological procedures as precipitation and complement fixation.

Immunofluorescence. Its specificity, sensitivity, rapidity, and relative simplicity make immunofluorescence a procedure of singular importance in the rapid diagnosis of viral infections. The prototypic example of immunofluorescence is the diagnosis of rabies, for which it has been the standard test for more than 20 years (see Chapter 30). It is now being used for a wide range of viruses. Immunofluorescence can be applied to smears and frozen sections of tissues or organs.

Two alternative staining procedures are used: (1) direct immunofluorescence, in which the antiviral antibody is conjugated to the fluorescent dye, fluorescein, and (2) indirect ("sandwich") immunofluorescence, in which an antiimmunoglobin specific for the animal species providing the antiviral antibody is conjugated to fluorescein (Fig. 13-1). For instance, an acetone-fixed smear or frozen tissue section is treated with virus-specific antibody (prepared, say, in rabbits), then rinsed before the second antibody, a fluorescein-conjugated anti-rabbit immunoglobulin made in goats, is added. Indirect procedures have two significant advantages over direct-staining procedures:

1. If antibodies to different viruses are raised in a single animal species, e.g., rabbits, then only a single conjugated antibody is required.
2. The amount of bound labeled antibody is greatly augmented, hence the method is much more sensitive. Although simple in principle, the effective use of immunofluorescence demands careful attention to many technical details if false positive results are to be avoided.

In addition to immunofluorescent staining of specimens taken directly from clinical cases, the method is an important adjunct in the identification of viruses isolated in cell cultures. It may also be used in reverse, for the detection of antibody in serum. Slides containing viral antigen, either smears, sections, or, more usually, cell cultures, are prepared in large numbers and stored at −70°C. For use, they are flooded with the serum under test and a second fluorescein-conjugated antispecies antibody is used to detect the bound antibody.

Special care needs to be exercised in the application of immunofluorescence to herpesviruses, in that some herpesvirus-infected cells are known to express Fc receptors on their plasma membrane; such receptors bind all IgG molecules, not only those with herpesvirus specificity, hence additional controls are required.

Immunoperoxidase Staining. An alternative method for locating and identifying viral antigen in infected cells employs an enzyme-labeled antibody. The procedure requires less expensive equipment than immunofluorescence—an ordinary light microscope is used—and produces a morphologically clearer, nonfading, permanent preparation. The procedures and principles are similar to those of immunofluorescence. The conjugated antibody, bound to antigen by a direct or indirect procedure, is detected by adding a substrate appropriate to the particular enzyme; in the case of peroxidase this is H_2O_2 mixed with a benzidine derivative which forms a colored, insoluble precipitate in the presence of enzyme. A disadvantage of the technique is that endogenous peroxidase present in the cells of many tissues, particularly leukocytes, produces false positive results. This problem can be circumvented by meticulous technique and adequate controls.

Radioimmunoassay. In radioimmunoassay the label is a radioactive element, commonly ^{125}I. The method is exquisitely sensitive, enabling viral antigens to be detected at concentrations as low as 10^{-12} *M*. Many protocols for radioimmunoassays have been devised. Both direct and indirect methods can be used, the principles being the same as for immunofluorescent staining (Fig. 13-2). Most are solid-phase procedures in

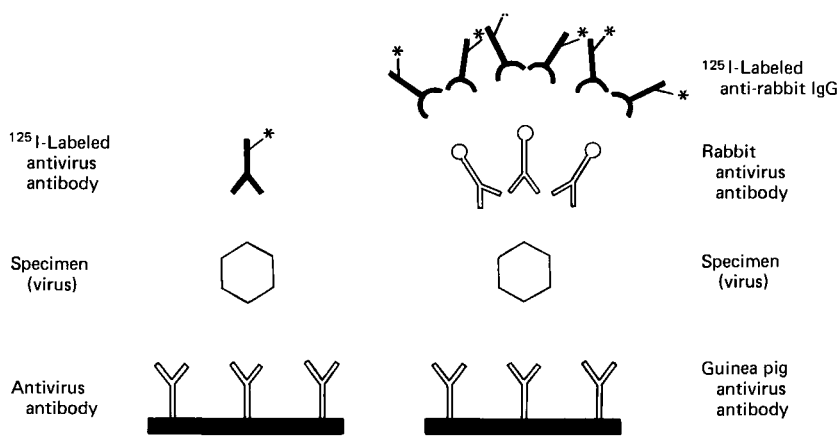

FIG. 13-2. *Radioimmunoassays for detection of virus and/or viral antigen. Left, direct. Right, indirect.*

which the "capture" antibody (or antigen) is bound to a solid substrate, typically a polystyrene tube or bead, or to the wells of a plastic microtiter plate. In the simplest format (Fig. 13-2, left) the sample suspected to contain virus or viral antigen is allowed to bind to the bound antibody, then after washing, ^{125}I-labeled antiviral antibody ("detector" antibody) is added. After a further washing, the bound labeled antibody is measured in a gamma counter. A more commonly used protocol is the indirect radioimmunoassay, in which the detector antibody is unlabeled but a further layer, ^{125}I-labeled anti-IgG, is added as "indicator" antibody. (The antiviral antibodies constituting the capture and detector antibodies must be raised in different animal species; see Fig. 13-2, right.)

Enzyme-Linked Immunosorbent Assays (ELISA). ELISA (also known as enzyme immunoassay, EIA) offers the same sensitivity as radioimmunoassay without the inherent disadvantages of expensive isotopes of short half-life and the need for safe handling and disposal and a costly gamma counter. The basic principles are similar to those of radioimmunoassay (Fig. 13-3). Antibody is bound to a solid phase, usually the wells of a microtiter tray. Samples suspected to contain antigen are added to the wells. After an appropriate reaction time, the wells are rinsed and a second virus-specific antibody that has been conjugated to an enzyme is added. After allowing this to bind, the contents of the well are rinsed and a substrate for the enzyme is added. The assay is read by

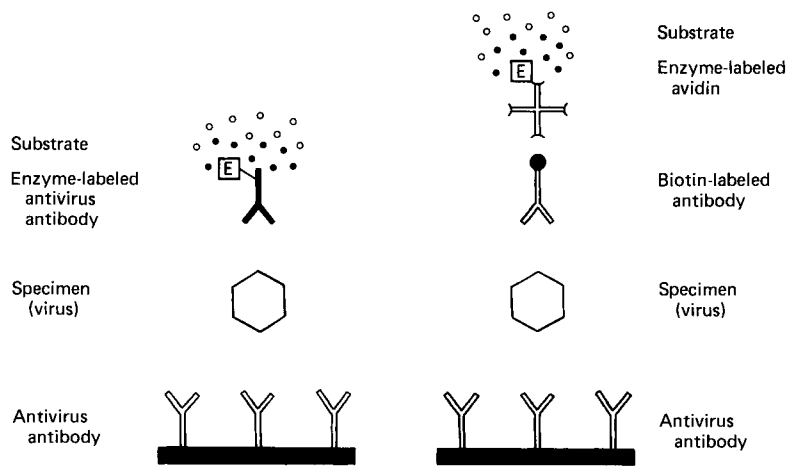

FIG. 13-3. *Enzyme-linked immunosorbent assay (ELISA) for detection of virus and/or viral antigen. Left, direct. Right, avidin–biotin.*

a color change in the substrate and can be made quantitative by serially diluting the antigen to obtain an end point or by photometrically reading the amount of color change, a reflection of the amount of enzyme-conjugated antibody bound. As in radioimmunoassay, there are many variations in protocol, e.g., exploiting the very high affinity of avidin for biotin (Fig. 13-3, right). Moreover, if antigen is bound to the plate first, the procedure is equally suitable for the detection and quantitation of viral antibody.

ELISA procedures have been developed for a wide variety of applications in veterinary medicine. At one level, kits have been marketed for the rapid diagnosis of a number of important viral diseases by veterinary practitioners themselves. At another level ELISA procedures have been automated by the introduction of automatic dispensing, washing, and spectrophotometric reading and recording instruments, that permit hundreds of samples to be processed in a day, e.g., in the testing of swine for pseudorabies antibodies.

Immunodiffusion (Precipitation-in-Gel). If wells are cut in agar and antibody and antigen are placed in separate wells, the two diffuse toward each other (immunodiffusion) and form visible bands of precipitate (Plate 13-6). Several ingenious applications of the procedure have been developed and the test is widely used for the diagnosis of some diseases of domestic animals (e.g., bovine leukemia, equine infectious anemia; see Chapter 31).

Complement Fixation. Although now considered too cumbersome a procedure for general use in the rapid detection of viral antigen, the complement fixation procedure is still employed for the rapid and specific detection of foot-and-mouth disease viral antigen in vesicular fluid, providing both rapid diagnosis and specific typing of the virus involved.

Direct Detection of Viral Nucleic Acid

If dsDNA is separated ("melted") into single strands by heat or alkali treatment, the single strands will, under appropriate conditions, reanneal to each other, or competitively to an identical or related complementary strand. If the original DNA is labeled with either ^{32}P or ^{35}S, it may be used as a probe for the detection of related DNA in infected cells. This procedure, known as *in situ* hybridization, can be made even more specific by using probes that are shorter than the full-length genome. It can also be made more sensitive by maximizing the amount of label incorporated into the probe. Hybridization is detected by autoradiography. Recently, nonradioactive hybridization procedures, based on the

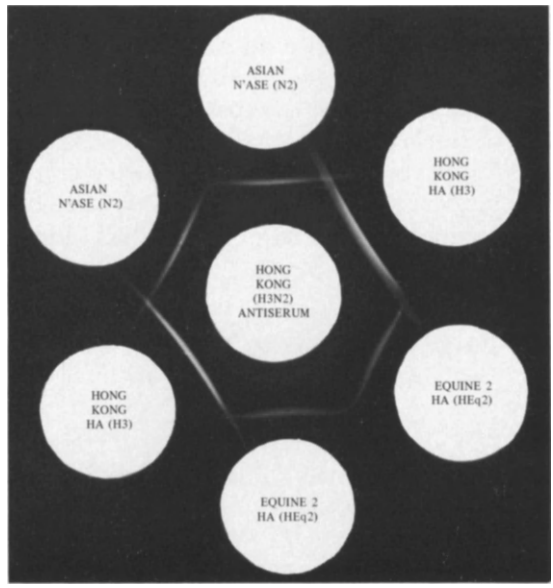

PLATE 13-6. *Immunodiffusion test. Example illustrates its use to analyze relationships between envelope antigens of influenza A virus. Center well: antiserum to Hong Kong influenza virus (H3N2). Peripheral wells, purified antigens: Hong Kong hemagglutinin (H3), Asian neuraminidase (N2), and equine 2 hemagglutinin (HEq2). Antiserum to Hong Kong virus contains antibodies to all the antigens tested. Note (1) two pairs of antigens (N2 and HEq2) each show fusion of precipitin lines ("reaction of identity"), (2) neuraminidase N2 and hemagglutinin H3 show complete crossing over of precipitin lines ("reaction of complete nonidentity"), and (3) equine (HEq2) and Hong Kong (H3) hemagglutinins show partial fusion of lines ("reaction of partial identity") indicating serological cross-reactivity. (Courtesy Dr. R. G. Webster.)*

incorporation of biotin-conjugated nucleotides into the DNA probe, have been developed; avidin, which binds strongly to biotin, is subsequently added. The avidin is detected by ELISA, immunofluorescence, or immunoperoxidase staining.

In situ hybridization procedures are particularly useful when viral DNA is present in cells but is not expressed, as with integrated retroviral DNA, or episomal DNA in some papovavirus-infected cells. Probes can be made highly specific by selection from a collection of cloned fragments of the whole viral genome. The probes are labeled by *in vitro* nick translation procedures, and are then applied to nitrocellulose blot transfers of the animal tissue (active hybridization) or to nitrocellulose blots

taken from gels on which viral nucleic acid has been separated (Southern blotting), or from nitrocellulose onto which viral nucleic acid-containing samples have been spotted (dot–blot hybridization). These procedures have proved of great value in virus research, but it remains to be seen to what extent nucleic acid probes and hybridization procedures displace other methods for rapid diagnosis of viral infections. It may have advantages over virus isolation in the case of viruses that are noncultivable, slow growing, dangerous, or nonviable as a result of suboptimal conditions of transport or storage.

DETECTION AND MEASUREMENT OF ANTIVIRAL ANTIBODIES

Detection of viral antibody can be used for the diagnosis of viral infections, either in individual animals or in populations. The method is particularly useful in the latter context, since serum samples are readily obtained with simple equipment, in contrast to special requirements, time, and effort needed for collecting samples for virus isolation. Furthermore, tests for antibody such as ELISA lend themselves to automation, so that large numbers of samples can be tested. They form the basis of epidemiological surveys and of control and eradication programs, but have major limitations in diagnosis.

For diagnosis in the individual animal, paired sera are tested for specific viral antibody, the first sample being taken when the animal is first examined (acute-phase serum), and the second sample 2–4 weeks later (convalescent-phase serum). A rise in antibody titer between the first and second samples is a basis, albeit in retrospect, for a specific viral diagnosis. Sometimes the demonstration of antibody in a single serum sample is diagnostic of current infection, e.g., with retroviruses and herpesviruses, since these viruses establish lifelong infections. However, in such circumstances there is no assurance that the persistent virus was responsible for the disease under consideration.

Detection of antiviral antibody in presuckle newborn cord or venous blood provides a basis for specific diagnosis of *in utero* infections. It was used, for example, in showing that Akabane virus was the cause of arthrogryposis–hydranencephaly in calves (see Chapter 29). Since transplacental transfer of immunoglobulins is rare in domestic animals (see Table 9-3), the presence of either IgG or IgM is indicative of exposure of the fetus to antigen.

Serological methods based on the detection of virus-specific IgM may also be used for the specific diagnosis of recent viral infection, since antibodies of the IgM class appear first after primary infection and de-

clines to relatively low levels, compared to IgG, by about 3 months after infection. However, the method has not yet been much exploited in veterinary medicine.

Technical advances such as miniaturization (microtiter plates), automation for large numbers of samples, monoclonal antibodies, and the development of diagnostic kits such as latex agglutination assays for detecting specific IgM, have resulted in a revolution in the approach to diagnostic serology in human medicine. The costs, coupled with the technical problems associated with the large number of animal hosts and their many viruses, have delayed the development of these procedures in veterinary medicine, but their use can be expected to expand considerably in the future. However, screening programs to establish regional or national prevalence rates for particular viruses, based on detection of specific antibody in single serum samples, are an essential feature in defining the epidemiology of viruses of domestic animals.

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CHAPTER 14

Immunization against Viral Diseases

The methods of reducing the impact of viral infections on animals of economic importance include a range of management practices such as test and slaughter, hygiene and sanitation, and immunization. The most generally applicable way of preventing viral diseases is by immunization, and the control of a large number of diseases of animals by immunization is probably the outstanding achievement of veterinary medicine in this century. Although we still have not fully utilized vaccines of traditional type, the field has been catapulted to a new plane of promise by the application of new technologies including the use of recombinant DNA, site-directed mutagenesis, synthetic peptides, and bacterial, yeast, and mammalian expression systems.

There are important differences between immunization practices in humans and animals. Except in developing countries, economic constraints are of little importance in human medicine, but very important in most areas of veterinary practice. There is far greater agreement within and between countries about the safest and most efficacious vaccines

TABLE 14-1
Types of Viral Vaccines

Live-virus vaccines	
Virulent	Delivered by an unnatural route or at a safe age
Attenuated	Naturally occurring or derived by serial passage in eggs or cell culture
	Genetic manipulation possible: temperature-sensitive, cold-adapted, or deletion mutants; or gene reassortants
Inactivated vaccines	Partially purified suspensions of virions, inactivated by chemical or physical treatment
Protein vaccines	
Subunit	Partially purified protective protein extracted from virions
"Cloned"	Protein derived by recombinant DNA technology, in bacteria, yeast, or mammalian cell line
Synthetic peptide	Critical epitopes of protective protein synthesized chemically
Virus-vectored vaccines	A virus, e.g., vaccinia virus, is used as a vector for the gene specifying the protective antigen

to be used in human vaccination than there is with vaccines destined for use in animals. At the international level, the World Health Organization exerts for human vaccine usage an impressive degree of persuasive leadership and maintains a number of programs such as the Expanded Programme on Immunization. This leadership is not matched for animal vaccine usage by its sister agency, the Food and Agriculture Organization. And within countries, far more latitude is allowed in the manufacture and use of vaccines for animal diseases than has ever been allowed by national regulatory authorities, such as the Food and Drug Administration of the United States, for human vaccines. Finally, even a very small number of vaccine-associated illnesses or deaths constitute a major objection to the use of a vaccine in humans whereas in animals mild disease (and even, in the past, occasional deaths) can be tolerated if the vaccine is effective and the potential cost of failure to control the disease is sufficiently high.

Viral vaccines have traditionally been classified into two broad categories: live-virus and inactivated (Table 14-1). Most live-virus vaccines are attenuated mutants, selected for their relative avirulence. They must nevertheless be capable of replicating in the host and eliciting an immune response similar to that occurring after natural infection, for that is the rationale of their use. Inactivated vaccines, in contrast, are produced by chemically or physically destroying the infectivity of the vir-

ulent wild-type virus, while retaining its immunogenicity. A refinement is to use subviral components, or to purify or synthesize the particular protein known to elicit neutralizing antibody.

The great majority of vaccines currently used in veterinary practice have been developed empirically. These are the major focus of this chapter, but we also briefly outline some of the modern approaches to vaccine production, because future developments lie in this direction.

ATTENUATED LIVE-VIRUS VACCINES

If they can be developed and are safe, live-virus vaccines consisting of attenuated mutants (see Chapter 5) are probably the best of all vaccines. Several of them have been dramatically successful in reducing the incidence of important diseases of animals and humans. Most live-virus vaccines are injected subcutaneously or intramuscularly, but some are delivered orally, and a few by aerosol. The vaccine virus replicates in the recipient, eliciting a lasting immune response, but causes little or no disease. In effect, a live-virus vaccine produces a subclinical infection.

Naturally Occurring Viruses as Vaccines

The original vaccine (*vacca* = cow), introduced by Jenner in 1798 for the control of human smallpox, utilized cowpox virus, a natural pathogen of the cow. This virus produced only a mild lesion in humans but, because it is antigenically related to smallpox virus, it conferred protection against the severe human disease. More recently the same principle has been applied to other diseases, such as the protection of chickens against Marek's disease with a herpesvirus of turkeys, the use of bovine rotavirus by the oral route to protect piglets against porcine rotavirus infection, and the use of naturally occurring "lentogenic" strains of Newcastle disease virus to protect chickens against virulent ("velogenic") strains.

Virulent viruses given by an unnatural route have been also used as vaccines, e.g., avian infectious laryngotracheitis virus via the cloaca (see Chapter 19), or orf virus by scarification of the skin of the inguinal region in sheep. Such a procedure minimizes the severity of disease, but results in immunity. Alternatively, a virulent virus, e.g., avian infectious bursal disease virus, may be administered to adult birds (at which age it causes no damage) in order to provide protection for their chicks via maternal antibody.

Artificially Attenuated Viruses

Most attenuated live-virus vaccines have been derived empirically, by serial passage of the wild-type virus through one or more types of cell culture, sometimes after prior adaptation to laboratory animals or eggs. In the process, the accumulation of a large number of mutations generally leads not only to more vigorous growth in the particular type of cultured cell used, but also to progressive loss of virulence for the original host. It is important to "fix" the degree of attenuation, so that the vaccine virus retains the capacity to replicate sufficiently to produce the desired level of immunity (even if it also causes mild illness in a few vaccinated animals), but does not cause damaging morbidity.

For most viruses, we are still ignorant about what genes are associated with virulence (see Chapter 5), and in only a few cases has the avirulence of an attenuated live-virus vaccine been characterized in terms of its altered pathogenesis in the vaccinated animal.Sometimes the vaccine strain appears to have lost its capacity to infect important target organs, e.g., the brain, with canine distemper vaccine.

An alternative approach in developing attenuated vaccine strains has been to select temperature-sensitive mutants (see Chapter 5); however, there have been problems with reversion to virulence. Temperature-sensitive mutant vaccines have been developed for bovine infectious rhinotracheitis, bovine respiratory syncytial disease, and bovine virus diarrhea. Cold-adapted mutants appear to be more stable, but they are often of low immunogenicity.

Genetic engineering can be used to construct deletion mutants by excising a sequence of nucleotides from a gene that is not essential for viral replication but contributes to virulence (e.g., the thymidine kinase gene in herpesviruses). Since such mutants do not revert, they are attractive candidates for attenuated live-virus vaccines. Moreover, site-directed mutagenesis (see Chapter 5) now permits the introduction of prescribed nucleotide substitutions at will. Thus, when more becomes known about particular genes involved in virulence, it will be possible to construct vaccines with any designated nucleotide sequence.

Among viruses with segmented genomes, such as orthomyxoviruses and rotaviruses, attenuated reassortants may be constructed. Eggs or cultured cells are coinfected with a "master" strain of documented low virulence, together with the virulent field strain bearing the particular antigens against which protection is sought. Though widely used for constructing vaccines against human influenza, this approach has had little use in veterinary medicine, except on a limited scale with equine and avian influenza and bluetongue viruses.

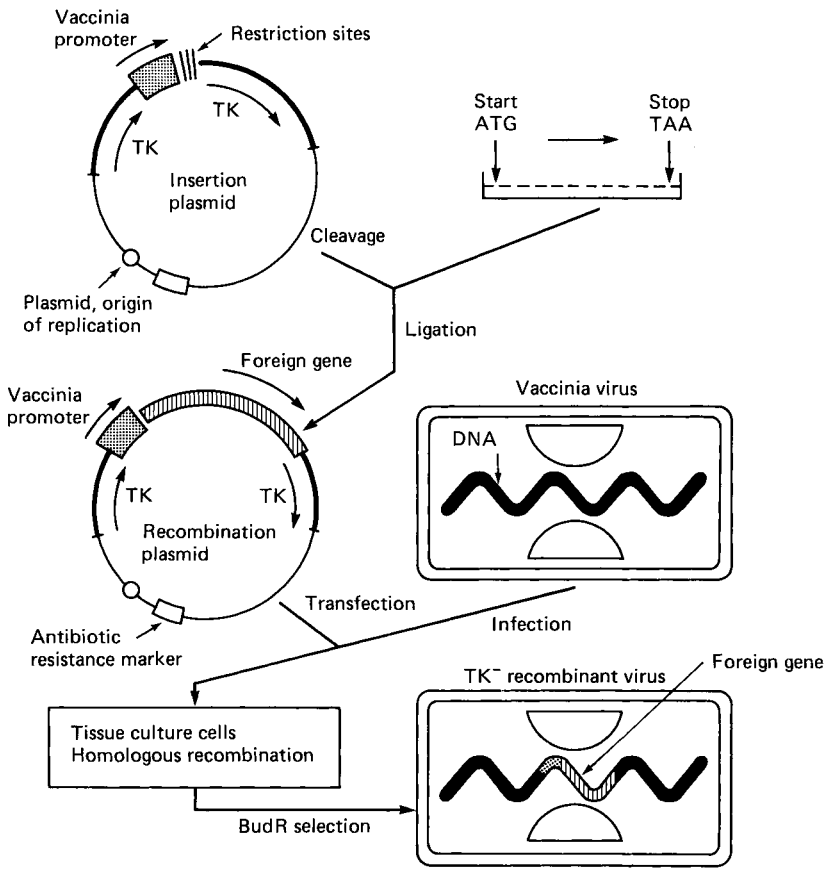

FIG. 14-1. *Method of constructing a vaccinia virus vector carrying a selected gene from another virus. TK, Thymidine kinase gene of vaccinia virus; BudR, bromodeoxyuridine. (Courtesy Dr. B. Moss.)*

Virus-Vectored Vaccines

A novel method potentially of wide applicability in veterinary medicine invokes the use of a virus with a large DNA genome, such as a poxvirus, herpesvirus, or adenovirus, as a vector to carry the genes for the "protective" antigens of other viruses. The prototype developed in 1983 was vaccinia virus incorporating the gene for hepatitis B surface antigen (Fig. 14-1). Analogous vaccinia–vesicular stomatitis, vaccinia–rabies, and vaccinia-transmissible gastroenteritis recombinant viruses have been shown to protect animals against challenge with the corresponding virulent

viruses. It has also been shown that genes for critical protective antigens from several unrelated viruses can be incorporated into the genome of vaccinia virus, and vaccination of animals with these compound hybrid vector vaccines produces good antibody responses to all of the corresponding viruses. Other viruses are also being developed as vectors. Fowlpox virus is a logical choice for avian vaccines, and a limited amount of experimental work has shown that adenoviruses and herpesviruses have advantages in terms of long-term antigen expression.

Although a great deal of research and development needs to be done and each putative virus-vectored vaccine must be carefully examined for its immunogenic and protective potential, this approach opens up the possibility of tailor-made vaccines for the range of viral and bacterial and perhaps parasitic infections common in each species of domestic animal in any particular country. This is clearly an approach of great potential and is being actively explored.

INACTIVATED VACCINES

Inactivated vaccines are made from virulent virus by destroying its infectivity while retaining its immunogenicity. Being noninfectious, such vaccines are safe, but they need to be injected in very large amounts to elicit an antibody response commensurate with that attainable by a much smaller dose of attenuated live-virus vaccine. Normally, even the primary course comprises two or three injections, and further ("booster") doses may be required at intervals over the succeeding years to revive waning immunity.

The most commonly used inactivating agents are formaldehyde, β-propiolactone and the ethylenimines. Formaldehyde is still used in some countries to produce foot-and-mouth disease vaccine, but since inactivation is sometimes incomplete, it is being replaced by other inactivating agents, for example, ethylenimine. One of the advantages of β-propiolactone, now used in the manufacture of human rabies vaccines, for example, is that it is completely hydrolyzed within hours to a nontoxic product.

Inactivated vaccines tend to be expensive because of the large amount of virus that needs to be injected. Recent efforts have therefore been directed toward scaling up the commercial production of virus, e.g., by using continuous cell lines capable of growing as individual cells in suspension culture, or as monolayers on "microcarrier" beads suspended in fermentation tanks.

Because virions in the center of aggregates may be protected from the

effects of chemical inactivation, it is important that aggregates be removed prior to the inactivation treatment. Failure to do so has resulted in vaccine-derived disease. Outbreaks of foot-and-mouth disease have been traced to such failure, and in 1955 a notorious human tragedy had a similar cause: an early batch of Salk poliovirus vaccine, containing residual live virus, was responsible for a number of cases of paralytic poliomyelitis.

ISOLATED IMMUNOGENS

The logical extension from using whole inactivated virions as vaccines is to remove all nonessential components of the virion and inoculate only the relevant immunogen, namely the particular protein against which neutralizing antibodies are directed. Such "subunit" vaccines are in use against influenza and hepatitis B in humans, and have been shown to be feasible against a wide range of other viruses, including foot-and-mouth disease virus.

Viral Proteins from Genes Cloned by Recombinant DNA Technology

Recombinant DNA technology provides a means of producing cheaply, on an industrial scale, large amounts of viral protein that can be readily purified. Details of this technology were given in Chapter 5. The complete genomes or selected genes of many vertebrate viruses have been cloned in prokaryotic or eukaryotic cells. When these genes are expressed, the resultant proteins are harvested from host cells or are released into supernatant culture fluids. Experiments are being carried out with foot-and-mouth disease virus VP1 protein produced in this way.

Synthetic Peptides

The amino acid sequence of a viral protein can be deduced from the nucleotide sequence of the viral nucleic acid or can be determined directly. Parts of the protein, oligopeptides, can then be synthesized chemically. Now that techniques are available for locating the antigenic sites on viral proteins (see Chapter 9 and Fig. 9–5), it is possible to synthesize short peptides corresponding to the critical antigenic determinants to which neutralizing antibodies bind. Of particular interest is the possibility that relatively invariate ("conserved"), even buried sequences, not normally immunogenic when presented *in situ* in the virion, may be capable in isolation of eliciting neutralizing antibodies

which may then have the added advantage of cross-protection against heterologous serotypes.

This new approach merits further research, although it must be emphasized that all antigenic determinants are conformational, i.e., they require the three-dimensional shape that they assume in the intact protein molecule. Since short synthetic peptides lack this tertiary conformation, most antibodies raised against them are incapable of binding to virions, hence the neutralizing titer may be orders of magnitude lower than that induced by inactivated vaccine, or even by the purified intact protein. Nevertheless, synthetic peptides have been shown to elicit neutralizing antibodies against foot-and-mouth disease virus, rabies virus, and certain other animal pathogens. Moreover, peptides incapable of inducing protective levels of antibody may prime an animal to respond anamnestically when subsequently boosted with a subimmunizing dose of virus.

Immunopotentiation by Adjuvants and Liposomes

The immunogenicity of inactivated vaccines, and to an even greater extent isolated viral proteins or peptides, needs to be considerably enhanced if they are to maximally effective. Such immunopotentiation may be achieved by emulsification with an *adjuvant* and/or incorporation in *liposomes*.

Adjuvants are materials that are added to vaccines to potentiate the humoral and/or cellular immune response so that a greater amount of antibody is produced, a lesser quantity of antigen is required, and fewer doses need to be given. Their mechanisms of action are still uncertain but include (1) prolonged retention and slow release of antigen, (2) activation of macrophages, leading to secretion of lymphokines and attraction of lymphocytes, and (3) mitogenicity for lymphocytes. The adjuvants most widely used in animals are alum and mineral oils. For some vaccines the immunogen is adsorbed to alum (aluminum hydroxide gel), but the resulting immune response is not particularly prolonged, hence booster injections are required. Other adjuvants are being developed, but none has yet come into general use. Attention has now turned to simple, chemically defined, preferably synthetic substances with a known mode of action. One of the most interesting is the active component of the mycobacterial component of Freund's complete adjuvant, a muramyl dipeptide derivative. Muramyl dipeptide and its derivatives can be coupled to synthetic antigen or incorporated into liposomes.

Liposomes consist of lipid membranes into which proteins can be incorporated. When purified viral envelope glycoproteins are used, the resulting "virosomes" (or "immunosomes") somewhat resemble the

original enveloped virion. This ingenious trick enables one not only to reconstitute viruslike structures lacking viral nucleic acid and other extraneous material, but also to select nonpyrogenic lipids and to incorporate substances with adjuvant activity, thus regaining much of the immunogenicity lost when the viral glycoprotein was removed from its original milieu. Viral proteins can also be assembled into other types of micelles to restore immunogenicity.

ANTIIDIOTYPE ANTIBODIES

The antigen-binding site of every antibody molecule contains a unique amino acid sequence known as its *idiotype.* Antibody can be raised against this idiotype and is known as an antiidiotype antibody. Because antiidiotype antibody is capable of binding to the same idiotype as binds the corresponding epitope on the original antigen, it may be surmised that antiidiotype antibody mimics the conformation of that epitope. Therefore, antiidiotype raised against a neutralizing monoclonal antibody to a particular virus could conceivably be used as a vaccine. The antibodies it would elicit would bind to and neutralize the virus. In fact, antiidiotype antibodies raised against antibodies to hepatitis B surface antigen or reovirus S1 capsid antigen do elicit an antiviral antibody response upon injection into animals. Moreover, antiidiotype antibodies raised against the receptor on a T lymphocyte line specific for Sendai virus have been reported to elicit a cytotoxic T cell response in mice, which protects them against viral challenge.

It is still far from clear that this points the way to a novel vaccine strategy. First, the generality of these initial findings needs to be confirmed. Second, it is crucial to establish whether antiidiotype antibody, when administered as an immunogen, elicits the same type of immune response as does antigen, i.e., that it achieves the right balance of humoral and cell-mediated immunity rather than, say, a suppressor T cell response.

ATTENUATED LIVE-VIRUS VERSUS INACTIVATED VACCINES

The relative merits and disadvantages of attenuated live-virus and inactivated vaccines are summarized in Table 14-2 and discussed below.

Immunological Considerations

The object of immunization is to protect against disease, not necessarily to prevent infection. If, as immunity wanes over months or years

TABLE 14-2
Advantages and Disadvantages of Attenuated Live-Virus and Inactivated Vaccines

Parameter	Vaccine type	
	Attenuated live-virus	Inactivated
Route of administration	Natural[a] or injection	Injection
Antigen per dose	Low	High
Cost	Low	High
Number of doses needed	Single[b]	Multiple
Need for adjuvant	No	Yes
Duration of immunity	Many years	Months or years[c]
Antibody response	IgG; IgA[d]	IgG
Cell-mediated response	Good	Uncertain
Heat lability[e]	Yes[f]	No
Interference	Occasional[g]	No
Side effects	Occasional mild signs	Occasional local or general reactions
Dangerous in pregnant animals	Some	No
Reversion to virulence	Possible	No

[a]Oral or respiratory, in certain cases.
[b]For some live vaccines a second dose may be required.
[c]But satisfactory with some inactivated vaccines.
[d]IgA if delivered via oral or respiratory route.
[e]Especially in hot climates.
[f]Stabilizers added to vaccine, plus maintenance of "cold chain" delay inactivation.
[g]If administered by oral or respiratory route.

following active immunization, or the weeks following passive immunization, infection with wild-type virus does occur, the infection is likely to be subclinical, but will boost immunity. This is a frequent occurrence in farm animals and birds in crowded pens, continuously exposed to an environment contaminated with certain common viruses.

Acquired immunity to many respiratory viruses is so poor even following natural infection that it is perhaps unrealistic to expect any respiratory viral vaccine to be effective in preventing infection. In such cases, however, a reasonable objective may be to prevent serious (lower respiratory) disease.

It has been argued that clinical or subclinical infection is Nature's way of immunizing, that by and large this is extremely effective, inducing lifelong immunity following systemic infection (see Chapter 9), and that attenuated live-virus vaccines, preferably delivered via the natural

route, are obviously the nearest approach to this ideal. In general, IgA is considered to be the most important class of immunoglobulin relevant to the prevention of infection of mucosal surfaces, such as those of the intestinal, respiratory, genitourinary, and ocular epithelia. One of the great advantages of an attenuated live-virus oral vaccine, such as Newcastle disease vaccine, is that by virtue of its replication in the intestinal tract, it leads to prolonged synthesis of local IgA. This in turn prevents the subsequent replication of wild-type virus and deprives the virus of hosts in which to circulate subclinically. Such a regime may make feasible the prospect of local eradication of the virus, as well as the disease, from the population.

A second, perhaps even more crucial reason for favoring attenuated live-virus vaccines is that they are more effective in eliciting cell-mediated immunity. Although it is clear that neutralizing antibody is the key to prevention of establishment of infection, there is good evidence that T lymphocytes, particularly T_c cells, play a crucial role in recovery from many infections. T_c cells are activated by viral antigen presented on the surface of infected cells in association with MHC antigens (see Chapter 9). The differential responses of T_s, T_h, T_d, and T_c cells to viral antigens presented in the form of attenuated live virus, inactivated virions, or soluble protein, respectively, merits fuller investigation.

A further major reason for favoring vaccines that elicit a T lymphocyte response is that T cells display broader cross-recognition of related viral strains than do B cells. Such cross-immunity would be advantageous where several viral serotypes circulate simultaneously or sequentially (antigenic drift).

Safety

Properly prepared and tested, both attenuated live-virus and inactivated vaccines are safe in immunocompetent animals, as are purified, synthetic, or cloned proteins. Licensing authorities have become extremely vigilant and have insisted on rigorous safety tests for residual live virulent virus in "inactivated" vaccines.

There are certain potential problems relating to safety which are unique to attenuated live-virus vaccines and must be overcome for every product before licensing.

Underattenuation. Some attenuated live-virus vaccines have been associated with significant disease in some recipients. Attempts to attenuate virulence further by additional passages in cultured cells have not always been successful, in that further attenuation has often been accompanied by a decline in the capacity of the virus to replicate in the host, with a corresponding loss of immunogenicity.

Most modern vaccines are satisfactory from the point of view of safety, and such side effects as do occur with current animal viral vaccines are not a significant disincentive to immunization, provided that owners are adequately informed in advance and perceive that the benefits of immunization are substantially greater than the risks. However, it is important that attenuated live-virus vaccines should be used only in the species for which they were produced; for example, canine distemper vaccine can cause fatalities in Mustelidae.

Genetic Instability. A quite different problem occurs in the case of vaccine strains with an inherent tendency to revert toward virulence during replication in the recipient or in contacts to whom the vaccine virus has spread. Most vaccine viruses are incapable of such spread, but in the case of those that do, there may be an accumulation of back-mutations that gradually leads to a restoration of virulence. The principal example of this phenomenon is the very rare reversion to virulence of poliovirus type 3 oral vaccine in humans, but temperature-sensitive mutants of bovine virus diarrhea virus have also proved to be unstable.

Contaminating Viruses. Since vaccines are grown in animals or cells derived from them, there is always a possibility that a vaccine will be contaminated with another virus from that animal or from the medium used for culturing its cells. Probably the most famous example, which led to restrictions on international trade in vaccines and sera, still in effect today, was the introduction into the United States in 1908 of foot-and-mouth disease virus, as a contaminant of the smallpox vaccine produced in calves. The use of embryonated eggs to produce vaccines for use in chickens poses obvious problems, since so many flocks carry retroviruses that are passed in the egg. Likewise, porcine parvovirus has been recognized as a common contaminant of primary porcine cell cultures and of crude preparations of trypsin, often used for the preparation of cell cultures of all species. The risk is not confined to attenuated live-virus vaccines, since some contaminating viruses are more resistant to inactivation than the vaccine virus.

The danger of contamination of attenuated live-virus vaccines with adventitious viruses has triggered considerable debate about which types of cultured cells should be licensed for use as substrates for the production of human and animal vaccines. If primary cell cultures are to remain legally acceptable for this purpose, the minimum requirement should be that the animals be bred in captivity, preferably under specific pathogen-free conditions, e.g., closed colonies of rabbits, or eggs from leukosis-free flocks, and that the cultured cells be rigorously tested for all possible endogenous viruses. In general, however, primary cell

cultures have been replaced for vaccine production by well-characterized diploid strains or continuous cell lines. Such strains or lines can be subjected to comprehensive screening, certified as safe, then frozen for storage and distribution to vaccine manufacturers on request.

Adverse Effects in Pregnant Animals. Attenuated live-virus vaccines are not generally recommended for use in pregnant animals, since they may be abortigenic or teratogenic. For example, many infectious bovine rhinotracheitis vaccines are abortigenic, and feline panleukopenia, hog cholera, bovine virus diarrhea, and bluetongue vaccines are teratogenic.

Interference and Activation

Attenuated live-virus vaccines delivered by mouth or nose depend critically for their efficacy on replication in the intestinal or respiratory tract, respectively. Interference can occur between different attenuated live viruses contained in the vaccine formulation (e.g., with bluetongue vaccines), or between the vaccine virus and enteric or respiratory viruses with which the animal to be vaccinated happens to be infected at the time. Canine parvovirus infection may be immunosuppressive to such an extent that it interferes with the response of dogs to vaccination against canine distemper. On the other hand, vaccination of cattle with inactivated foot-and-mouth disease vaccine may activate a latent infection with infectious bovine rhinotracheitis virus.

Heat Lability

Attenuated live-virus vaccines are vulnerable to inactivation by high ambient temperatures, a particular problem in the tropics. Since these countries are also, in the main, those with underdeveloped veterinary services, formidable problems are encountered in maintaining refrigeration ("the cold chain") from manufacturer to the point of delivery, e.g., animals in remote rural areas. To some extent the problem has been alleviated by the addition of stabilizing agents to the vaccines, and by packaging them in lyophilized (freeze-dried) form, for reconstitution immediately before administration.

PASSIVE IMMUNIZATION

Instead of actively immunizing with viral vaccines it is possible to confer short-term protection by the intramuscular administration of antibody, as immune serum or immunoglobulin purified therefrom. Homologous immunoglobulin is usually preferred, because heterologous pro-

tein provokes an immune response which can manifest itself as serum sickness or even anaphylaxis. Pooled normal immunoglobulin can be relied upon to contain reasonably high concentrations of antibody against all the common viruses that cause systemic disease in the respective species. Higher titers occur in "convalescent" immunoglobulin from donor animals that have recovered from infection with the virus in question, and such specific immune globulin is the preferred product if commercially available, e.g., for canine distemper and canine parvovirus infection.

Vaccination of the dam is practiced in selected situations in order to provide the newborn with passive (maternal) immunity via antibodies present in the egg (in birds) or in colostrum and milk (in mammals). This is particularly important for diseases in which the major impact occurs during the first few weeks of life, when active immunization of the newborn cannot be accomplished early enough. With avian encephalomyelitis and infectious bursal disease, this strategy is employed for the further reason that the attenuated live-virus vaccines themselves are pathogenic in young chicks.

VACCINATION POLICY

Economic Considerations

Cost–benefit factors are critical in determining the usage of veterinary vaccines. For example, good vaccines are available for the control of many diseases of swine and poultry, but their costs limit their use to large producers, and the diseases remain enzootic elsewhere. Economic constraints are most evident in developing countries. For example, they have led to the abandonment of vaccination against rinderpest in sub-Saharan Africa, in spite of the fact that a concerted vaccination program could well lead to regional elimination and eventually to global eradication of this virus (see Chapter 27).

Because of huge potential markets, together with somewhat less stringent licensing requirements, veterinary vaccines are generally much cheaper than those used in humans. Some avian viral vaccines cost less than 1 cent per dose; many human (and some veterinary) viral vaccines cost dollars. Consumption of veterinary vaccines worldwide is estimated to be about 10^{11} doses per year, for a livestock population of about 10^{10}. Examination of vaccine production data for the United States and South Africa indicates that (1) the poultry industry accounts for over 90% of all doses of veterinary viral vaccines used in the United States, and (2) the pattern of vaccine usage differs strikingly from country to

country; many of the principal diseases against which vaccines are employed in South Africa are exotic to the United States.

The decision to use a vaccine is governed by a complex equation, balancing expected extra profit against costs and risks. If the disease is lethal or causes major economic losses, the need for immunization is clear; both the owners and the vaccine-licensing authorities will accept a risk of occasional quite serious side effects. If, on the other hand, the disease is perceived as trivial, or economically unimportant, no side effects will be countenanced. Where equally satisfactory vaccines are available against a particular disease, considerations such as cost and ease of administration tip the balance, e.g., toward vaccines administered via drinking water in poultry.

Continuation of routine immunization after the risk of disease in an area has almost vanished, but the virus has not been totally eradicated, is psychologically difficult to sustain. Yet it is essential, because reduction in circulation of wild-type virus leaves unimmunized animals highly susceptible, by removing the protective effect of repeated subclinical infections. For example, canine distemper has reemerged among dogs in Switzerland, due to gradual discontinuation of vaccination. Within a country, legislation for compulsory immunization against particular diseases is perhaps the most effective single measure for maintaining protection in the apparent absence of disease.

Vaccination Schedules

The available range of alternative vaccines, inactivated and attenuated live-virus, often in multivalent formulations and often with somewhat different recommendations from the manufacturers regarding immunization schedules, means that the practicing veterinarian is often required to make critical and technically complex decisions about vaccine choice and usage. Vaccine formulations, such as those available for canine distemper/hepatitis/parvovirus/leptospirosis in dogs, or in beef cattle, infectious bovine rhinotracheitis/bovine virus diarrhea/-parainfluenzavirus 3, with or without a variety of bacterial vaccines, confer major practical advantages in minimizing the number of visits the small-animal owner must make to the veterinarian, or the number of inoculations needed in farm animals. Their use leads to the more extensive use of vaccines of secondary importance—a mixed blessing. Unlike the situation in human medicine, where there is wide national and international agreement on the optimum vaccines and schedules for vaccination for all the common viral diseases of childhood, and very few vaccine manufacturers, there is no such consensus in veterinary medi-

cine and there are many vaccine manufacturers, each of whom promotes his own products. Hence we cannot provide tables showing the optimum schedules for immunization of various domestic animals without including so many annotations as to render them of little value. However, it will be useful to outline some of the principles underlying decisions on vaccination schedules.

Optimal Age for Vaccination. The risk of most viral diseases is greatest in young animals. Vaccines are therefore given during the first 6 months of life. Maternal antibody, whether transferred transplacentally or, as in domestic animals, in the colostrum, inhibits the immune response of the newborn to vaccines. Optimally, vaccination should be delayed until the titer of maternal antibody in the young animal has declined to near zero. However, waiting may leave the animal defenseless during the resulting "window of susceptibility." This is life-threatening in crowded, highly contaminated environments. There are a number of approaches to handling this problem in different animal species, but none is fully satisfactory.

Since the titer of passively acquired antibody in the circulation of a newborn animal is proportional to that in the dam's blood, and the rate of its subsequent clearance in different animal species is known, it is possible to estimate, for any given maternal antibody titer, the age at which no measureable antibody remains in the offspring. This can be plotted as a nomograph, from which the optimal age of vaccination against any particular disease can be read (Fig. 14-2). The method is not much used but could be considered for exceptionally valuable animals.

In practice, relatively few vaccine failures are encountered if one simply follows the instructions from the vaccine manufacturers who have used averaged data on maternal antibody levels and rate of IgG decay in that animal species to estimate an optimal age for vaccination. Commonly it is recommended, even in the case of live vaccines, that a number of doses of vaccine be administered, say at monthly intervals, to cover the window of susceptibility in animals with particularly high maternal antibody titers. This precaution is even more relevant to multivalent vaccine formulations, because of the differences in maternal antibody titers against each virus.

Dam Vaccination. The aim of immunization is generally thought of as the protection of the vaccinee. This is usually so, but in the case of certain vaccines (e.g., those for equine abortion virus, rotavirus infection in cattle, parvovirus infection in swine, infectious bursal disease of chickens) the objective is to protect the vaccinee's offspring. This is achieved by vaccination of the pregnant animal or laying hen, such that

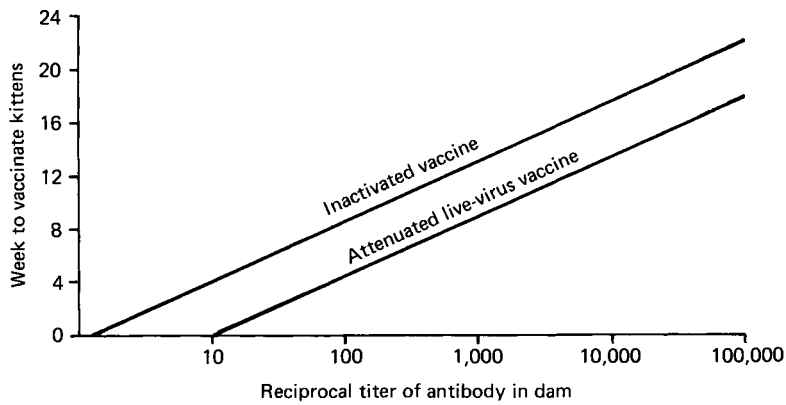

FIG. 14-2. *Nomograph indicating optimum times for vaccinating kittens with feline panleukopenia vaccines, based on the antibody titer of dam and a half-life of IgG in the kitten of 9.5 days. [Data from F. W. Scott, C. K. Csiza, and J. H. Gillespie,* J. Am. Vet. Med. Assoc. **156,** *439 (1970).]*

the level of maternal antibody transferred in the colostrum and milk or in the egg ensures that the offspring have a protective level of antibody during the critical early days of their lives. Since many attenuated live-virus vaccines are abortifacient or teratogenic, inactivated vaccines are used for dam vaccination.

Herd Effects of Vaccination. Under some circumstances an important auxiliary objective of a vaccination program may be to protect unvaccinated members of the population (e.g., against some avian virus diseases), either by natural spread of attenuated vaccine virus, or by reducing the circulation of wild-type virus.

Available and Recommended Vaccines. The types of vaccines available for each viral disease (or the lack of any satisfactory vaccine) are discussed in each chapter of Part II of this book and summarized in the tables in Chapter 35. An important general observation is that there are obvious geographical variations in the requirements for particular vaccines, e.g., between the United States and South Africa or between countries in Europe where vaccination against foot-and-mouth disease is practiced, and the United Kingdom and United States where it is not. There are also different requirements appropriate to various types of husbandry, e.g., in cattle—for dairy cattle, beef cows and their calves, or cattle assembled in feedlots, and in poultry—for breeders, commercial layers, and broilers. From time to time professional organizations

publish articles on recommended vaccination schedules for various animal species; we refer to some recent publications of the American Veterinary Medical Association on this matter in "Further Reading."

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CHAPTER 15

Epidemiology of Viral Infections

Viruses survive in nature only if they are able to pass from one host to another, whether of the same or another species. Viral epidemiology is the study of the factors that determine the frequency and distribution of viral diseases in an animal population. In the broadest sense, epidemiology may be viewed as a part of population biology, involving mainly environmental (ecological) factors, but also genetic factors, in both the virus (e.g., new strains of influenza virus) and the host (differences in genetic resistance of different receptive hosts).

The terms incidence and prevalence are used to describe quantitative aspects of the occurrence of infections in populations. The *incidence* of infection (or of disease) is defined as the proportion of a population contracting that infection (or disease) during a specified period (usually a year), whereas *prevalence* refers to the proportion infected (or sick, or immune) at a particular point in time. Comparisons of incidence and prevalence at different times and places (incidence and prevalence rates) are made by relating the appropriate numerator to a denominator which may be as general as the total population of the animal species con-

cerned (in a region or country), or may be specified as the susceptible population at risk (exposed and susceptible, the latter usually being defined as those lacking antibodies).

Deaths from a disease can be categorized in two ways: as a cause-specific mortality rate (the number of deaths from the disease in a given year, divided by the total population at midyear), usually expressed as per 100,000; or the case fatality rate (the percentage of those animals with a particular disease that die from it). These rates vary with age, sex, genetic constitution, immune status, and nutrition.

A disease is said to be *enzootic* (*endemic* in humans) when there are continuous chains of transmission in the region involved; *epizootics* (*epidemics*) are peaks in disease incidence. The size of the peak required to constitute an epizootic is arbitrary and is related to the background enzootic rate, the morbidity (frequency of illness), and the anxiety that the disease arouses; e.g., a few cases of velogenic Newcastle disease in poultry might be regarded as an epizootic, whereas a few cases of infectious bronchitis would not be. A *panzootic* (*pandemic*) is a worldwide epizootic.

TRANSMISSION OF VIRUSES

Transmission involves both the entry of viruses into the body and, after replication, their shedding from the body surfaces (see Chapter 7).

Direct transmission involves actual contact between an infected and a susceptible animal, such as by licking or rubbing. Two special cases of direct transmission are contacts occurring during coitus (sexually transmitted diseases) and those occurring between mother and the fetus via the placenta (*transplacental*). There are several modes of *indirect transmission*. Respiratory infections are transmitted both by contact and by *droplets* (*aerosols*) emitted during coughing or sneezing. Such aerosols contain large droplets which settle before evaporating and microdroplets which evaporate before settling, and thus produce dry *droplet nuclei* less than 5 μm in diameter. Droplets may travel only a meter or so, while droplet nuclei may remain airborne for long periods and hence travel long distances—many kilometers if wind and other weather conditions are favorable.

Enteric infections are often transmitted via a *fecal–oral cycle*, that may include fecal contamination of food and water supplies; diarrheic feces may also splash to give rise to aerosols or dry to give rise to infected airborne dust. Indirect transmission may also occur via *fomites*, i.e., any object that may be contaminated by a virus, such as bedding, harness, grooming or surgical equipment, multiple-use syringes, vehicles, books,

and clothing. Some diseases are transmitted via milk (e.g., caprine arthritis encephalitis virus), meat-contaminated garbage (e.g., vesicular exanthema virus, hog cholera virus), or dander (Marek's disease virus). Some are spread by bite (e.g., rabies virus), while a number of viruses are transmitted by *arthropod vectors* (see p. 300).

Veterinarians (or other persons) may themselves be responsible for the transmission of viruses. Such *iatrogenic* infections include transmission of equine infectious anemia virus via multiple-use syringes and of reticuloendotheliosis virus via contaminated Marek's disease vaccine. *Nosocomial infections* are those acquired while in a hospital or clinic. During the peak incidence of canine parvovirus in the late 1970s many pups brought to veterinary hospitals acquired canine parvovirus there. Kennel cough and feline respiratory virus infections are also often noso-

TABLE 15-1
Common Routes of Transmission of Viruses of Animals

Virus family	Routes
Papovaviridae	Contact, skin abrasion
Adenoviridae	Respiratory, fecal–oral
Herpesviridae	Sexual (e.g., equine coital exanthema)
	Respiratory (e.g., infectious bovine rhinotracheitis)
	Transplacental (e.g., canine herpesvirus)
African swine fever virus	Fecal–oral, respiratory, arthropod
Poxviridae	Contact (e.g., orf, cowpox)
	Arthropod (mechanical, e.g., myxoma virus)
	Contact, respiratory (e.g., sheeppox)
Parvoviridae	Fecal–oral, respiratory, contact, transplacental
Picornaviridae	Fecal–oral, respiratory (*Enterovirus*)
	Respiratory (*Aphthovirus, Rhinovirus*)
Caliciviridae	Respiratory, fecal–oral, contact
Togaviridae	Arthropod (*Alphavirus*)
	Respiratory, fecal–oral, transplacental (e.g., hog cholera)
Flaviviridae	Arthropod
Orthomyxoviridae	Respiratory
Paramyxoviridae	Respiratory, contact
Coronaviridae	Fecal–oral, respiratory, contact
Arenaviridae	Contact with contaminated urine, respiratory
Bunyaviridae	Arthropod (Rift Valley fever)
Rhabdoviridae	Arthropod and contact (vesicular stomatitis), animal bite (rabies)
Retroviridae	Contact, *in ovo* (germ line)
Birnaviridae	Fecal–oral, water (fish)
Reoviridae	Fecal–oral (*Rotavirus* and *Reovirus*)
	Arthropod (*Orbivirus*)

TABLE 15-2
Factors Affecting Survival of Viruses in Nature

Factor	Example
Population size	Canine distemper virus
	Respiratory viruses
Virulence and transmissiblity	Myxoma virus
Antigenic variation	Influenza viruses
	Foot-and-mouth disease viruses
Subclinical infections	Enteroviruses, many others
Persistent infections	Herpesviruses
Vertical transmission	Retroviruses
Reservoir hosts	Alphaviruses
Resistant virus	Parvoviruses

comial. Certain viruses are vertically transmitted, either as virions via the placenta or the egg, or as provirus integrated in the DNA of the gamete (germ-line transmission; see Fig. 31-2). Some viruses are transmitted by several routes; others are transmitted in nature exclusively by one route (Table 15-1).

SURVIVAL OF VIRUSES IN NATURE

Some of the factors that affect the survival of viruses in nature are set out in Table 15-2 and are discussed below.

Transmissibility and Virulence

The best-documented example of how the virulence of a virus may directly affect the probability of transmission is myxomatosis of rabbits (see Chapter 5). Here mechanical transmission by biting arthropods is most effective when the diseased rabbit maintains highly infectious skin lesions for a long period. Very virulent strains of myxoma virus kill rabbits too quickly and very attenuated strains cause lesions that heal too rapidly, so that viruses at either extreme of the virulence range do not survive in nature.

Subclinical Infections

Survival of a virus in nature depends on the maintenance of serial infections, i.e., a chain of transmission; the occurrence of disease is neither required nor necessarily advantageous. Infection without recognizable disease is called *subclinical* or *clinically inapparent*. Overall, sub-

clinical infections are much more common than those that result in disease. Their relative frequency accounts for the difficulty of tracing chains of transmission, even with the help of laboratory aids. Although clinical cases may be somewhat more productive sources of virus than subclinical infections, the latter are more numerous and, because they do not restrict the movement of infected animals, can be an important source of viral dissemination. In most acute infections, whether clinically apparent or not, virus is shed at highest titers during the late stages of the incubation period, before the influence of the host immune response takes effect.

The infrequency of subclinical infections in some diseases, such as rinderpest or velogenic Newcastle disease, is an important factor in the implementation of control programs, because it makes surveillance and quarantine an efficient strategy for interrupting chains of transmission.

Persistent Infections

Persistent viral infections, whether or not they are associated with episodes of clinical disease, also play an important role in the perpetuation of many viruses in nature. For example, persistent virus shedding by an animal can introduce virus into a population of susceptible animals all of which have been born since the last episode of acute infections. This transmission pattern is important for the survival of bovine virus diarrhea virus, hog cholera virus, equine arteritis virus, and the herpesviruses.

Sometimes the effects of persistent infections in the production of disease and the transmission of infection are dissociated. Thus togaviruses and arenavirus infections seem to have little adverse effect on their reservoir hosts (arthropods, birds, and rodents), but virus shedding and transmission are very efficient. On the other hand, persistent infection of the central nervous system with canine distemper virus is of no epidemiological significance, since no infectious virus is shed; infection of the central nervous system may have a severe effect on the dog, but is of no consequence for the virus.

Infection with viruses of some viral families is characteristically associated with continuous or intermittent shedding; certain other viruses cause acute infections which are associated with transient intense shedding. Table 15-3 lists some of the more important groups of viruses that are associated with persistent or recurrent virus shedding.

Population Size

It is self-evident that the long-term survival of a virus requires that it should continue to be transmitted from one host to another. In general,

TABLE 15-3
Some Viral Infections Associated with Persistence

Family	Virus	Comments
Herpesviridae	Infectious bovine rhinotracheitis virus	Persistence in nerve ganglia
	Pseudorabies virus	Recurrent shedding in oral and/or genital secretions
Unclassified	African swine fever virus	Persistence in hematopoietic system
		Persistence in infected ticks
Picornaviridae	Foot-in-mouth disease virus	Sometimes, persistence in pharynx of ruminants, but transmission from persistently infected cattle is rare and therefore mainly important as possible means of introduction of virus into a disease-free area
Togaviridae	Hog cholera virus	Persistence in hematopoietic system
	Bovine virus diarrhea virus	Congenital persistent infection with chronic shedding after birth
Arenaviridae	All species	Long-term excretion in urine of reservoir rodent hosts
Retroviridae	Bovine leukemia virus	Persistence in hematopoietic system
	Visna/maedi virus	Persistence in central nervous and respiratory systems
Reoviridae	Bluetongue viruses	Persistence in infected *Culicoides*

for rapidly and efficiently transmitted viruses such as the respiratory viruses, survival of the virus requires that the susceptible host population is very large. A virus may disappear from a population because it exhausts its potential supply of susceptible hosts. Depending on the breeding characteristics (population turnover rate), duration of immunity, and the pattern of virus shedding, the *critical population size* varies considerably with different viruses and with different host species.

Acute Infections. The most precise data on the importance of population size in acute, nonpersistent infections come from studies of measles, which is a cosmopolitan human disease caused by a virus which is related to rinderpest and canine distemper viruses. Measles has long

been a favorite disease for modeling epidemics, because it is one of the few common human diseases in which subclinical infections are rare and clinical diagnosis is easy. Persistence of measles virus in a population depends on a continuous supply of susceptible hosts. Analyses of the incidence of measles in large cities and in island communities have shown that a population of about half a million persons is needed to ensure a large enough annual input of new susceptible hosts, by birth or immigration, to maintain measles virus in the population.

Because infection depends on respiratory transmission, the duration of epidemics of measles is correlated inversely with population density. If a population is dispersed over a large area, the rate of spread is reduced and the epidemic will last longer, so that the number of susceptible persons needed to maintain transmission chains is reduced. On the other hand, in such a situation a break in the transmission chain is much more likely. When a large proportion of the population is initially susceptible, the intensity of the epidemic builds up very quickly and attack rates are almost 100% (a *virgin-soil epidemic*). There are many examples of similar transmission patterns among animal viruses, but the quantitative data are not as complete as those for measles. Exotic viruses, i.e., those which are not present in a particular country or region, represent the most important group of viruses with a potential for causing virgin-soil epizootics.

The history of rinderpest in cattle in Africa in the early twentieth century shows many parallels with measles in isolated human populations. When it was first introduced into virgin populations in various parts of Africa in the late nineteenth and early twentieth centuries, the initial impact was devastating. Cattle and wild ruminants of all ages were susceptible, and the mortality was so high that in northern Tanzania the ground was so littered with the carcasses of cattle that a Masai tribesman commented that "the vultures had forgotten how to fly." The development of effective vaccines in the 1920s changed the epidemiology of rinderpest, leading to a period in the 1960s when its global eradication was anticipated. Unfortunately, in the 1970s vaccination programs in West Africa were poorly maintained and by the 1980s the disease had once more become rampant and the cause of major losses in many parts of Africa. The cyclical nature of the occurrence of such diseases is determined by several variables, including the rate of buildup of susceptible animals, introduction of the virus, and environmental conditions which promote viral spread.

Persistent Infections. Although acute herpesvirus infections are not unlike acute measles or rinderpest, each has a much smaller critical

population size, as small as a single farm, kennel, or cattery. This is because herpesviruses establish lifelong latent infections, from which virus may be reactivated and initiate infection in susceptible animals born or introduced into the population since the last acute cases (see Chapters 11 and 19).

Vertical Transmission

Vertical transmission refers to the transfer of virus from parent to offspring, usually before birth, but occasionally in the immediate perinatal period. Other modes of transmission are designated *horizontal*. Vertical transmission of a virus may occur via the germ line, via the egg (especially in birds), across the placenta, or during the perinatal period via milk. Vertical transmission may be associated with congenital disease, and occurs in all domestic animals and with viruses belonging to several families (see Table 7-4): all arenaviruses, several herpesviruses, parvoviruses, and retroviruses, some orbiviruses and togaviruses, and a few bunyaviruses and coronaviruses may be transmitted in this way. Germ-line transmission (i.e., via the egg or sperm) occurs only with certain retroviruses, in which the viral genome is integrated as provirus in the DNA of the gametes (see Chapter 31).

Zoonoses

Because most viruses are host-restricted, most viral infections are maintained in nature within populations of the same or related species. However, there are a number of viruses that may have multiple hosts and spread naturally between several different species of vertebrate hosts, e.g., rabies and eastern equine encephalitis viruses. The term *zoonosis* is used to describe multiple-host infections that are transmissible from animals to humans (Tables 15-4 and 15-6). Most viral zoonoses are caused by arboviruses (see Table 15-6). The non-arthropod-borne zoonoses listed in Table 15-4 are primarily infections of domestic or wild animals transmissible only under special conditions to humans engaged in activities involving close contact with animals.

SEASONAL VARIATIONS IN DISEASE INCIDENCE

Many viral infections show pronounced seasonal variations in incidence. In temperate climates, arbovirus infections transmitted by mosquitoes or sandflies occur mainly during the summer months, when vectors are most numerous and active. Infections transmitted by ticks

TABLE 15-4
Non-Arthropod-Borne Viral Zoonoses

Family	Virus	"Reservoir" host	Mode of transmission to humans
Poxviridae	Cowpox virus	Cattle, ? rodents	Contact, through skin abrasions
	Monkeypox virus	Monkey, squirrel	
	Pseudocowpox virus	Cattle	
	Orf virus	Sheep, goats	
Herpesviridae	B virus	Monkey	Animal bite
Bunyaviridae	Hantaan virus	Rodents	Contact with rodent urine
Rhabdoviridae	Rabies virus	Terrestrial mammals and bats	Animal bite, scratch
	Vesicular stomatitis virus	Cattle	Contact with oral secretions, vesicular fluid
Filoviridae	Ebola, Marburg viruses	Unknown	Contact; iatrogenic (injection) human-to-human spread
Orthomyxoviridae	Influenza A virus	Swine, horses, birds	Respiratory
Arenaviridae	Lymphocytic choriomeningitis virus	Rodents	Contact with rodent urine
	Junin virus		
	Machupo virus		
	Lassa virus		

occur most commonly during the spring and early summer months. Other biological reasons for seasonal disease include both virus and host factors. Influenza viruses and poxviruses survive better in air at low rather than at high humidity, and in aerosols all viruses survive better at lower temperatures. It has also been suggested, without much supporting evidence, that there may be seasonal changes in the susceptibility of the host, perhaps associated with changes in the physiological status of nasal and oropharyngeal mucous membranes.

More important in veterinary medicine than any natural seasonal effects are the changes in housing and management practices that occur in different seasons. Housing animals such as cattle and sheep for the winter often increases the incidence of respiratory and enteric diseases. These diseases often have obscure primary etiologies, usually viral, followed by secondary infections caused by opportunistic pathogens (see

Chapter 10). In such cases, infectious disease diagnosis, prevention, and treatment must be integrated into an overall system for the management of facilities as well as husbandry practices. In areas where animals are moved—e.g., to feedlots or seasonally to distant pasturage—there are two major problems: animals are subjected to the stress of transportation and the diseases associated with stress, and they are brought into contact with new populations carrying and shedding different infectious agents. Often summer pasturage is at high altitude, adding the stress of pulmonary vascular dysfunction and pulmonary edema to the insult of respiratory virus infections. Secondary *Pasteurella* pneumonia, shipping fever, is not limited to animals subjected to the stress of transportation to feedlots.

In areas of the world where cattle are moved hundreds of miles each year, such as in the Sahelian zone of western Africa, viral diseases such as rinderpest are associated with the contact between previously separate populations brought about by this traditional husbandry practice. In southern Africa, the communal use of waterholes during the dry season promotes the exchange of viruses such as foot-and-mouth disease virus between different species of wildlife, and in certain circumstances between wildlife and domestic animals.

EPIDEMIOLOGICAL ASPECTS OF IMMUNITY

Immunity from prior infection or from vaccination plays a vital role in the epidemiology of viral diseases. Two examples are given here: (1) canine distemper, caused by a monotypic virus, for which immunity is very effective, and (2) feline respiratory disease due to feline calicivirus and feline rhinotracheitis virus (a herpesvirus), in which lasting immunity does not follow either natural infection or vaccination. In industrialized countries, widespread vaccination of puppies with attenuated live canine distemper virus vaccine has sharply diminished the incidence of canine distemper and of its complications, old dog encephalitis and hard-pad disease.

Feline respiratory infection in a cattery is a good example of the failure of immunity, for disease occurs not only in previously unexposed cats, but also in persistently infected cats. Both feline calicivirus and feline viral rhinotracheitis virus produce persistent infections. Only partial immunity follows primary infection with either of these viruses. The succession of respiratory problems experienced in many catteries reflects a series of minor epizootics. Reactivation and recurrent infection leading to transmission to young kittens accounts for the maintenance of feline

rhinotracheitis virus, whereas long-term carrier status and continuous or intermittent shedding account for the transmission of feline calicivirus (possibly assisted by the existence of antigenic variants of the virus).

Seroepidemiology

Seroepidemiology is useful in veterinary public health operations and in research. Because of the expense of collecting and properly storing sera, advantage is taken of a wide range of sources, such as abattoirs, culling from overstocked wildlife populations, and vaccination programs. Such sera can be used to determine the prevalence or incidence of particular infections, to evaluate eradication and immunization programs, and to assess past history when a "new" virus is discovered.

Estimates of Prevalence. Traditional surveillance is based on the reporting of clinical disease. However, examination of appropriate numbers of sera by appropriate serological methods gives a more accurate index of the prevalence of a particular virus. By detecting antibodies to selected viruses in various age groups of the population, it is possible to determine how effectively enzootic viruses have spread, or how long it has been since the last appearance of epizootic viruses.

Estimates of Incidence. When pairs of serum specimens are obtained from individual animals several weeks apart, the initial appearance of antibody in the second specimen or a rise in antibody titer (usually a fourfold or greater rise is considered significant) indicates recent infection. Likewise, the presence of specific IgM antibody (or, with some infections, significant titers of complement-fixing antibody) in a single serum sample often indicates recent infection. Correlation of serological tests with clinical observations makes it possible to determine the ratio of clinical to subclinical infections.

Evaluation of Eradication or Immunization Programs. Serological surveys to detect infected breeding herds of swine have been of major importance in hog cholera eradication campaigns. Vaccination programs were restricted to fattening herds, so that serological surveys could be used to monitor breeding herds and destroy these if they were infected. Serological surveys are also valuable in determining how well immunization programs have succeeded in various populations. For example, the gradual decrease in the percentage of animals with antibodies to rinderpest virus heralded the reemergence of epidemic rinderpest in Africa in the late 1970s.

Emergence of "New" Diseases or Viruses. Sometimes "new" viruses are discovered for which no clinical disease is recognized, e.g., bluetongue virus 20 in Australia in 1977 and bluetongue virus 2 in Florida in 1983. Serological surveys can be used to determine their distribution. Serology is also valuable in determining retrospectively the geographical and *secular distribution* (i.e., the distribution over time) of newly discovered viruses.

ANALYTICAL EPIDEMIOLOGY

Analytical epidemological techniques are used to investigate the relationships between cause and effect and to evaluate risk factors of disease. There are two basic methods, the *case–control study* and the *cohort study*. In the case–control study, investigation starts after the disease has occurred and it attempts to identify the cause; thus it is a *retrospective study*, going back in time to determine cause. This is the most common type of study. Although it does not require the creation of new data or records, it does require careful selection of the control group, matched to the test group so as to avoid bias. The advantages of the retrospective study are that it lends itself to quick analysis and it is relatively inexpensive to carry out.

In cohort studies, investigation starts with a presumed cause and a population exposed to the causative virus is followed into the future to identify correlated resulting effects—a *prospective study*. This type of study requires the creation of new data and records, and the careful selection of the control group to be as similar as possible to the exposed group, except for the absence of contact with the presumed causative virus. It usually does not lend itself to quick analysis as groups must be followed until disease is observed, which makes such studies expensive. However, when cohort studies are successful, proof of cause–effect relationship is often incontrovertible.

The production of congenital defects by Akabane virus in cattle provides examples of both the case–control and prospective (cohort) types of epidemiological studies. Epizootics of congenital defects in calves, characterized by deformed limbs and abnormal brain development referred to as congenital arthrogryposis–hydranencephaly (see Chapter 29), occurred in Australia in the 1950s and 1960s, but the cause was not identified. During the summer and early winter months from 1972 to 1975, approximately 42,000 calves were born with these congenital defects in central and western Japan, causing significant economic loss. Japanese scientists postulated that the disease was infectious but were

unable to isolate a virus from affected calves. However, when precolostral sera from such calves were tested for antibody to a number of viruses, antibody to Akabane virus, an arbovirus (Simbu serogroup, family *Bunyaviridae*), which was first isolated from mosquitoes in Akabane Prefecture in 1959, was found in almost all sera tested. A retrospective serological survey indicated a very high correlation between the geographical distribution of the disease and the presence of antibody to the virus, suggesting that Akabane virus was the etiological agent of congenital arthrogryposis–hydranencephaly in cattle.

Prospective studies were then organized. Sentinel herds were established (see long-term herd studies, below) in Japan and Australia, and it was soon found that the virus could be isolated from fetuses obtained by slaughter or caesarian section for only a short period after infection, thus explaining the earlier failures in attempts to isolate virus after calves were born. Experimental inoculation of pregnant cows with Akabane virus during the first two trimesters of pregnancy induced congenital abnormalities in calves similar to those seen in natural cases of the disease; clinical disease was not seen in the cows.

Vaccine Trials

The immunogenicity, potency, safety, and efficacy of vaccines are first studied in laboratory animals, followed by small-scale closed trials in the definitive animal species, and finally in large-scale field trials. Such studies employ epidemiological methods rather like those of the cohort (prospective) study described above. There is no alternative way to evaluate new vaccines, and the design of field trials has now been developed so that they yield maximum information with minimum risk and cost. Even with this system, however, a serious problem may be recognized only after a vaccine has passed into commercial use. This occurred after the introduction of attenuated live-virus vaccines for infectious bovine rhinotracheitis in the United States in the 1950s. Surprisingly, the vaccines had been in use for 5 years before it was recognized that abortion was a common sequel to vaccination (see Chapter 19).

Long-Term Herd Studies

Another kind of epidemiological investigation that can provide etiological information and data on the value of vaccines or therapeutic agents is the long-term herd study. Because of the present advanced state of diagnostic and serological virology, such studies now yield a much greater array of valuable data than was possible a few years ago, but they are very expensive and require long-term dedication of both

personnel and money. When used principally for the detection of virus in an area, such investigations are referred to as *sentinel studies*, and are widely used for studying the prevalence of arbovirus infections. For example, sentinel chickens are used for the early detection of eastern equine and St. Louis encephalitis viruses in the southern United States. When used for the estimation of the value of vaccines or therapeutic agents, long-term herd studies have the exceptional advantage that they include all of the variables attributable to the entire husbandry system.

MOLECULAR EPIDEMIOLOGY

The term molecular epidemiology has been applied to the use of molecular biological methods for epidemiological investigation of viral diseases. All the techniques described in Chapter 13 can be used. With DNA viruses and retroviruses, restriction endonuclease fingerprinting or mapping provides a means of identification of viral strains with a specificity that often surpasses serological methods, e.g., with pseudorabies virus. With viruses that have segmented genomes, like orbiviruses and rotaviruses, polyacrylamide gel electrophoresis provides a method of genome analysis that is a valuable supplement to serological typing of these viruses. The genome of a poliovirus isolate recovered from a paralyzed (or normal) person can be sequenced to determine whether it is a wild-type strain, an attenuated vaccine strain, or a vaccine strain that has reacquired neurovirulence as a result of subsequent mutations. Other techniques such as nucleic acid hybridization and oligonucleotide fingerprinting can also distinguish strains of virus within the same serotype. For example, the 1981 outbreak of foot-and-mouth disease in the United Kingdom was traced by oligonucleotide fingerprinting to the presence of live virus in an inactivated vaccine (see Chapter 23). Monoclonal antibodies provide a powerful method of distinguishing viruses that cannot be readily differentiated by serology employing polyclonal antibodies. This method has been particularly useful in elucidating the relationships between rabies virus and rabieslike rhabdoviruses and in distinguishing geographical variants of rabies virus. We can expect to see all these techniques become incorporated into standard epidemiological investigations in the future; properly used, they become tools for much more precise analyses of the role of specific viruses in particular disease outbreaks.

MATHEMATICAL MODELING

From the time of William Farr, who studied both medical and veterinary problems in the 1840s, mathematicians have been interested in

"epidemic curves" and secular trends in the incidence of infectious diseases. With the development of mathematical modeling techniques there has been a resurgence of interest in the population dynamics of infectious diseases. Models are now being developed that allow estimates of (1) the patterns of disease transmission, and (2) the critical population size for animal viruses with short or long incubation periods, persistent or recurrent infectivity, and/or age-dependent pathogenicity.

Computer modeling also provides useful insights into the effectiveness of disease control programs. In this regard, most attention has been given to the potential national and international spread of exotic diseases in susceptible populations. Figure 15-1 illustrates results obtained in modeling the spread of foot-and-mouth disease across the United States, commencing at the stage where the disease becomes well established and traditional control measures (quarantine, slaughter, disinfection) are no longer effective. The model suggests that under these conditions—the "worst scenario" model—60% of the cattle herds in the United States could become infected within a period as short as 30 weeks. In the absence of vaccination, the disease would increase again in incidence after 60 weeks and begin a series of enzootic cycles.

Models such as this make no special claim for reliability, but their construction and use brings a number of issues into focus. The results are often unexpected, pointing to the need for better data and different strategies for disease control.

EPIDEMIOLOGY OF ARTHROPOD-BORNE VIRAL DISEASES

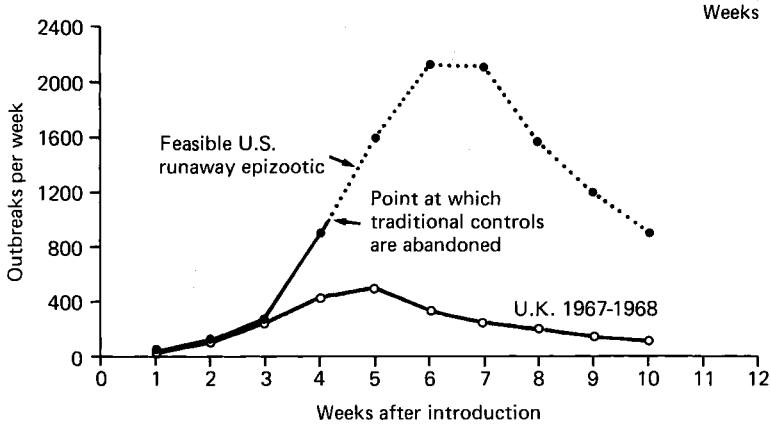
Arboviruses have two classes of hosts: vertebrate and invertebrate. Over 400 arboviruses are known, of which at least 66 cause disease in domestic animals or humans (Tables 15-5 and 15-6). Fourteen of these pathogenic arboviruses are tick-borne, and 52 are transmitted by mosquitoes, phlebotomine flies (sandflies), or *Culicoides* spp. (midges).

Arthropod transmission may be *mechanical*, as in myxomatosis and fowlpox, in which mosquitoes act as "flying pins," or, more commonly, *biological*, involving replication of the virus in the arthropod vector. The arthropod vector acquires virus by feeding on the blood of a viremic animal. Replication of the ingested virus, initially in the insect gut, and its spread to the salivary gland takes several days (the *extrinsic incubation period*); the interval varies with different viruses and is influenced by ambient temperature. Virions in the salivary secretions of the vector are injected into animal hosts during all blood meals. Arthropod transmission provides a way for a virus to cross species barriers, since the same arthropod may bite birds, reptiles, and mammals that rarely or never come into close contact in nature.

A

	Dissemination rate										Peak
Actual U.K. incidence	4.52	2.13	1.82	1.21	0.67	0.66	0.86	0.71	0.66	0.53	0.79	5.5
Possible U.S. incidence	4.52	3.54	2.47	1.83	1.35	1.00	0.75	0.75	0.75	0.75	0.75	7.0

Weeks



B

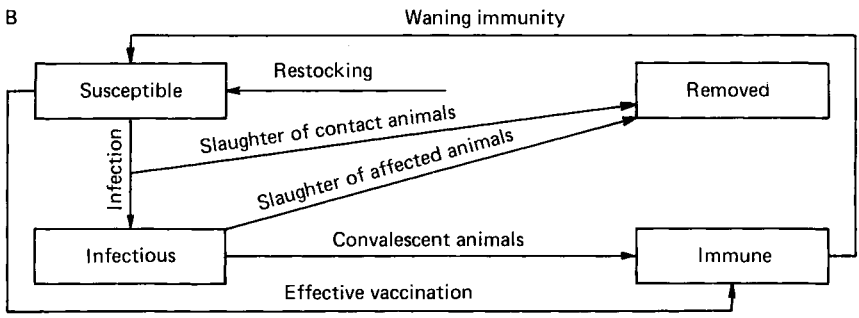


FIG. 15-1. *(A) A state transition model of epizootic foot-and-mouth disease. In constructing this model, the aim was to simulate the spread of foot-and-mouth disease across the United States commencing at the stage where the disease became established and traditional control measures (quarantine, slaughter, and disinfection) were no longer effective. The model was based on data collected from the 1967–1968 epizootic of foot-and-mouth disease in the United Kingdom during which outbreaks of disease were recorded on 2364 farms. The objective was to simulate a major epizootic. The state transition model is a general model that permits a variety of disease control strategies to be examined. It illustrates several key characteristics of a useful model: (1) its pathways are intuitively acceptable, (2) it behaves in a biologically and mathematically logical way (i.e., it is sensitive to appropriate variables), (3) it mimics real-life situations, and (4) it is simple enough to be rigorously tested, but complex enough to represent the system being studied. (From W. M. Miller, 1979.) (B) Pathways of transitions in the model. The basic unit is the herd (or farm), which is in one of four mutually exclusive categories: "susceptible,"*

Ecology of Arboviruses

Most arboviruses have localized natural habitats in which specific receptive arthropod and vertebrate hosts are involved in the viral life cycle. Vertebrate reservoir hosts are usually wild mammals or birds; domestic animals and humans are rarely involved in primary transmission cycles, although the exceptions to this generalization are important (e.g., Venezuelan equine encephalitis virus in horses, yellow fever and dengue viruses in humans). Domestic animal species and humans are in most cases infected incidentally, for example by the geographical extension of a reservoir vertebrate host and/or a vector arthropod.

Most arboviruses that cause periodic epizootics have ecologically complex enzootic maintenance cycles; often these enzootic cycles involve different arthropod as well as different vertebrate hosts from those involved in epizootic cycles. Enzootic cycles provide for the amplification of virus and therefore are critical in dictating the magnitude of epizootics. Enzootic cycles are generally poorly understood and inaccessible to effective control measures.

When arthropods are active, arboviruses replicate alternately in vertebrate and invertebrate hosts. A problem that has concerned many investigators has been to understand what happens to these viruses during the winter months in temperate climates, when the arthropod vectors are inactive. An important mechanism for "overwintering" is *transovarial transmission* from one generation of arthropods to the next. This is necessarily associated with *transstadial transmission* (from one larval stage to the next). Transovarial transmission has long been known to occur with the tick-borne flaviviruses and has more recently been discovered to occur with some mosquito-borne bunyaviruses and flaviviruses. For example, some bunyaviruses are found in high northern latitudes where the mosquito breeding season is too short to allow virus survival by horizontal transmission cycles alone; many of the first mosquitoes to emerge each summer carry virus as a result of transovarial

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"infectious," "immune," or "removed/dead." Each week the probability of each transition is calculated and the number of herds in each category during the next week are derived. A key factor in determining the probability of a susceptible herd becoming infected in a particular week is the dissemination rate. *This depends on a number of factors such as topography, weather, husbandry, animal movement, and quarantine. The dissemination rate used for the "scenario" shown in the figure is based on that calculated for the 1967–1968 epidemic in the United Kingdom. With a dissemination rate diminishing at a rate only slightly more slowly than in the United Kingdom, a situation whereby the traditional controls would be abandoned* could *be reached 4–5 weeks after introduction of the virus to the United States.*

TABLE 15-5

Arthropod-Transmitted Viral Diseases of Domestic Animals

Family	Subfamily, genus, or virus	Disease	Domestic animals	Vector	
				Species	Replication[a]
Unclassified	African swine fever virus	African swine fever	Swine	Tick	+
Poxiviridae	*Avipoxvirus*	Fowlpox, pigeonpox, etc.	Poultry, pigeons	Mosquito, other	–
	Leporipoxivirus	Myxomatosis	Rabbit	Mosquito, flea, other	–
Togaviridae	*Alphavirus*	Eastern equine encephalitis	Horse	Mosquito	+
		Western equine encephalitis	Horse	Mosquito	+
		Venezuelan equine encephalitis	Horse	Mosquito	+
		Getah virus disease	Horse	Mosquito	+
Flaviviridae	*Flavivirus*	Louping ill	Sheep	Tick	+
		Wesselsbron disease	Sheep	Mosquito	+
		Japanese encephalitis	Swine	Mosquito	+
Bunyaviridae	*Phlebovirus*	Rift Valley fever	Sheep, cattle	Mosquito	+
	Bunyavirus (Akabane)	Arthrogryposis–hydraencephaly	Sheep, cattle	Mosquito	+
	Nairovirus	Nairobi sheep disease	Sheep	Mosquito	+
Reoviridae	*Orbivirus*	Bluetongue	Sheep, cattle	*Culicoides*	+
		Ibaraki infection	Cattle	?	+
		Epizootic hemorrhagic disease of deer	Deer	*Culicoides*	+
		African horse sickness	Horse	*Culicoides*, mosquito	+
		Equine encephalosis	Horse	?	+
Retroviridae	*Lentivirinae*	Equine infectious anemia	Horse	Biting flies	–

[a]Only viruses that replicate in the vector are classed as arboviruses.

TABLE 15-6
Arthropod-Borne Viral Zoonoses

Family	Genus	Virus	Principal vertebrate hosts	Arthropod vector
Togaviridae	*Alphavirus*	Chikungunya	Mammals	Mosquitoes
		Eastern equine encephalitis	Birds	
		Western equine encephalitis	Birds	
		Ross River	Mammals	
		Venzuelan equine encephalitis	Mammals	
Flaviviridae	*Flavivirus*	Japanese encephalitis	Birds and pigs	Mosquitoes
		Murray Valley encephalitis	Birds	
		Yellow fever	Primates	
		Kyasanur Forest disease	Primates	Ticks
		Louping ill	Mammals	
		Omsk hemorrhagic fever	Mammals	
		Powassan	Mammals	
		Tick-borne encephalitis	Mammals and birds	
Bunyaviridae	*Bunyavirus*	California encephalitis La Crosse Tahyna	Mammals	Mosquitoes
	Phlebovirus	Sandfly fever	Gerbils	Sandflies
		Rift Valley fever	Mammals	Mosquitoes
	Nairovirus	Crimean–Congo hemorrhagic fever	Mammals	Ticks
Reoviridae	Unclassified	Colorado tick fever	Mammals	Ticks

transmission, and the pool of virus is rapidly amplified by horizontal transmission in mosquito–mammal–mosquito cycles.

Vertical transmission in arthropods does not explain all arbovirus overwintering, but other possibilities are still unproven or speculative. For example, hibernating vertebrates have been thought to play a role in overwintering. In cold climates, bats and some small rodents, as well as snakes and frogs, hibernate during the winter months; their low body temperature has been thought to favor persistent infection, with recrudescent viremia occurring when normal body temperature returns in the spring. Though demonstrated in the laboratory, this mechanism has never been proven to occur in nature.

Effects of Human Activities on Arbovirus Cycles. Many ecological changes produced by human activities disturb natural arbovirus life cycles and have been incriminated in the geographical spread or increased prevalence of the diseases they cause:

1. Population movements and the intrusion of humans and domestic animals into new arthropod habitats, notably tropical forests.
2. Deforestation, with development of new forest–farmland margins and exposure of domestic animals to new arthropods.
3. Irrigation, especially primitive irrigation systems, which pay no attention to arthropod control.
4. Uncontrolled urbanization, with vector populations breeding in accumulations of water and sewage.
5. Increased rapid and long-distance air travel, with potential for carriage of arthropod vectors.
6. Increased long-distance livestock transportation, with potential for carriage of viruses and arthropods (especially ticks).
7. New routing of long-distance bird migrations brought about by new man-made water impoundments.

As an example, horses may become infected in the eastern part of North America with eastern equine encephalitis virus when their pasturage is made to overlap the natural swamp-based mosquito–bird–mosquito cycle of this virus. Similarly, in Japan and southeastern Asian countries, swine may become infected with Japanese encephalitis virus and become important amplifying hosts when they are bitten by mosquitoes that breed in improperly irrigated rice fields.

Life Cycles of Arboviruses. Examples of the complexity of the life cycles of arboviruses are given in Chapters 20, 25, 29, 30, and 32. Mosquito-borne and tick-borne arboviruses have some features worth comment here.

Mosquito-Borne Encephalitis. Several togaviruses and bunyaviruses cause encephalitis in domestic animals (see Table 15-6). Most of these viruses cycle through wild birds or small mammals, with domestic animals being only incidental or "dead-end" hosts. However, in its epizootic cycle Venezuelan equine encephalitis virus can be maintained in a horse–mosquito–horse cycle, from which humans are easily infected by the same species of mosquitoes.

Tick-Borne Encephalitis. Central European tick-borne flavivirus encephalitis illustrates two features not found in mosquito-transmitted viral infections. First, transovarial infection in ticks is sufficient to ensure survival of the virus, independently of a cycle in vertebrates; vertebrate

infection serves to amplify the population of infected ticks. Second, transmission of this arbovirus from one vertebrate host to another can also occur by a mechanism not involving an arthropod at all, namely, via milk. Tick-borne encephalitis is widespread in central Europe and the eastern part of the Soviet Union. A variety of mammals may be infected in nature; small rodents are the most important. Goats, cows, and sheep are incidental hosts and sustain inapparent infections, but they excrete virus in their milk. Adult and juvenile animals may acquire the virus during grazing on tick-infested pastures, and suckling animals may be infected by drinking infected milk. Humans may be infected in two ways; they may enter natural foci of infection in pursuit of work or recreation and become infected by tick bite, or they may acqure infection from milk. All milkborne outbreaks have been associated with goat's milk.

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CHAPTER 16

Surveillance, Control, and Eradication of Viral Diseases

Nowhere in veterinary medicine is the adage "prevention is better than cure" more appropriate than in viral diseases. Apart from procedures for ameliorating symptoms—e.g., the administration of fluid in viral diarrheas, or using antibiotics to prevent secondary bacterial infections after viral respiratory diseases—there are no effective and practical treatments for viral diseases of domestic animals. However, there are several approaches to their prevention, control, and eradication. The most generally useful control measure is the use of vaccines (see Chapter 14). On farms, hygiene and sanitation are important methods of controlling fecal–oral infections, and vector control on a regional basis may be useful for arbovirus diseases. Exotic diseases may be excluded by national quarantine programs. Finally, either local, regional, national, continental, or global eradication may be considered. Countrywide eradication is widely practiced in developed countries if serious exotic diseases gain entry.

During the latter part of the present century there has been a revolu-

tion in systems of food animal management and production, and these changes have had profound effects on disease patterns and control. We begin this chapter by briefly setting some of these changes in perspective.

CHANGING PATTERNS OF ANIMAL PRODUCTION

Throughout much of the developed and all of the developing world, systems of animal production for food and fiber are traditional or extensive, typified by the grazing of sheep and cattle across vast areas in the Americas and Australia, or the movement of small herds of cattle or goats across the Sahel or in Somalia by nomadic tribes. Chickens and swine were penned and housed centuries ago, but what are termed intensive animal production systems, particularly for chickens and swine, were developed from the middle of this century. In the developed world, they are now almost the only method of production for these species, and similar intensive systems are used for some classes of cattle. Infectious diseases, particularly viral diseases, have often been the rate- and profit-limiting step in the development of such systems. Significant aspects of intensive animal production include the following:

1. The bringing together of large numbers of animals, often from diverse backgrounds, and confining them to limited spaces, at high density
2. Asynchronous turnoff of finished animals and the introduction of new animals to be finished
3. The care of large numbers of animals by few, sometimes inadequately trained, personnel
4. Elaborate housing systems, with complex mechanical services for ventilation, feeding, waste disposal, and cleaning
5. Limitation of the husbandry system to one species
6. Manipulation of natural biological rhythms (artificial daylight, estrus synchronization, etc.)
7. Use of very large batches of premixed, easily digestible foodstuffs
8. Improved hygienic conditions
9. Isolation of animal populations

Some figures from the United States illustrate the scale of operations of intensive animal production units. A large cattle feedlot operation in Colorado has at any one time 100,000 cattle held in 1-acre pens of 400 each, and 2.5 batches are turned off each year. A single farrowing house in Iowa may hold 5000 sows; on a fully integrated farrow-to-finish farm

there may at any one time be 50,000 swine, of which 45,000 are turned off and replaced 2.2 times a year. A dairy farm in California may milk 5000 cows. A single broiler house in Georgia may house 50,000 birds, and the farm comprises six such houses; several farms are often located in close proximity. The growing time for a broiler chicken is 9 weeks, so that 1.7 million chickens are produced per year. There are also intensive systems for producing vealer calves and lambs. Three consequences follow upon this situation:

1. These conditions favor the emergence and spread of enzootic infectious diseases, as well as opportunistic infections.
2. Such populations are at great risk from the introduction of nonenzootic viruses, although many are designed to provide reliable barriers against such introductions.
3. These conditions favor multiple infections working synergistically, further complicating diagnostic, preventative, and therapeutic measures.

None of the basic characteristics of intensive livestock production systems are going to change because of disease constraints; the economics of these systems is such that losses due to diseases are generally small relative to gains, due mainly to feed and labor efficiency. Nevertheless, there is great merit in improving these production systems by minimizing disease losses, and thereby increasing yields and lowering costs. The chief constraint for developing better health management in intensive livestock production systems is methodological, the solution requiring the introduction of modern epidemiological methods into the training and experience of veterinarians and other animal scientists concerned with livestock production.

In contrast, production of sheep, goats, and most cattle in developed countries still follows traditional methods, which pertain for all species in developing countries. In the latter, husbandry is still primitive, but requirements for animal products have greatly increased because of the continuously expanding human populations. For example, in the Sahel, installation of watering points has led to buildup of herds in good seasons, with disastrous results when droughts occur or when epizootic diseases enter the region. More frequent and extensive movement of stock and people exacerbates the spread of infectious diseases, especially in Africa where the large populations of wildlife harbor many viral diseases that affect introduced livestock. These are matters of national and international concern, not only for humanitarian reasons, but also because of the risk of the international transfer of exotic viruses of livestock and the disastrous consequences such importations could have on animal production industries in the developed countries.

The situation with companion animals is very different, but the risk of infectious diseases varies greatly between the single, mature-age household dog, cat, or pony and the large breeding establishments for these species, in which several hundred animals, of all ages, are kept and bred.

SURVEILLANCE

The implementation of disease control programs is critically dependent on accurate information about the existence and extent of occurrence of particular viral diseases. *Surveillance* of infectious diseases provides this basic information; it is the systematic and regular collection, collation, and analysis of data on disease occurrence. Its main purpose is to detect changes in trends or distribution of disease.

The need for data on the occurrence of infectious diseases has led to the concept of "reportable" (notifiable) diseases, whereby veterinary practitioners are required to report to a central authority such as the local or state veterinary authority. Clearly the list of such diseases must not be too large, or notification will be ignored. However, data provided by a system of notification influences decisions on resource allocation for the control of diseases and the intensity of follow-up. Such data can also provide useful information on strategies of prevention, especially by allowing calculations of cost–benefit ratios and indices of vaccine efficacy.

Sources of Surveillance Data

The methods of surveillance commonly used for animal diseases are notifiable disease reporting, laboratory-based surveillance, and population-based surveillance. The key to surveillance is the veterinary practitioner. Although any one practitioner may see only a few cases of a particular disease, data from many practitioners can be accumulated and analyzed to reveal trends in the occurrence of diseases. There are seven sources of information on disease incidence, not all of which are required for any one disease:

1. Morbidity and mortality data assessed through material submitted to national, state, and local diagnostic laboratories
2. Case and outbreak investigations linked to diagnostic laboratories
3. Analyses of vaccine manufacture and use
4. Reviews of local media reports of disease
5. Monitoring of virus activity by clinical, serological, and virological examination of animals presented for slaughter at abattoirs, tested for legal movement, or exposed as sentinels to detect arbovirus activity

6. Monitoring of arthropod populations and virus infection rates to detect arbovirus activity
7. Specific serological and virological surveys

Having collected data, it is important that it should be analyzed quickly enough to influence the institution of control measures. Dissemination of information—the provision of feedback from the state veterinary authority to the local veterinary practitioner—is a vital component of an effective surveillance system.

Investigation and Action in Disease Outbreaks

When there is a disease outbreak, it must first be recognized, hopefully at the level of primary veterinary care. This is not always easy when a disease emerges in a new setting. The only way that shortcomings in recognition can be avoided is by an elevation in the "index of suspicion," which is instilled as part of professional training. The strategy for investigation and action is dictated by the diagnosis itself. For diseases that are identified as endogenous or sporadically present in the given animal population, outbreaks are usually handled by veterinary practitioners working directly with livestock producers. For diseases that are identified as exotic or as having epizootic potential, most further investigation and action depends on available organization and expertise.

Early in an outbreak investigation is focused on: (1) identification of the etiological agent, (2) measurement of population susceptibility and acquired immunity, (3) quantitation of the magnitude of the outbreak and the rate of spread, and (4) a search for reservoirs and modes of transmission.

In usual enzootic circumstances, disease control may be based upon routine activities such as immunizations or control of arthropods; however, in epizootic circumstances, control programs more commonly require delivery of a whole package of resources, including technically trained personnel, special equipment, supplies, transportation, and funds. Resource needs might be of such magnitude as to support large-scale tasks such as the limitation of movement of animals or even the implementation of quarantine under national or international regulations.

HYGIENE AND SANITATION

Intensive animal husbandry leads to heavy contamination of the local environment with feces, urine, hair, feathers, etc. For viruses that are

thermostable, the buildup of such products provides a ready source of virus for infecting newly introduced livestock. To avoid this, many pork, veal, and poultry farmers operate an "all in, all out" management system, by which the animal houses are emptied, cleaned, and disinfected between groups of animals.

Hygiene and disinfection are most effective in the control of fecal–oral infections; they have less effect on the incidence of respiratory infections. In general, attempts to achieve "air sanitation" have failed and respiratory viral infections constitute the single most important group of diseases in intensive animal production systems.

Nosocomial Infections

In contrast with human medicine, nosocomial infections are uncommon in large-animal veterinary practice, since animals are usually treated on the farm; however, such infections are recognized in companion animal medicine. The use of appointment systems in veterinary practices reduces the risk of disease transmission in the waiting room; further, the preference of many clients to wait in their cars so that their animals become less excited also cuts down the risk of disease transmission. Veterinary clinics should require that all inpatients have current immunization records or receive booster immunization. Clinics should be designed for easy disinfection, with wash-down walls and flooring, and as few permanent fixtures as possible. They should also have efficient ventilation or air conditioning, not only to minimize odors but also to reduce the aerosol transmission of viruses. Frequent handwashing and decontamination of infected equipment is essential.

Disinfection and Disinfectants

Disinfectants are chemical germicides formulated for use on inanimate surfaces, in contrast to *antiseptics*, which are chemical germicides designed for use on the skin or mucous membranes. Disinfection of contaminated premises plays an important role in the control of diseases of livestock, especially where all-in, all-out management systems are used and when importations of exotic diseases occur.

Viruses of different families vary greatly in their resistance to disinfectants, enveloped viruses usually being much more sensitive than nonenveloped viruses. However, most modern disinfectants rapidly inactivate all viruses. Their effective action is influenced by access, and virus trapped in heavy layers of mucus or fecal material will not always be inactivated. Standard requirements by the United States Environmental Protection Agency specify that test viruses must be suspended in 5% serum

and dried on a hard surface before testing the efficacy of a disinfectant. There are special problems when surfaces cannot be cleaned thoroughly or where cracks and crevices are relatively inaccessible, as in old timber buildings or the fenceposts and railings of cattle and sheep yards.

Table 16-1 sets out some common disinfectants and their potential uses. The first five compounds listed can be used in animal quarters, although some are too expensive for large-scale use. The second four are of use primarily in the consulting room, hospital, or laboratory. Lye (2% NaOH) is cheap and has been the traditional disinfectant for large-scale disinfection of farm premises following outbreaks of foot-and-mouth disease and other exotic viral diseases. Hot water containing detergent and steam sterilization are also useful for the decontamination of premises.

CONTROL OF ARTHROPOD VECTORS

Control of arbovirus infections relies, where possible, on the use of vaccines, which provide the least expensive method because of the large areas and extended period over which vectors may be active. However, vector control is an important adjunct control strategy. For example, aerial spraying with ultralow-volume insecticides is used to prevent the establishment of mosquito populations carrying western equine encephalitis and St. Louis encephalitis viruses in some parts of North America.

Elimination of cracks and crevices in pig pens (in which soft ticks can hide) has been important in the control of African swine fever in Spain (see Chapter 20). A similar approach has been found to be successful in South Africa, where swine are double-fenced to avoid contact with warthogs. In the case of many other arbovirus infections, vector arthropods breed over too wide an area to make vector control feasible. In Australia and New Zealand, spraying of the luggage bays and passenger cabins of overseas aircraft with insecticides reduces the chances of intercontinental transfer of exotic arthropods, whether infected or not.

VACCINATION

The foregoing methods of control of viral diseases are based on reducing the chances of infection. The most generally effective method of control, vaccination, is directed at making individual animals resistant to infection; if practiced widely, vaccination reduces or may even interrupt transmission.

As outlined in Chapter 14, and discussed at length in the chapters of

TABLE 16-1
Commercially Available Disinfectants Used to Inactivate Viruses[a]

Disinfectant	Uses	Remarks
Sodium hypochlorite (Chlorox, chlorize)	Drinking water, food and utensils, dairies, spot disinfection	Highly effective, but high protein concentrations interfere; inexpensive, nontoxic, rapid action
Detergent iodophores (Betadine, Wescadine, Redene)	Same as sodium hypochlorite	Action based on slow release of iodine and detergent action; less affected by high protein concentrations than sodium hypochlorite; expensive
Formaldehyde (Formalin)	Laundry, bedding surfaces, and as vapor for surface sterilization	Low power of penetration, but useful for terminal disinfection; irritating, hypersensitivity develops
Phenol derivatives (Lysol, Dettol, Staphene, Sudol)	2.5% Aqueous solution for hands, examination tables, cages, hospital surfaces	Efficacy depends on concentration and temperature; high protein concentrations interfere
Chlorhexidine (Hibitane, Nolvasan)	Wide range, examination tables, cages, hospital surfaces	Little affected by body fluids, soap, organic compounds; expensive
Ethylene dioxide	For heat-sensitive medical supplies, plastic isolators	Toxic and explosive except as mixture, 10% with 90% CO_2, which is available commercially as compressed gas
Glutaraldehyde (Cidex)	Cold sterilization of instruments with lenses	2% Solution buffered with sodium bicarbonate is virucidal in 10 minutes at pH 7.5–8.5; expensive
Alcohol (ethyl, isopropyl)	Hands, thermometers	Moderately virucidal only in high concentrations (70–80%); ethanol preferable to methanol or isopropanol; nontoxic
Quaternary ammonium compounds (Zephiran, Roccal, Savlon)	Zephiran (benzalkonium chloride) used for cleansing wounds	Not very effective against many viruses; high protein concentrations interfere

[a]Based on data supplied by Professor J. Storz.

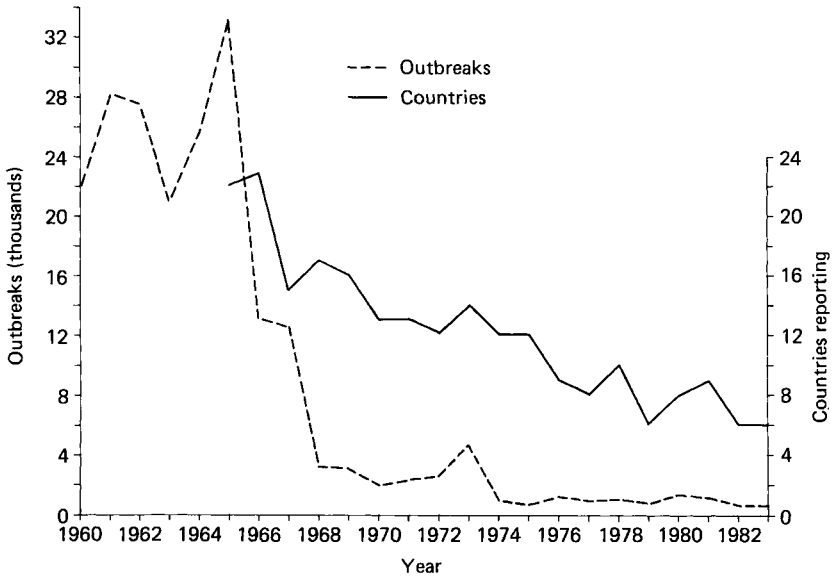

FIG. 16-1. *Foot-and-mouth disease in Europe, 1960–1983, showing numbers of outbreaks and numbers of countries reporting cases each year. The reduction since the mid-1960s is due to the effective use of trivalent inactivated vaccines (types A, O, and C). Over half the outbreaks each year since 1969 and some 80% each year since 1977 have occurred in Turkey. (Data from the European Commission for the Control of Foot-and-Mouth Disease,* In *"Animal Health Yearbook," Food and Agriculture Organization, Rome, 1984.)*

Part II, there are now effective vaccines for many viral diseases of animals. These vaccines are especially effective against diseases with a viremic phase and caused by monotypic viruses, such as canine distemper and infectious bovine rhinotracheitis viruses. It has proved much more difficult to immunize effectively against infections of the digestive or respiratory tracts, or the skin. The effect of vaccination programs in reducing the incidence of canine distemper in urban communities and foot-and-mouth disease in European livestock has been dramatic (Fig. 16-1).

QUARANTINE

Originally introduced in the fifteenth century for the control of plague, quarantine was used by the English colonists in North America in 1647 to try to prevent the entry of yellow fever and smallpox. Quaran-

tine proved effective in keeping smallpox out of Australia in the nineteenth and early twentieth centuries, and in delaying the entry of pandemic human influenza into that country in 1919. However, with the onset of air travel and the consequent arrival of passengers before the end of the incubation period of most viral diseases, quarantine as applied to human diseases became much less effective.

In contrast to the increasing deregulation of human movement, movement of domestic animals across international and even state borders can be regulated, at least in the industrialized countries, where there are appropriate veterinary services and regulatory infrastructure. Thus, quarantine remains a cornerstone in many animal disease control programs. A period of quarantine, with or without specific etiological or serological testing, is usually a requirement for the importation of animals from another country, and similar requirements may be enforced within a country or local area to assist in the control or eradication of specific infectious agents.

As international movement of live animals for breeding purposes and exhibition has increased, so also has the risk of introducing disease. Before the advent of air transport, the duration of shipment usually exceeded the incubation period of most diseases, but this is no longer the case. With the ever-increasing value of livestock, national veterinary authorities have tended to adopt stricter quarantine regulations to protect their livestock industries. Complete embargoes on importation are imposed for some animals by some countries. The original concept of quarantine, where animals were simply isolated and observed for disease for a given period of time, is now augmented by extensive laboratory testing designed to detect previous exposure to selected viruses or a carrier state. Laboratory testing requirements are set down in detailed protocols and supported by national legislation.

While dog and cat quarantine has been a successful method for preventing the introduction of many diseases, such as rabies into Australia, New Zealand, and Great Britain, other diseases may be introduced in animal products (e.g., foot-and-mouth disease in meat products) or by virus-infected arthropods (e.g., bluetongue). It must also be recognized that most countries have land boundaries with their neighbors and cannot easily control human and wildlife movement, e.g., the movement of rabies via foxes in Europe (see Fig. 30-1). For countries with long land borders, quarantine is difficult to enforce. To help overcome this problem, most countries have agreed to notify each other through the Office International des Epizooties (OIE) in Paris, of the disease status of their livestock. Although the recognition of internationally acceptable criteria for reporting the presence of specific diseases remains a problem, the

system usually provides countries with an opportunity to take appropriate action, e.g., increased vigilance along a national boundary and maintenance of vaccine stocks. However, there is still a long way to go in developing standards for testing animals and controlling animal movement. The problems are often social, economic, and political rather than scientific. For example, smuggling of exotic birds may play a significant role in the introduction of Newcastle disease and fowl plague viruses.

ERADICATION OF VIRAL DISEASES

Control, whether by vaccination alone, or by vaccination plus the various methods of hygiene and sanitation aimed at lessening the chance of infection, is an ongoing process, which must be maintained as long as the disease is of economic importance. If a disease can be eradicated, so that the virus is no longer present anywhere except in microbiologically secure laboratories, control measures are no longer required. However, eradication of a disease that is enzootic demands a major financial commitment for a long period, often decades, if success is to be achieved, even on a national scale. Close cooperation between veterinary services and the farming industry is essential. To achieve such cooperation, the veterinary services of a country must justify their proposals by cost–benefit analyses and consult with all interested organizations. As the control program proceeds, they must ensure feedback of information on progress (or problems) directly to those involved, or via the media.

Eradication on a countrywide scale has been achieved for foot-and-mouth disease in a number of countries in which it was once established: Japan, the United Kingdom, the United States, Mexico, and the countries of Central America. Rinderpest, once a devastating disease of cattle in Europe, was finally eradicated from that continent in 1949, and rabies was eradicated from the United Kingdom in 1901 and again in 1922, after reintroduction of the disease during the First World War.

So far, global eradication has been achieved for only one disease, and that is a disease of humans. The last endemic case of smallpox occurred in Somalia in October 1977. Global eradication was achieved by an intensified effort that involved a high level of international cooperation and made use of a potent and very stable vaccine that was easy to administer. However, mass vaccination alone could not have achieved eradication of the disease from the densely populated tropical countries where it remained endemic in the 1970s, because it was impossible to achieve the necessary high level of vaccination. The effective strategy was to

TABLE 16-2

Biological Characteristics Enhancing Chances of Eradication

Characteristic	Human smallpox	Canine distemper	Newcastle disease	Bovine rinderpest	Foot-and-mouth disease
Reservoir host in wildlife	No	No	Yes	Yes	Yes, in Africa
Persistent infection occurs	No	No	No	Yes	Yes, especially in buffalo
Subclinical cases occur	No	No	Yes	Unusual	Yes
Number of serotypes	1	1	1	1	7
Infectivity during prodromal stage	No	Yes	Yes	Yes	Yes, very infectious
Vaccine					
Effective	Yes	Yes	Yes	Yes	Usually but type-specific polyvalent formulation used
Heat stable	Yes	Moderately	Moderately	No	Yes
Number of doses	1	1	2	1	2, then annually or semiannually
Early containment of outbreak possible	Yes	No	Sometimes	Yes	Difficult
High level of public concern	Yes	No	Yes	Yes	Yes

combine vaccination with *surveillance and containment,* by which cases were actively sought out, isolated, and their contacts vaccinated, first in the household and then at increasing distances from the index case.

The achievement of global eradication of smallpox led to discussions as to whether any other diseases could be eradicated worldwide. Smallpox was unusual in that, given an effective vaccine, all of its biological characteristics enhanced the possibility of global eradication (Table 16-2). Yet this was achieved only after an immense and sustained international effort.

The biological characteristics that would render more likely the eradication of viral diseases of livestock are: (1) no wildlife reservoir host, (2) no reservoir or carrier host, (3) lack of recurrent disease with virus shedding, (4) only one or a few stable serotypes, and (5) an effective vaccine. No less important is the level of public concern, for any eradication program requires a sustained commitment of human and financial resources. Applying these criteria, it becomes obvious that rinderpest could be eradicated throughout the world. In fact, several developed nations of the world eradicated the disease by a *test and slaughter* ("stamping out") policy before vaccines were available—even in some cases before the viral etiology of the disease was recognized. In the 1960s, when an effective pan-African vaccination program was in force, the prospect of global eradication of rinderpest was seriously discussed. Unfortunately, as the incidence of disease fell so did the momentum of the control program, and by 1984 Africa was again experiencing large-scale epizootics of rinderpest.

The possibilities of global eradication of the other diseases listed in Table 16-2 are more remote. A low level of public concern, and infectivity in the prodromal stage make the eradication of canine distemper difficult, and the existence of a widely dispersed wildlife reservoir makes the global eradication of Newcastle disease impossible. Foot-and-mouth disease has the largest constellation of unfavorable features, balanced in the developed countries by the very high level of concern about its presence or importation. However, by using test and slaughter policies—in conjunction with quarantine, licensed movement of animals, and, in some diseases, vaccination—several important viral diseases, such as sheeppox, hog cholera, and velogenic Newcastle disease, have been eradicated from most of the developed and some of the developing nations. Whether any widespread disease of domestic animals eventually proves to be globally eradicable depends biologically on whether there is a major wildlife reservoir, and politically on the setting of priorities for the use of human and financial resources, on an international scale.

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CHAPTER 17

Papovaviridae

The family *Papovaviridae* includes two genera, *Papillomavirus* and *Polyomavirus*. Papillomaviruses are naturally tumorigenic, causing papillomas (warts), which occur in many different species (Table 17-1). Warts have been recognized in humans from time immemorial and in animals for centuries; equine papillomas were described in the ninth century by a stablemaster of the Caliph of Baghdad. Their viral etiology was established in 1907. Only those affecting cattle, horses, and dogs are likely to require veterinary attention. They are commonly seen in young animals as areas of simple hyperplasia or as benign neoplasms and usually regress spontaneously. However, in association with certain cofactors, bovine, human, and rabbit papillomaviruses may produce carcinomas.

Papillomaviruses have not yet been grown in cultured cells, which until recently restricted study to the pathology of the lesions, ultrastructure of the virion, and experiments on transmission. However, a few years ago it became possible to clone restriction fragments of papillomavirus DNAs obtained from virions purified from papillomas. This has made molecular hybridization, heteroduplex mapping, and DNA se-

TABLE 17-1
Diseases Caused by Papillomaviruses

Virus[a]	Principal species affected	Disease
Bovine papillomavirus		
Type 1	Cattle	Cutaneous fibropapilloma
	Horse	Sarcoid
Type 2	Cattle	Cutaneous fibropapilloma
	Horse	Sarcoid
Type 3	Cattle	Cutaneous papilloma
Type 4	Cattle	Alimentary tract papilloma (may become malignant)
Type 5	Cattle	Teat fibropapilloma ("rice grain")
Type 6	Cattle	Teat papilloma ("frond")
Ovine papillomavirus	Sheep	Cutaneous fibropapilloma
Equine papillomavirus	Horse	Cutaneous papilloma
Porcine genital papillomavirus	Swine	Cutaneous papilloma
Canine oral papillomavirus	Dog	Oral papilloma
Cottontail rabbit papillomavirus[b] (*Sylvilagus floridanus*)	Rabbit	Cutaneous papilloma (may become malignant)
Human papillomavirus (32 types)	Human	Cutaneous and mucosal papillomas (some become malignant)

[a]In addition, papillomaviruses have been recovered from several species of deer, from birds, and from hamsters, in all of which they cause small cutaneous papillomas or fibropapillomas.

[b]Shope papillomavirus was much used in early studies of tumorigenic viruses.

quencing possible. This has greatly increased our knowledge of the papillomaviruses and has stimulated renewed interest in their mechanism of tumorigenesis.

Polyomavirus infections are not associated with clinical disease in their natural hosts, but certain of these viruses are tumorigenic when inoculated into newborn rodents. They grow readily in cell cultures, and during the 1960s and 1970s mouse polyomavirus and SV40 were intensively studied by tumor virologists. The genus *Polyomavirus* does not contain any recognized pathogens of veterinary importance.

PROPERTIES OF PAPILLOMAVIRUSES

The virion of papillomaviruses has a nonenveloped icosahedral capsid, approximately 55 nm in diameter, with 72 capsomers (Table 17-2).

TABLE 17-2
Properties of Papovaviruses

Nonenveloped icosahedral virion, 45[a] or 55[b] nm in diameter
Genome: circular, supercoiled dsDNA, 5[a] or 7–8[b] kbp
Replicate in nucleus
Integrated[a] or episomal[b] DNA may be tumorigenic

[a] *Polyomavirus.*
[b] *Papillomavirus.*

Both "empty" and "full" virus particles are seen by electron microscopy (Plate 17-1). Tubular and other aberrant morphological forms are common. The genome of papillomavirus is a double-stranded, covalently closed, circular, supercoiled DNA molecule, varying in size from 6.7 kbp for bovine papillomavirus 3 to 8.2 kbp for canine oral papillomavirus. So far, some 10 polypeptides have been identified by polyacrylamide gel electrophoresis, with major capsid proteins of 50–63K. Papillomaviruses are ether resistant, acid stable, and thermostable.

Although antisera to intact virions of different papillomaviruses show no cross-reactivity, wide serological cross-reactivity can be demonstrated with antisera to detergent-disrupted virions, indicating that there are conserved epitopes on internal proteins. There is only a limited sequence homology between the DNAs of papillomaviruses of different species, but substantial sequence homologies between those derived

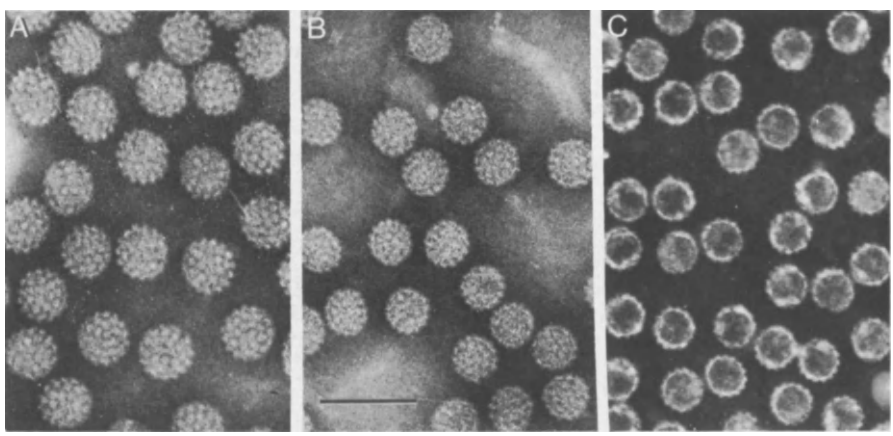

PLATE 17-1. Papovaviridae. *(A)* Papillomavirus. *(B)* Polyomavirus. *(C)* Polyomavirus, *empty virions (bar = 100 nm).(Courtesy Dr. E. A. Follett.)*

from any given species (human or bovine, for example). Sequence homology has been used to distinguish different "types" among the papillomaviruses infecting a given species. The convention has been adopted that to be classified as a new type, there should be less than 50% homology between the DNAs and significant serological differences in reciprocal assays. Six types of bovine papillomaviruses and 32 types of human papillomaviruses have been identified so far.

The bovine papillomaviruses can be divided into two groups. Bovine papillomavirus 1, 2, and 5 are related serologically, have the same genome size, and share DNA sequences. Viruses of the other group, bovine papillomavirus 3, 4, and 6, have a smaller genome and also have DNA sequences in common. The two groups are only distantly related.

VIRAL REPLICATION

Since papillomaviruses have not been grown in cultured cells, little is known about their replication. In skin lesions, mature virions can be seen only in the outer keratinizing epithelial cells.

Although they do not replicate *in vitro*, bovine papillomavirus 1 and 2 will transform cells, as will their DNAs. In contrast to other tumorigenic or transforming DNA viruses, papillomavirus DNA is not integrated into the cellular genome, but remains episomal. Fragments of the bovine papillomavirus 1 genome have been cloned and subsequently expressed in *E. coli*, and antisera to some of the proteins expressed inhibit *in vitro* transformation of cells by bovine papillomavirus 1. Bovine papillomavirus 1 DNA and a subgenomic fragment of it (the 69% transforming fragment) have been used as eukaryotic vectors for foreign DNA.

PATHOGENESIS AND IMMUNITY

Based on host response, papillomaviruses can be subdivided according to tissue tropism and histology of the lesion. There is a good correlation between histopathological appearance and viral type as determined by DNA sequence (Table 17-3). The cells of the dermal layer of a papilloma proliferate excessively, but neither virions nor viral antigen can be detected in the proliferating cells, although many copies of the viral DNA are present. Viral antigen and virions can be detected only in the keratinized cells at the surface of the papilloma.

TABLE 17-3
Host Responses to Papillomaviruses

Host response	Virus
Group 1	
Neoplasia of cutaneous stratified epithelium (cutaneous papilloma)	Bovine 3 and 6, equine, and cottontail rabbit papillomaviruses[a]
Group 2	
Hyperplasia of normal nonstratified squamous epithelium or metaplastic squamous epithelium	Bovine 4[b] and canine oral papillomaviruses
Group 3	
Cutaneous papilloma with underlying fibroma of connective tissue	Bovine papillomavirus 1, 2, and 5

[a]May progress to squamous carcinoma.
[b]With cofactors, alimentary tract and bladder papillomas may progress to carcinoma.

Papillomas develop after the introduction of virus through abrasions of the skin. Infection of the epithelial cells results in hyperplasia of cells of the stratum spinosum with subsequent degeneration and hyperkeratinization. Clinically, the epithelium overlying the area of hyperplasia begins to proliferate 4–6 weeks after infection. In general, fibropapillomas persist for 4–6 months before spontaneous regression; multiple warts regress simultaneously. The level of antibody does not appear to be correlated with either growth or regression of papillomas, and the mechanisms inducing regression are unknown.

BOVINE PAPILLOMATOSIS

Warts are more commonly seen in cattle than any other domestic animal. Cattle of all ages are affected, but the incidence is highest in calves and yearlings.

Clinical Features

Warts caused by bovine papillomavirus 1 and 2 have a fibrous core covered to a variable depth with stratified squamous epithelium, the outer layers of which are hyperkeratinized. The lesions vary from small, firm nodules to large cauliflowerlike growths; they are grayish to black in color, and rough and spiny to touch. Large fibropapillomas are sub-

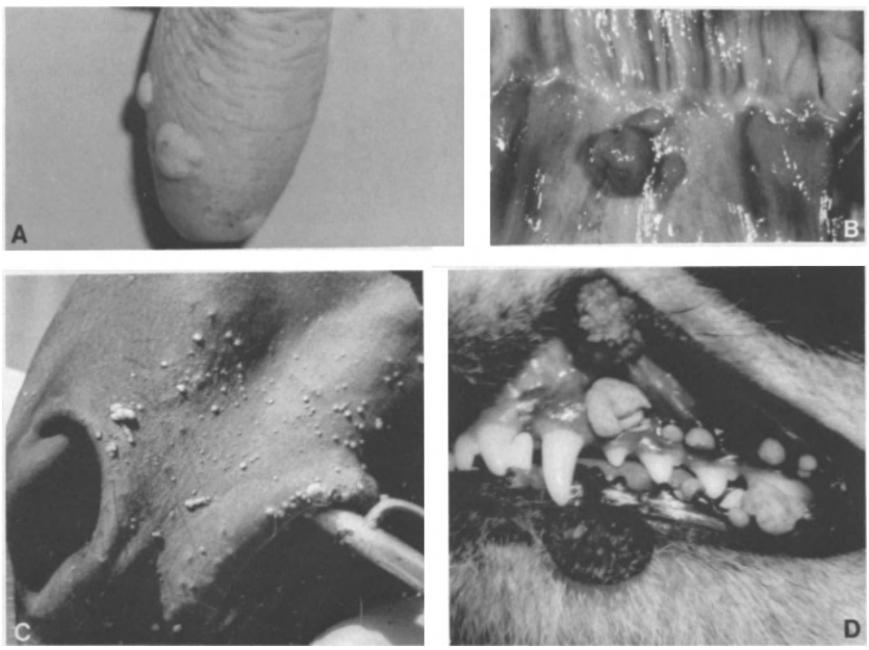

PLATE 17-2. *Warts affecting different species. (A) Bovine teat warts—fibropapilloma (bovine paillomavirus 5). (B) Bovine bladder papilloma (bovine papillomavirus 4). (C) Equine papillomatosis. (D) Canine oral papillomatosis (D, courtesy Dr. L. E. Carmichael).*

ject to abrasion and may bleed. Fibropapillomas can occur on the udder and teats and around the genitalia, but are most common on the head, neck, and shoulders.

Cutaneous papillomas, lacking a fibrous core, are also seen in cattle. They are caused by bovine papillomavirus 3 and have a tendency to persist. This type of wart is usually flat with a broad base, in contrast to fibropapillomas which protrude and are often pedunculated.

In upland areas of Scotland and northern England, papillomas due to bovine papillomavirus 4 occur commonly in the alimentary tract and in the urinary bladder (Plate 17-2B) of cattle, and may progress to squamous cell carcinomas. Ingestion of bracken fern (*Pteridium aquilinum*) appears to be a major contributing factor (cocarcinogen) in the transition from benign papilloma to invasive carcinoma of the alimentary tract or bladder, the latter leading to so-called chronic endemic hematuria.

Papillomas on the teats are common in dairy cattle throughout the world. Generally they are of little concern, but large lesions may inter-

fere with milking. Pedunculated fibropapillomas ("frond papillomas") caused by bovine papillomavirus 6 and "rice grain" papillomas, caused by bovine papillomavirus 5, may also occur on the teats of cattle (Plate 17-2A).

Laboratory Diagnosis

The clinical appearance of papillomas is characteristic and laboratory diagnosis is seldom necessary. Virus particles can be found by electron microscopic examination of lesion biopsies.

Epidemiology

Virus is transmitted between animals by contaminated halters, nose leads, grooming equipment, rubbing posts, and other articles contaminated by contact with diseased cattle. Cattle that have been groomed for show may have extensive lesions. The disease is more common in housed cattle than in cattle at pasture.

Prevention and Control

Despite their wide use, there is little evidence that autologous vaccines are effective. The disease is self-limiting, and apart from surgical removal of fibropapillomas on the teats of milking cattle, veterinary intervention is rarely justified. Carcinoma associated with alimentary and urinary tract papillomatosis can be prevented by restricting or eliminating the ingestion of bracken fern.

EQUINE PAPILLOMATOSIS AND SARCOIDS

Warts due to equine papillomavirus occasionally appear as small, elevated, keratinized papillomas around the lips and noses of horses (Plate 17-2C). They generally regress after a few months. Warts that interfere with the bit or bridle can be surgically removed.

Sarcoids are naturally occurring skin tumors of horses that have the histological appearance of a fibrosarcoma. Although they do not metastasize, they persist for life and are locally invasive, often recurring after surgical removal. Horses are susceptible to experimental infection with bovine papillomavirus 1 and 2, and the tumors produced are similar to sarcoids. Bovine papillomavirus DNA sequences have been detected in high copy number by molecular hybridization in both experimental and natural lesions. These data, together with the observation that sarcoids can occur in epidemic form, suggest that bovine papillomavirus 1 and 2 may be the cause of equine sarcoids.

CANINE PAPILLOMATOSIS

Warts in dogs usually begin on the lips and can spread to the buccal mucosa, tongue, palate, and pharynx before regressing spontaneously (Plate 17-2D). The lesions occasionally become extensive, requiring veterinary attention.

PAPILLOMATOSIS IN OTHER SPECIES

Classical studies of viral tumorigenesis were carried out 40–50 years ago with the Shope rabbit papilloma virus. These papillomas often progress to carcinoma in both naturally infected cottontail rabbits and experimentally infected laboratory rabbits. Papillomaviruses of other species are of little importance in veterinary medicine.

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CHAPTER 18

Adenoviridae

In 1953 Rowe and his colleagues, having observed that explant cultures of human adenoids degenerated spontaneously, isolated a new virus which they named "adenovirus." The next year Cabasso and his colleagues demonstrated that the etiological agent of infectious canine hepatitis was an adenovirus. Subsequently many serotypes of adenoviruses, each of which appeared to be highly host specific, were isolated from humans and many other species of animals and birds, usually from the upper respiratory tract but sometimes from feces. Most of these viruses produce subclinical infections, with occasional evidence of upper respiratory disease, but the canine and avian adenoviruses are associated with a variety of important diseases, including hepatitis (Table 18-1).

PROPERTIES OF ADENOVIRUSES

The morphology of the virion is shown in Plate 18-1 (see also Fig. 1-1). The capsid is an icosahedron 70–80 nm in diameter, composed of 252

TABLE 18-1
Animal Diseases Associated with Adenoviruses

Animal species	Number of serotypes	Disease
Horses	2	Usually asymptomatic or mild upper respiratory disease; generalized disease in foals with congenital immunodeficiency
Cattle	9	Usually asymptomatic, or mild upper respiratory disease
Swine	4	
Sheep	6	
Goats	2	
Dogs	2	Infectious canine hepatitis (type 1) Infectious canine tracheobronchitis (type 2)
Birds	11	
Chickens		Rarely, inclusion body hepatitis and egg drop syndrome
Turkeys		Bronchitis, marble spleen disease, enteritis
Ducks		Rarely, duck hepatitis
Quail		Bronchitis

capsomers: 240 hexamers which occupy the faces and edges of the 20 equilateral triangles of an icosahedron and 12 pentamers which occupy the vertices. From each pentamer projects a fiber 20–50 nm in length, with a terminal knob. There are several other minor structural proteins, some of which are associated with the capsid and some with the core, which also contains the viral genome (see Fig. 1-1A). This is linear dsDNA, 30–37 kbp in size, with inverted terminal repeats (see Fig. 1-3A). This molecule, in association with a 55K protein covalently linked at each 5′ terminus, is infectious.

Many adenoviruses agglutinate red blood cells, hemagglutination occurring when the tips of the fiber bind to an appropriate receptor on the surface of the erythrocyte. The species of erythrocytes agglutinated and the optimal conditions of assay have been empirically established for each viral species or serotype. Once established, hemagglutination and hemagglutination inhibition assays provide the simplest and most convenient assays for virus and antibody.

Classification

Classification is based primarily on antigenic relationships. Restriction endonuclease fragment patterns of the viral DNA are useful for detailed comparisons of strains. Shared antigenic determinants associated with

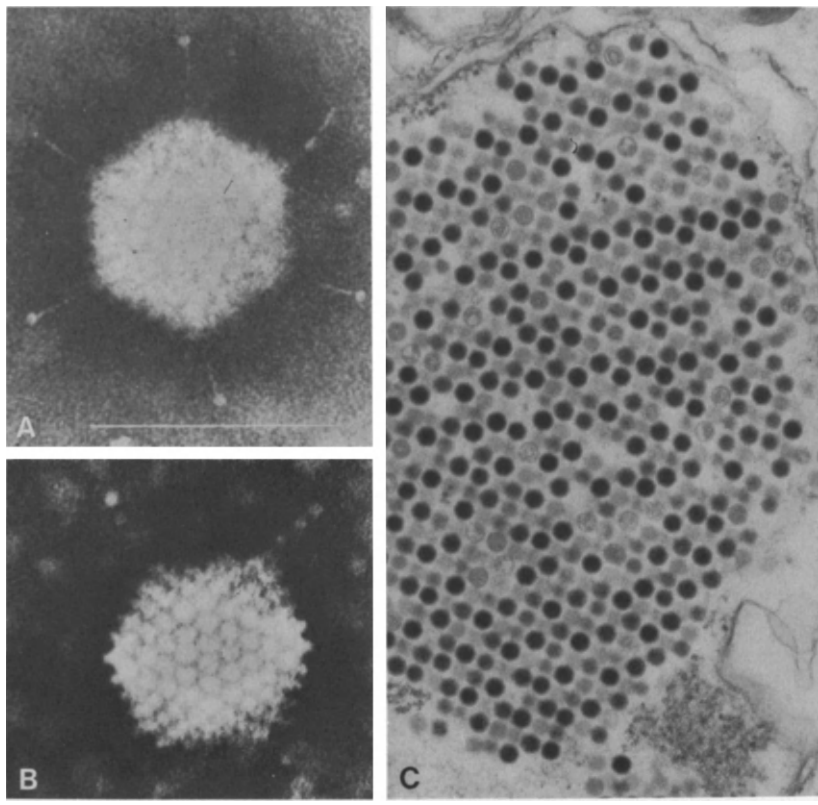

PLATE 18-1. Adenoviridae. *(A and B) Negatively stained preparations of* Mastadenovirus h5. *(A) Virion showing the fibers projecting from the vertices. (B) Virion showing the icosahedral array of capsomers. Capsomers at the vertices (pentons) are surrounded by five nearest neighbors, all the others (hexons) by six. (C) Section showing crystalline array of mature virions of* Mastadenovirus h7 *in nucleus of a human fibroblast cell (bar = 100 nm). [A and B from R. C. Valentine and H. G. Periera,* J. Mol. Biol. **13,** *13 (1965); C, courtesy of Dr. A. K. Harrison.]*

the inner part of the hexamers define two genera: *Mastadenovirus,* the mammalian adenoviruses, and *Aviadenovirus,* the avian adenoviruses. There are also genus-specific antigenic determinants on the pentamer. Type-specific antigenic determinants, which are defined by neutralization and hemagglutination inhibition assays, are located on the outward-facing surface of the hexamers. The fiber contains type-specific determinants, which can also be detected by neutralization assays. Al-

though the fiber binds to specific cell receptors during absorption, antibody to the fiber or fiber–pentamer complex is only weakly neutralizing.

The number of adenovirus serotypes within each animal species is shown in Table 18-1. Some 41 different types are recognized in humans and certain serotypes, which can be grouped together, tend to be associated with particular clinical syndromes. The number of serotypes defined for each domestic animal species is smaller, although 11 are recognized in birds (which of course encompass many species) and 9 in cattle.

VIRAL REPLICATION

Adenoviruses apparently enter the cell by direct translocation across the plasma membrane. The pentons are removed in the cytoplasm and the core migrates to the nucleus. In the nucleus the genome is transcribed according to a complex program (see Chapter 4). RNA transcribed from five separate regions, situated on both strands of the DNA, is spliced, then translated into about a dozen, mainly nonstructural, early proteins. Viral DNA replication, using the 5'-linked 55K protein as primer, proceeds from both ends by a strand displacement mechanism. Following DNA relication, late mRNAs are transcribed; these are translated into structural proteins, which are made in considerable excess. Virions are assembled in the nucleus, where they form crystalline aggregates (Plate 18-1C) which can be seen by light microscopy as intranuclear inclusion bodies. Shutdown of host macromolecular synthesis occurs progressively during the second half of the replication cycle. Virions are released by cell lysis.

PATHOGENESIS AND IMMUNITY

Most of the adenoviruses listed in Table 18-1 are causes of acute, mild, or subclinical respiratory disease, which is usually confined to the upper respiratory tract but may include conjunctivitis and bronchopneumonia. Some adenoviruses cause mild or subclinical enteric disease. All infections with adenoviruses are associated with long periods of latency, and adenoviruses can often be recovered from the pharyngeal lymphoid tissue of apparently healthy animals.

In contrast to the usual mild effects of most adenoviruses, canine adenovirus 1 causes a severe generalized disease of dogs, and several other adenoviruses are pathogenic in immunodeficient animals. These include generalized infections in certain Arabian foals with primary se-

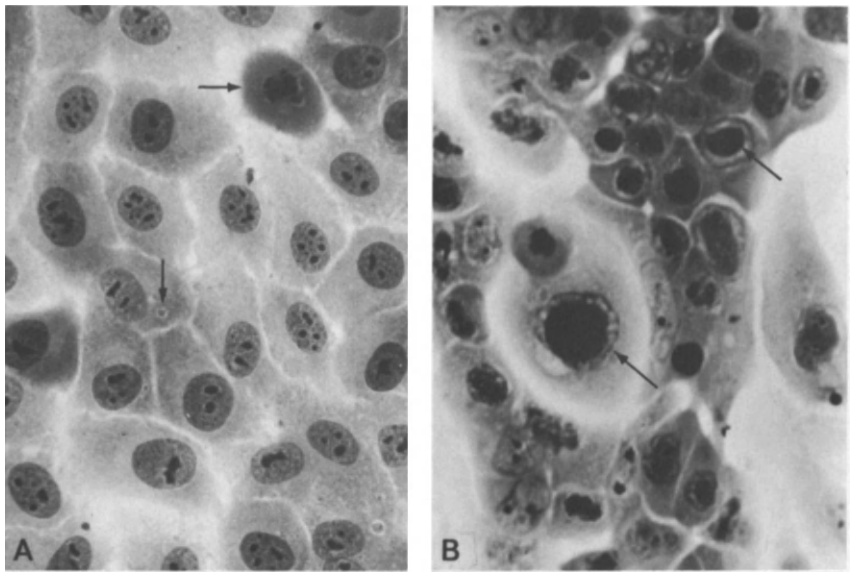

PLATE 18-2. *Cytopathic effects induced by adenoviruses (H and E stain; ×400). (A) Normal monolayer of HEp-2 cells. Horizontal arrow, cell in mitosis. Vertical arrow, phagocytosed cell debris, not to be confused with viral inclusion body. (B) Cytopathic effects induced by adenovirus in HEp-2 cells. Note distended cells containing basophilic intranuclear inclusions (arrows), which consist of masses of virions (see Plate 18-1C). Threads of chromatin sometimes radiate from the nuclear inclusions to the periphery of the nucleus. (Courtesy I. Jack.)*

vere combined immunodeficiency and in avian species whose immune systems are compromised by other infections, such as avian leukosis (see Chapter 31) or infectious bursal disease (see Chapter 33).

LABORATORY DIAGNOSIS

Virus can be isolated from swabs, fecal samples, or tissue homogenates by inoculation of cell cultures derived from the homologous species (Plate 18-2). Cytopathic changes are usually evident on first passage, although for some more slowly growing viruses a second passage is required. Some enteric adenoviruses are difficult to grow in cell cultures. Hemagglutination inhibition and neutralization assays are universally used for typing viral isolates.

EPIDEMIOLOGY AND CONTROL

Adenoviruses are highly species specific. Persistent, subclinical, productive infection permits the survival of the virus and provides a source of infection for each new generation of animals. For most adenoviruses the pharyngeal region, particularly the associated lymphoid tissues, is the site of persistent infection, which is concomitant with the presence of antibody. Enteric adenoviruses presumably persist by similar mechanisms, probably in gut-associated lymphoid tissues. Transmission occurs by droplet or a fecal–oral route. Following localization of the virus in the kidney, urine is an important mode of excretion of canine adenovirus 1.

Only the canine adenoviruses cause sufficiently troublesome diseases to warrant vaccination, and attenuated live or inactivated canine adenovirus 1 or 2 vaccines are widely used for the control of canine hepatitis and canine respiratory disease. Avian adenoviruses may be important contaminants of live vaccines produced in eggs or avian cell cultures.

EQUINE ADENOVIRUS INFECTIONS

Most adenovirus infections in horses produce an asymptomatic or mild upper respiratory tract disease. However, certain Arabian foals which suffer from primary severe combined immunodeficiency disease, in which there is an almost total absence of both T and B cells, are particularly susceptible to adenoviruses. As maternal antibody wanes, these foals are increasingly susceptible to a wide range of pathogens before they die, which invariably occurs within 3 months. Among these diverse pathogens, the dominant role of equine adenovirus 1 in the total disease process is intriguing. In addition to the extensive adenovirus bronchiolitis and pneumonia, the virus destroys a wide range of other tissues, particularly the pancreas and salivary glands, but also renal, bladder, and gastrointestinal epithelium.

CANINE ADENOVIRUS INFECTIONS

The two diseases caused by canine adenoviruses are much the most important adenovirus infections. Infectious canine hepatitis, caused by canine adenovirus 1, was first recognized as fox encephalitis. As well as causing hepatitis in dogs, canine adenovirus 1 may cause respiratory or ocular disease, encephalopathy, chronic hepatitis, and interstitial nephritis. Canine adenovirus 2 causes respiratory disease: tonsillitis, pharyngitis, tracheitis, bronchitis, and bronchopneumonia.

Infectious Canine Hepatitis

Most infections with canine adenovirus 1 are asymptomatic, a situation probably enhanced by the introduction of vaccination. The virus is acquired as a nasooral or conjunctival infection, and is a recognized cause of pharyngitis and tonsillitis. It is one of the several causes of "kennel cough," although in this context it is probably less important than canine adenovirus 2. Occasionally generalization via the bloodstream occurs, with destruction of vascular endothelium and mesothelium, leading to petechial hemmorrhages in many tissues and sometimes massive destruction of hepatocytes, resulting in a peracute fatal disease. In addition to the initial respiratory disease, the pattern of systemic canine adenovirus 1 infection is divisible into three overlapping syndromes, which are usually seen in pups less than 6 months old: (1) peracute disease in which the dog is found dead either without apparent preceding illness or after an illness lasting only 3 or 4 hours, (2) an acute illness which may or may not be fatal, with fever, depression, loss of appetitie, vomiting, bloody diarrhea, petechial hemorrhages of the gums, pale mucous membranes, and jaundice, or (3) mild cases.

Postmortem findings may include edematous lymph nodes, excess, and sometimes bloody fluid in the abdominal cavity, hemorrhages in many tissues, and jaundice. The liver may show slight enlargement, congestion, and yellowish mottling, and invariably histological examination reveals characteristic inclusion bodies in hepatocytes (Plate 18-3A).

In the convalescent stages of natural infection, or 8–12 days after vaccination with attenuated live canine adenovirus 1, corneal edema (*"blue eye"*) is occasionally observed (Plate 18-3B). Though clinically dramatic and alarming, especially after vaccination, the edema usually re-

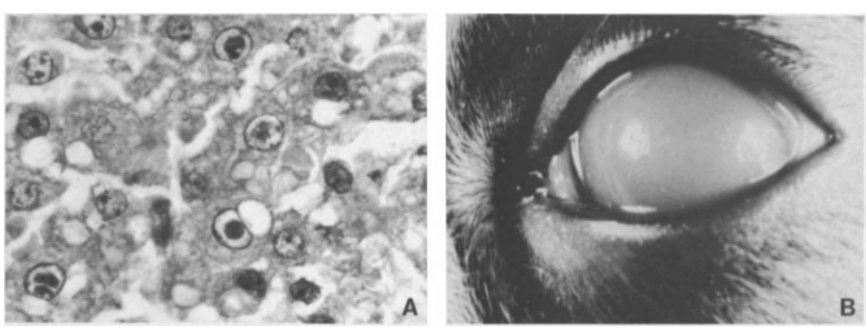

PLATE 18-3. *Canine adenovirus 1 infections. (A) Inclusion bodies within hepatocytes in peracute infectious canine hepatitis. (B) "Blue eye" in a pup 9 days after vaccination with canine adenovirus 1 vaccine. (B, courtesy Dr. L. E. Carmichael.)*

solves after a few days without consequence. The edema is due to virus–antibody complexes, deposited in the small blood vessels of the ciliary body, interfering with normal fluid exchange within the cornea. A similar pathogenesis underlies glomerulonephritis due to canine adenovirus 1. Infection of the kidney is associated with viruria, which is a significant mode for transmission.

Both killed and attenuated live canine adenovirus 1 vaccines are in general use. Attenuated live canine adenovirus 2 vaccines are also used. The antigenic relationship between canine adenoviruses 1 and 2 is sufficiently close for the canine adenovirus 2 vaccine to be cross-protective; it has the advantage that it does not cause corneal edema.

AVIAN ADENOVIRUS INFECTIONS

Adenoviruses commonly infect chickens and other avian species including quail, turkeys, ducks, geese, and pheasants. Initially, avian adenoviruses were considered to be nonpathogenic for domestic poultry, although they were lethal following inoculation of chick embryos. However, they are now recognized as a cause or contributing cause of a number of diseases of chickens, including respiratory disease, enteritis, inclusion body hepatitis, egg drop syndrome, atrophy of the bursa of Fabricius, and a hemorrhagic–aplastic anemia syndrome. Turkey adenoviruses cause respiratory disease, marble spleen disease, and enteritis in poults. Hepatitis in ducks may be caused by an adenovirus.

Although the recovery of the virus and the accompanying pathological changes, as seen in inclusion body hepatitis of chickens, suggest that adenoviruses play a specific role in the production of disease, these varied syndromes are difficult or impossible to reproduce experimentally with adenoviruses alone. There is a strong suspicion that the pathogenicity of adenoviruses is enhanced by immunodeficiency, particularly that caused by intercurrent infections with agents such as avian leukosis or infectious bursal disease viruses.

Adenoviruses persist as subclinical infections in breeding flocks and contaminate eggs and cell cultures used as substrate for the production of vaccines, hence they have caused concern as contaminants of avian live virus vaccines.

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CHAPTER 19

Herpesviridae

More than 80 herpesviruses have been characterized, and they have been found in insects, reptiles, and amphibia as well as in virtually every species of bird and mammal that has been investigated. At least one major disease of each domestic animal species except sheep is caused by a herpesvirus, including such important diseases as infectious bovine rhinotracheitis, pseudorabies, and Marek's disease.

Herpesvirus particles are fragile and do not survive well outside the body. In general, transmission requires close contact, particularly the kinds of physical contact that bring moist epithelial surfaces into apposition, e.g., coitus, or licking and nuzzling as between mother and offspring or members of a litter. In large, closely confined populations, such as are found in a cattle feedlot, multiple farrowing unit, cattery, or broiler chicken run, sneezing and short-distance aerosol spread are major modes of transmission.

The classification of viruses within the family *Herpesviridae* is complex and not yet fully resolved. Three subfamilies have been constructed: *Alphaherpesvirinae*, comprising the rapidly growing cytolytic viruses, *Be-*

taherpesvirinae, the slowly growing "cytomegalic" viruses, and *Gammaherpesvirinae,* viruses which grow in lymphocytes and may transform them to the malignant state. Many herpesviruses have not yet been allocated to a subfamily.

Herpesviruses survive from generation to generation by establishing latent infections, from which virus is periodically reactivated and shed, and in some betaherpesvirus and gammaherpesvirus infections, shedding may be continuous.

Many herpesviruses are highly host specific, but some alphaherpesviruses, such as pseudorabies virus and B virus, may affect a range of species.

PROPERTIES OF HERPESVIRUSES

The herpesvirus virion is enveloped and about 150 nm in diameter (Table 19-1). The DNA genome is wrapped around a fibrous spoollike core, the fibers of which are anchored to the inner side of the surrounding capsid. The capsid is an icosahedron, 100 nm in diameter, composed of 162 hollow capsomers: 150 hexamers and 12 pentamers (see Plate 19-1B). Surrounding the capsid is a layer of globular material, known as the "tegument," which is enclosed by a typical lipoprotein envelope, which carries the glycoprotein peplomers (Plate 19-1A). Because of the nature of the envelope, the virion is somewhat pleomorphic and can range in diameter from 120 to 200 nm.

The virion contains more than 20 proteins, of which 6 are present in the nucleocapsid, 2 being DNA-associated. The glycoproteins, of which there are at least five, are located in and may project as peplomers from the envelope. One of the peplomer glycoproteins possesses Fc receptor activity and it binds normal IgG. Other structural proteins are tegument-associated. Antigenic relationships are complex. There are some shared antigens within the family, but different species have distinct envelope glycoproteins, the antigenicity of which is stable.

TABLE 19-1
Properties of Herpesviruses

Enveloped virions, 120–200 nm (usually about 150 nm) in diameter
Icosahedral capsid with 162 capsomers
Peplomers 8 nm long in envelope
Linear dsDNA genome, 120–220 kbp
Replicates in nucleus; acquires envelope by budding through nuclear membrane
All species produce latent infections

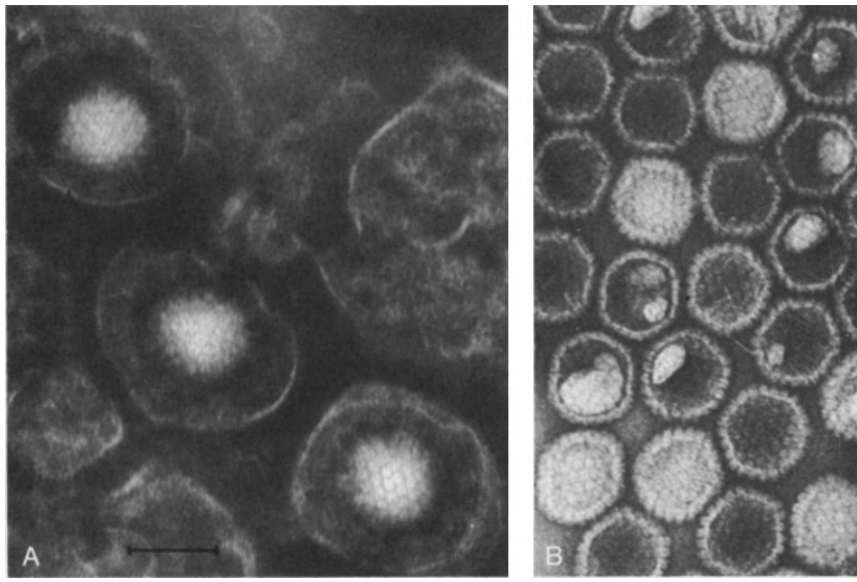

PLATE 19-1. Herpesviridae. *Negatively stained preparations of the prototype herpesvirus, herpes simplex virus type 1. (A) Enveloped particles. (B) Icosahedral capsids with 162 capsomers (bar = 100 nm). (Courtesy Dr. E. L. Palmer.)*

The herpesvirus genome consists of a linear dsDNA molecule which is infectious under appropriate experimental conditions. There is a remarkable degree of variation in the composition, size, and structure of herpesvirus DNAs. The percentage of guanine plus cytosine varies between 32 and 74% (for canine herpesvirus and pseudorabies virus, respectively), a range which far exceeds that found in the DNAs of all eukaryotes. Their size varies between 121 and 227 kbp for bovine mammillitis virus and human cytomegalovirus, respectively. Some aspects of the variation in structure of the DNAs of some alphaherpesviruses are illustrated in Fig. 19-1.

Physical maps based on ordering of restriction endonuclease fragments are available for many herpesviruses, and DNA fragment patterns are proving increasingly useful for the intratypic analysis of herpesviruses of particular species.

Classification

Subdivision of the family into subfamilies is based on biological properties. Classification into genera on the basis of the genome arrangement and serological reactivity has just begun.

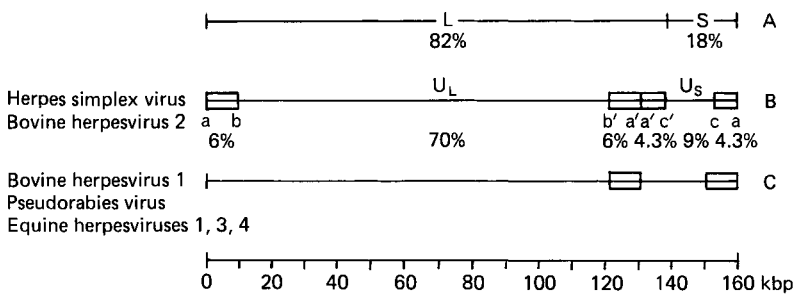

FIG. 19-1. *The DNA of alphaherpesviruses consists of two covalently linked components designated long (L) and short (S), which comprise approximately 82 and 18% of the genome, respectively (A). Both (B), one (C), or neither of these two components is bracketed by inverted repeat sequences. The nonrepeat, unique sequences are designated U_L and U_S. When present, the repeat sequences allow the unique regions to invert relative to the other such that isomers of the DNA are formed, and are found in equimolar amounts within a virus population. Where both U_L and U_S are bracketed by repeat sequences, four isomers are found. Where only U_S is bracketed by repeat sequences, two isomers are found. (B) Location and extent (percentage of DNA) in repeat sequences of herpes simplex virus and bovine herpesvirus 2. (C) Location of repeat sequences of equine herpesviruses 1, 3, and 4, pseudorabies virus, and bovine herpesvirus 1.*

Alphaherpesvirinae. The members of this family are classified on the basis of a moderately wide host range, rapid growth and lysis of infected cells, and capacity to establish latent infections primarily but not exclusively in nerve ganglia.

Betaherpesvirinae. Most members have a restricted host range. The replicative cycle is long and the infection progresses slowly in culture; cell lysis occurs several days after infection. Infected cells often become enlarged (cytomegalia). The virus can be maintained in latent form in secretory glands, lymphoreticular tissue, kidneys, and other tisses.

Gammaherpesvirinae. The host range is narrow. All members replicate in lymphoid cells, and some also cause cytocidal infections in epithelial and fibroblastic cells. Viruses in this subfamily are specific for either T or B lymphocytes, which are transformed to tumors. Latent virus is frequently demonstrable in lymphoid tissue.

VIRAL REPLICATION

Herpesvirus replication can be illustrated best by considering one of the well-studied alphaherpesviruses, such as herpes simplex virus (Fig.

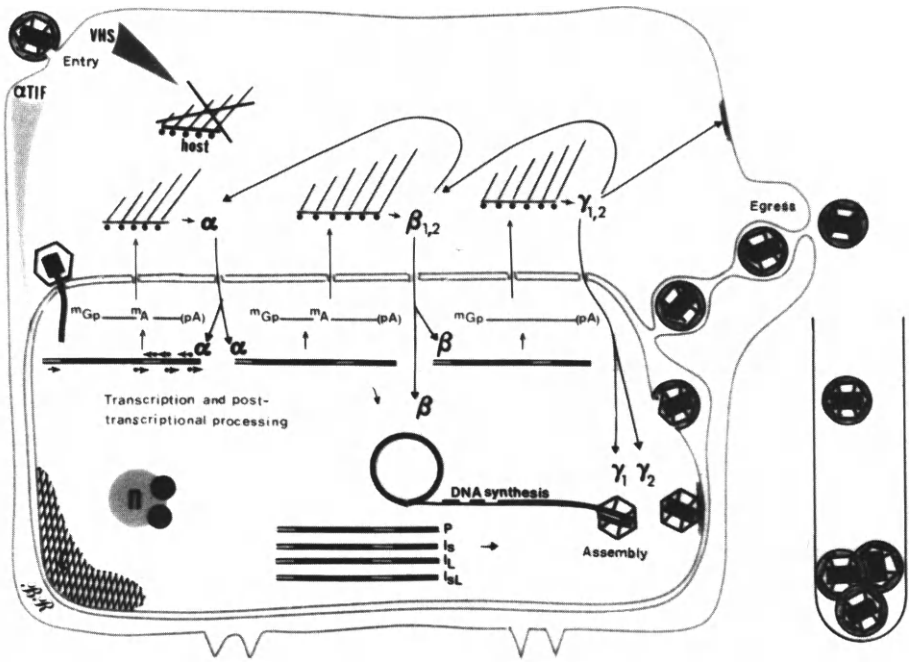

FIG. 19-2. *Schematic diagram of the major features in the replication of a herpesvirus. For explanation see text. (Courtesy Dr. B. Roizman.)*

19-2). Betaherpesviruses and gammaherpesviruses probably follow a similar pattern, but replicate more slowly. Following adsorption to host cell receptors via the glycoprotein peplomers of the envelope, the nucleocapsid penetrates to the cytoplasm either by fusion of the envelope to the cell membrane or via a phagocytic vacuole. A DNA–protein complex is then freed from the nucleocapsid and enters the nucleus.

At least three classes of mRNA, α, β, and γ, are transcribed by the cellular DNA-dependent polymerase II in a coordinated, regulated, and sequentially ordered cascade. Thus α (immediate early) RNAs, when appropriately processed to mRNAs, are translated to α proteins, which initiate transcription of β (early) mRNAs, whose translated products, β (early) proteins, suppress transcription of further α mRNAs. Viral DNA relication then commences, utilizing some of the α and β proteins as well as host cell proteins. The program of transcription then switches once again, and the resulting γ (late) mRNAs, which are transcribed from sequences situated throughout the genome, are translated into the γ proteins. More than 70 virus-coded proteins are made during the

cycle; many α and β proteins are enzymes, whereas most of the γ proteins are structural. Intricate controls must regulate expression at the level of both transcription and translation.

Viral DNA is replicated in the nucleus utilizing a "rolling circle" mechanism; circularization of the genome is brought about by bonding of the terminal sequences (see Fig. 1-3B). Newly synthesized DNA is spooled into preformed immature capsids. Host cell DNA, RNA, and protein synthesis declines concomitantly with viral biosynthesis and ceases 3–5 hours after infection.

Maturation involves the encapsidation of DNA into nucleocapsids and the association of nucleocapsids with altered areas of the inner layer of the nuclear membrane, followed by envelopment during the process of budding. Mature virions accumulate within vacuoles in the cytoplasm, and may be released slowly by vacuolar membrane fusion and exocytosis or by cytolysis. Virus-specific proteins, possibly similar to those that become associated with the nuclear membrane and determine sites for nucleoprotein binding, are also found in the plasma membrane, where they are involved in cell fusion, may act as Fc receptors, and are presumed to be targets for immune cytolysis. Each infected cell produces up to 10^5 virus particles.

Intranuclear inclusion bodies are characteristic of herpesvirus infections and can usually be found both in tissues from herpesvirus-infected animals and in appropriately fixed and stained cell cultures.

PATHOGENESIS AND IMMUNITY

Collectively and individually, herpesviruses are versatile pathogens. Transmission is generally associated with close contact of moist surfaces, but droplet infection is also common. Many alphaherpesviruses produce localized lesions, particularly of mucosal surfaces of the respiratory and genital tracts or the skin, that are characterized by the sequential production of vesicles, pustules and shallow ulcers which become covered by a pseudomembrane and heal after 10–14 days, usually without scar formation.

Generalized alphaherpesvirus infection, characterized by foci of necrosis in almost any organ or tissue, is seen when young animals (less than 3 months of age) are infected without the protection provided by maternal antibody. In older animals a mononuclear cell-associated viremia may result in transfer of virus across the placenta, leading to abortion; focal necrotic lesions are found throughout the fetus. Betaherpesviruses are associated with respiratory and generalized disease while gammaherpesviruses may produce tumors.

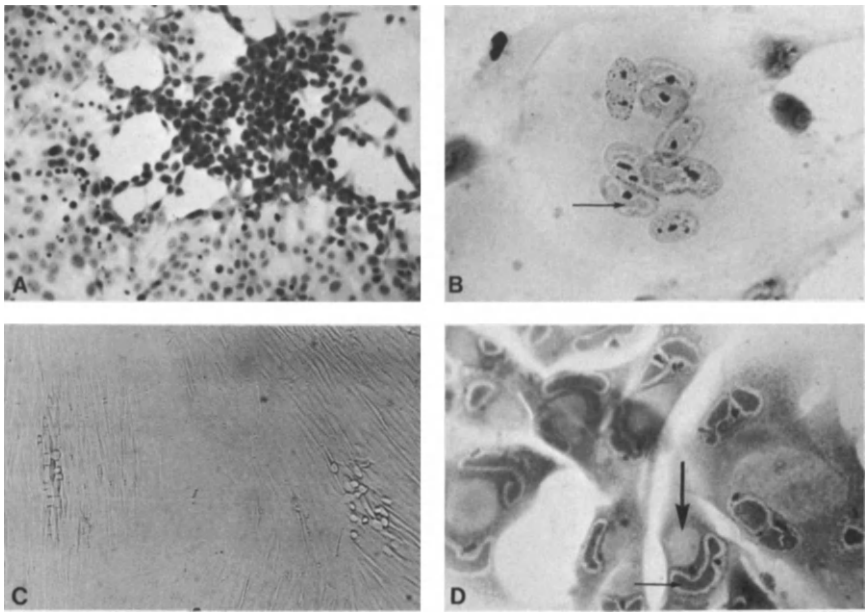

PLATE 19-2. *Cytopathic effects induced by herpesviruses. Alphaherpesvirus in (A) HEp-2 cells, showing early focal cytopathic effect (top right), and (B) kidney cells (H and E stain, ×228), showing multinucleated giant cell containing acidophilic intranuclear inclusions (arrow). Cytomegalovirus in (C) embryo fibroblasts (unstained, ×35), showing two foci of slowly developing cytopathic effect, and (D) (H and E stain, ×220), showing giant cells with acidophilic inclusions in the nuclei (small arrow) and cytoplasm (large arrow), the latter being characteristically large and round. (Courtesy I. Jack.)*

Persistent infection with periodic or continuous shedding is believed to occur in all animal herpesvirus infections. In alphaherpesvirus infections, multiple copies of viral DNA are demonstrable either as episomes or integrated into host cell chromosomal DNA of latently infected neurons. Reactivation is periodic and may be associated with stress, such as is occasioned by intercurrent disease, shipping, cold, or crowding. Shedding of virus in nasal, oral, or genital secretions provides the source of infection for other animals, including mother to offspring. In domestic animals reactivation is frequently subclinical, partly because the sites involved, on nasal or genital mucosae, are not readily observed. Some betaherpesviruses and gammaherpesviruses cause a persistent, cell-associated viremia and appear to be shed continuously via epithelial surfaces.

Both humoral and cell-mediated immune responses are generated

during herpesvirus infections. Neutralizing antibody is primarily directed at envelope glycoproteins, and reaches a maximum titer about 14 days after infection. Early neutralizing antibody can be demonstrated only in the presence of complement; later complement is not required but its presence enhances the titer four- to eightfold. Following primary infection, antibody titers may drop to nondetectable levels, but with advancing age titers are generally high, presumably as a consequence of recurrent infections. In adult animals antibody titers tend not to rise in association with syndromes such as abortion or encephalitis.

Viral antigens expressed on the surface of infected cells are targets for cell-mediated immune lysis. Although both antibody and cell-mediated immune responses develop following primary infection, they are without effect on virus in latently infected neurons, or in preventing its egress via sensory nerve pathways to sites of epithelial infection. However, in recurrent episodes viral spread at epithelial sites is restricted to contiguous cells, so that recurrent lesions are usually milder than the primary lesion.

Herpesvirus infections are much more severe following primary infection of young animals that have lost or lack maternally derived antibodies.

DIAGNOSIS

Rapid diagnostic methods include electron microscopy of vesicular fluid or scrapings and immunofluorescent staining of smears or tissue sections. Viral isolation and characterization provide a definitive diagnosis. Herpesviruses are most readily grown in cell cultures derived from their natural host species. With adaptation, the host range of a particular herpesvirus in cell culture can be extended, although for some betaherpesviruses, only cells from the natural host species are permissive. Alphaherpesviruses produce a rapid cytopathic effect, some species producing syncytia, and characteristic eosinophilic intranuclear inclusion bodies are readily demonstrated in infected cells. Betaherpesviruses and gammaherpesviruses are slowly cytopathogenic in cell culture but produce similar intranuclear inclusion bodies.

TREATMENT

Herpesvirus diseases are the only viral infections for which it is necessary to mention the possible use of chemotherapy, although this would be feasible only for individual valuable companion animals. Antiher-

TABLE 19-2
Herpesviruses of the Subfamily Alphaherpesvirinae *That Cause Diseases in Domestic Animals*

Virus	Disease
Bovine herpesvirus 1	Infectious bovine rhinotracheitis; infectious pustular vulvovaginitis; infectious balanoposthitis; abortion
Bovine herpesvirus 2	Bovine mammillitis; pseudolumpyskin disease
Caprine herpesvirus 1	Conjunctivitis, respiratory disease
Pseudorabies virus	Pseudorabies
Equine herpesvirus 1	Abortion
Equine herpesvirus 4	Rhinopneumonitis
Equine herpesvirus 3	Coital exanthema
Canine herpesvirus 1	Hemorrhagic disease of pups
Feline herpesvirus 1	Feline viral rhinotracheitis
Avian herpesvirus 1	Infectious laryngotracheitis
Duck herpesvirus 1	Duck plague
B virus	Inapparent in monkeys; paralysis in humans

petic drugs such as 5-iodo-2′-deoxyuridine (Iodouridine), cytosine arabinoside (Cytarabine), and acycloguanosine (Acyclovir) represent a progression in the development of increasingly effective drugs for the treatment of human herpesvirus diseases. Such drugs are not used or seriously contemplated for use in veterinary medicine, with the possible exception of the treatment of feline herpesvirus 1 corneal ulcers.

DISEASES CAUSED BY ALPHAHERPESVIRUSES

Viruses of this subfamily cause infections of veterinary importance in a wide range of animals (Table 19-2).

Infectious Bovine Rhinotracheitis–Infectious Pustular Vaginitis

The synonyms for infection with bovine herpesvirus 1 (infectious pustular vulvovaginitis, infectious pustular balanoposthitis, coital exanthema, and infectious bovine rhinotracheitis) identify this virus as a cause of genital and respiratory disease, but they do not fully encompass the spectrum of diseases it can cause in cattle, which include, in addition, conjunctivitis, abortion, encephalitis, enteritis, and a generalized disease of newborn calves.

A genital disease of cattle called coital exanthema was described in

Europe in the nineteenth century. The availability of surplus grain and a growing demand for grain-finished beef in the United States in the 1950s resulted in a rapid expansion of feedlots, particularly in California and Colorado. Prominent among a variety of new disease syndromes recognized in these cattle was an acute respiratory disease, "infectious bovine rhinotracheitis," from which a herpesvirus was isolated. Comparison of the pathogenic and serological properties of herpesviruses isolated from infectious bovine rhinotracheitis and from infectious pustular vulvovaginitis in dairy cattle in the eastern United States showed that the viruses were indistinguishable. Bovine herpesvirus 1 and the diseases it causes are now known to occur worldwide.

Clinical Features of Genital Disease. Infectious pustular vulvovaginitis is most commonly recognized in dairy cows. Affected cows develop fever, depression, anorexia, and stand apart, often with the tail held away from contact with the vulva; micturition is frequent and painful. The vulval labia are swollen, there is a slight vulval discharge, and the vestibular mucosa is reddened with many small pustules. Adjacent pustules usually coalesce to form a fibrinous pseudomembrane which covers an ulcerated mucosa. The acute stage of the disease lasts 4–5 days and uncomplicated lesions usually heal by 10 to 14 days. Many cases are subclinical or go unnoticed.

In bulls, the lesions of infectious balanoposthitis and the clinical course of disease are similar. Where lesions are extensive and acute (Plate 19-3), there is reluctance or complete refusal to serve. Semen from

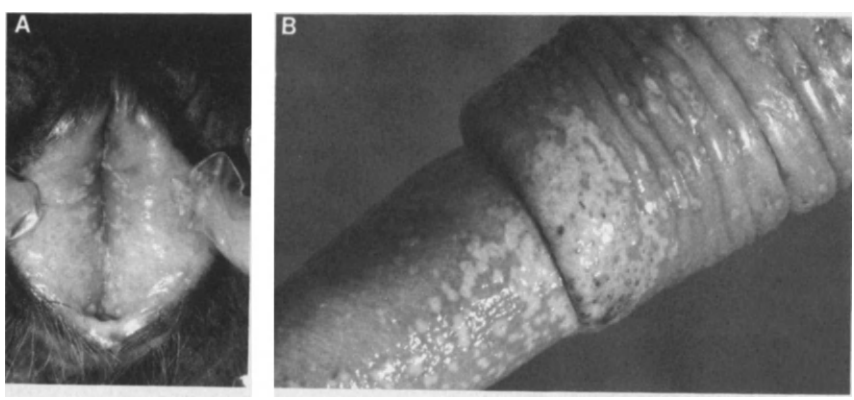

PLATE 19-3. *Genital disease caused by bovine herpesvirus 1. (A) Infectious pustular vulvovaginitis. (B) Infectious pustular balanoposthitis. [A, from D. F. Collings* et al., Vet. Rec. **91,** *214 (1972); B, from M. J. Studdert* et al., Am. J. Vet. Res. **105,** *303 (1964).]*

recovered bulls may be contaminated with bovine herpesvirus 1 as a result of periodic shedding of virus.

Despite the acute and sometimes severe nature of genital disease due to bovine herpesvirus 1, it is not a major cause of infertility or reproductive wastage. Cows may conceive to the service or artificial insemination from which they acquire infectious pustular vulvovaginitis, and pregnant cows that develop the infection rarely abort.

Clinical Features of Respiratory Infection. Infectious bovine rhinotracheitis occurs as a subclinical, mild, or severe disease. Morbidity approaches 100% and mortality may reach 10%, particularly if complications occur. Initial signs include fever, depression, inappetance, and a profuse nasal discharge, initially serous and later mucopurulent. The nasal mucosa is hyperemic, and lesions within the nasal cavity, which may be difficult to see, progress from focal pustular necrosis to large areas of hemorrhagic, ulcerated mucosa which is covered by a cream-colored diphtheritic membrane. The breath may be fetid. Dyspnea, mouth breathing, salivation, and a deep bronchial cough are common. Acute uncomplicated cases last 5–10 days.

Unilateral or bilateral conjunctivitis, often with profuse lacrimation, is a common clinical sign in cattle with infectious bovine rhinotracheitis but may occur in a herd as an almost exclusive clinical sign. Gastroenteritis may occur in adult cattle infected with bovine herpesvirus 1 and is a prominent finding in the generalized disease of neonatal calves, which is often fatal. Abortion may occur at 4 to 7 months gestation, after infection with bovine herpesvirus 1. The virus has also been reported to be associated with mastitis, in both Europe and North America.

Pathogenesis and Pathology. Genital disease may result from coitus or artificial insemination, although some outbreaks, particularly in dairy cows, may occur in the absence of coitus. Respiratory disease and conjunctivitis result from aerosol transmission. Within the animal, dissemination of the virus from the initial focus of infection probably occurs via a cell-associated viremia. Encephalitis is believed to result from direct neural spread from the nasal cavity, pharynx, and tonsils via the maxillary and mandibular branches of the trigeminal nerve. Lesions initially occur in the midbrain and later involve the entire brain.

Lifelong latent infection with periodic virus shedding is the usual consequence of bovine herpesvirus 1 infection and it is assumed that the sciatic and trigeminal ganglia are sites of latency following genital and respiratory disease, respectively. The administration of corticosteriods results in reactivation of the virus, and has been used as a means of detecting and eliminating carrier bulls in artificial insemination centers.

The lesions are initially focal areas of epithelial cell necrosis in which there is ballooning of epithelial cells; typical herpesvirus inclusions may be present in nuclei at the periphery of necrotic foci. There is an intense inflammatory response. Gross lesions are not observed in aborted fetuses, but microscopic necrotic foci are present in most tissues and the liver is consistently affected.

Epidemiology and Control. Genital and respiratory disease are rarely diagnosed in the same herd at the same time. Infectious bovine rhinotracheitis is an uncommon disease in range cattle, but is of major significance in feedlots. In cattle not in feedlots, the incidence of antibody varies between 10 and 35%. Primary infection often coincides with transport and introduction to a feedlot of young, fully susceptible cattle from diverse sources. Adaptation from range to feedlot conditions, and dietary changes, including the high-protein diet, contribute to a stressful environment which may potentiate disease, although in well-managed operations these factors are minimized, with a corresponding reduced incidence of severe disease. The damaged necrotic mucosa provides a substrate for bacterial infection in debilitated, stressed cattle, and contributes to the complex syndrome called "shipping fever" (see Chapter 10).

Iatrogenic transmission has occurred among bulls in artificial insemination centers when a common sponge has been used for washing the prepuce prior to semen collection. Virus may also be spread by artificial insemination.

A wide range of bovine herpesvirus 1 vaccines (mostly attenuated live-virus vaccines) are extensively used, alone or in formulations containing other viral vaccines. In 1983, 72 million doses of bovine herpesvirus 1 vaccine were manufactured in the United States, and such vaccines are also widely used throughout Europe. Although they do not prevent infection, vaccines contribute to a significantly reduced incidence of disease.

Bovine Herpesvirus 1-Like Infections in Other Ruminant Species

Alphaherpesviruses antigenically related to bovine herpesvirus 1 have been isolated from several ruminant species including red deer, reindeer, and buffalo. Clinical disease in these species has not been described. Very few of these viruses have been well characterized, but restriction endonuclease analysis of their DNAs suggests that each is species-specific and that these viruses do not commonly cross species barriers. Bovine herpesvirus 1 has been isolated occasionally from cases of vaginitis and balanitis in swine and from stillborn piglets.

Bovine Mammillitis and Pseudolumpyskin Disease

Two clinical forms of bovine herpesvirus 2 infections are known: lesions localized to the teats, occasionally spreading to the udder (bovine mammillitis) and a generalized skin disease (pseudolumpyskin disease). Bovine herpesvirus 2 was first isolated in 1957 from cattle in South African with a generalized lumpyskin disease. The disease was mild and its major significance lay in the need to differentiate it from a more serious lumpyskin disease found in South Africa and caused by a poxvirus (see Chapter 21). A similar herpesvirus was isolated from cattle with extensive erosions of the teats in Africa, and subsequently from similar lesions in cattle in many countries of the world.

Bovine herpesvirus 2 is antigenically related to human herpes simplex virus, and the DNAs of these viruses show 15% homology, compared with less than 6% homology between bovine herpesvirus 2 and bovine herpesvirus 1.

Clinical Features. Pseudolumpyskin disease has an incubation of 5 to 9 days and is characterized by a mild fever, followed by the sudden appearance of skin nodules—a few scattered lumps, or, in severe cases, many nodules on the face, neck, back, and perineum. The nodules have a flat surface with a slightly depressed center, and involve only the superficial layers of the epidermis, which undergo necrosis. Within 7 to 8 days the local swelling subsides, and healing, without scar formation, is complete within a few weeks.

In many countries, the generalized skin disease is not seen and bovine herpesvirus 2 is recognized only as a cause of mammillitis, but experimentally, virus isolated from cases of mammillitis can cause generalized skin disease. Lesions usually occur only on the teats (Plate 19-4), but in severe cases most of the skin of the udder may be affected. Occasionally heifers may develop fever, coinciding with the appearance of lesions. Milk yield may be reduced by as much as 10% as a result of difficulty in milking the affected cows and intercurrent mastitis.

Pathogenesis. The distribution of lesions in mammillitis suggests local spread. The generalized distribution of lesions in pseudolumpyskin disease suggests viremic spread, but viremia is difficult to demonstrate.

Diagnosis. The benign nature of pseudolumpyskin disease, the characteristic central depression on the surface of the nodules, the superficial necrosis of the epidermis, and the shorter course of the disease are helpful in differentiating the condition from true lumpyskin disease, in countries where both occur.

The clinical differentiation of the various conditions that affect the teats of cattle (see Plate 21.2) can be difficult; other virus diseases caus-

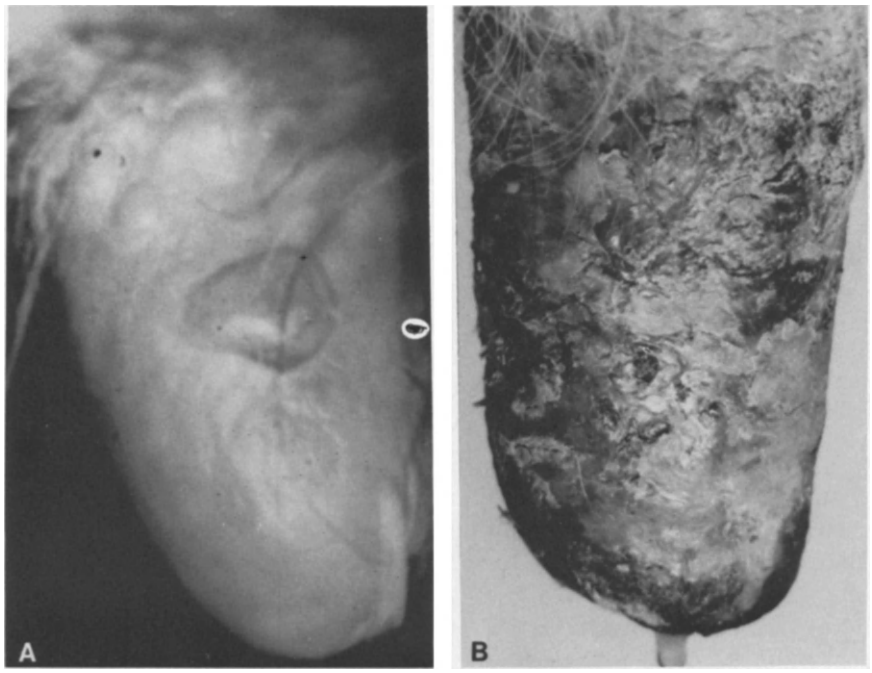

PLATE 19-4. *Bovine mammilitis caused by bovine herpesvirus 2. (A) Early vesicle. (B) Late crusted lesion. Compare with lesions of teats caused by poxviruses in Plate 21-2.*

ing teat lesions are warts, cowpox, pseudocowpox, vesicular stomatitis, and foot-and-mouth disease. For this reason it is advisable to examine the whole herd, as a comparison of the early developmental stages helps considerably in the diagnosis. Advanced lesions are often similar, irrespective of the cause.

Epidemiology. Pseudolumpyskin disease occurs most commonly in southern Africa, in moist low-lying areas, especially along rivers, and has its highest incidence in the summer months and early fall. Susceptible cattle cannot be infected by placing them in contact with diseased cattle if housed in insect-proof accommodation. It is therefore assumed that mechanical transmission of the virus by arthropods occurs, but attempts to identify the vectors have failed. Buffalo, giraffe and other African wildlife may be naturally infected with bovine herpesvirus 2.

Although milking machines were initially thought to be responsible for transmission of mammillitis in dairy herds, there is evidence of arthropod transmission. The infection may spread rapidly through a herd, but in some outbreaks disease is confined to newly calved heifers or

heavily pregnant cattle. Serological surveys suggest that many infections are subclinical.

Infections with Caprine Herpesvirus 1

Herpesviruses originally considered to be related to bovine herpesvirus 1 have been isolated from goats in association with a variety of clinical signs, including conjunctivitis and disease of the respiratory, digestive, and genital tracts, including abortion, in several parts of the world. The caprine virus is antigenically related to bovine herpesvirus 1 in a one-way cross-reaction. Caprine herpesvirus 1 is not infectious for cattle or for lambs, and its restriction endonuclease DNA fragment pattern is quite different from that of bovine herpesvirus 1.

Pseudorabies

This disease (synonym, Aujeszky's disease) is caused by an alphaherpesvirus and is primarily a disease of swine, which serve as a reservoir and the principal source of natural infection for a diverse range of secondary hosts, which include cattle, sheep, goats, dogs, and cats, and many feral species. The diverse host range is also demonstrated *in vitro;* cell cultures derived from almost any animal species support the replication of pseudorabies virus.

Pseudorabies is enzootic in swine in most parts of the world except Japan and Australia. The eradication of hog cholera from the United States and the United Kingdom (see Chapter 25) has brought pseudorabies to greater prominence, and it is now economically the most important viral disease of swine, causing multimillion-dollar losses each year in countries where it is found.

Clinical Features in Swine. In herds in which the disease is endemic, reactivation of virus occurs without obvious clinical signs, but the spread of the virus within a nonimmune herd may be rapid, the consequences of primary infection being markedly influenced by age and, in sows, pregnancy. Pruritus, which is such a dominant feature of the disease in secondary hosts such as cattle, is rare in swine.

Pregnant Sows. In nonimmune herds, up to 50% of pregnant sows may abort over a short period of time, due to rapid spread of infection from an index case or carrier. Infection of a sow before day 30 of gestation results in death and resorption of embryos—after that time, in abortion. Infection in late pregnancy may terminate with the delivery of a mixture of mummified, macerated, stillborn, weak, and normal swine, and some of these pregnancies may be prolonged for 2 or 3 weeks

beyond the normal gestation period. Up to 20% of aborting sows are infertile on the first subsequent breeding but do eventually conceive.

Piglets. Mortality rates among piglets born to nonimmune dams depend somewhat on their age, but approach 100%. Maternal antibody is protective, and disease in piglets born to recovered or vaccinated sows is greatly diminished in severity, with recovery the usual outcome.

Weaned, Growing, and Mature Swine. The incubation period is about 30 hours. In younger animals the course is typically about 8 days but may be as short as 4 days. Initial signs include sneezing, coughing, and moderate fever (40°C) which increases up to 42°C in the ensuing 48 hours. There is constipation during the fever, the feces are hard and dry, and vomiting may occur. Slight tremors of the tail and flank muscles may be observed by 96 hours. Pigs are listless, depressed, and tend to remain recumbent. By the fifth day central nervous system signs are more obvious; there is incoordination and pronounced muscle spasm, circling, and intermittent convulsions accompanied by excess salivation. By the sixth day swine are unable to stand; they become moribund and die within 12 hours. In mature swine the mortality rate is low, usually less than 2%, but there may be significant weight loss and poor growth rates after recovery.

Clinical Features in Secondary Hosts. Important secondary hosts include cattle ("mad itch"), dogs, and cats ("pseudorabies"). Disease in secondary hosts in sporadic and occurs where there is direct or indirect contact with swine. Infection is usually by ingestion, less commonly inhalation, and possibly via minor wounds. In cattle the dominant clinical sign is intense pruritus. Particular sites, often on the flanks or hindlimbs, are licked incessantly, there is gnawing and rubbing such that the area becomes abraded. Cattle may become frenzied. There is progressive involvement of the central nervous system; following the first signs, the course leading to death may be as short as a few hours and is never longer than 6 days.

In dogs, the frenzy associated with intense pruritus, paralysis of the jaws and pharynx accompanied by drooling of saliva, and plaintive howling simulates true rabies; however, there is no tendency for the dogs to attack other animals. In cats, the disease may progress so rapidly that pruritus is not observed.

Pathogenesis and Pathology. Following primary oral or intranasal infection of swine, virus replicates in the oropharynx. There is no viremia during the first 24 hours and it is difficult to demonstrate at any time. However, within 24 hours virus can be isolated from various cranial nerve ganglia and the medulla and pons, to which virions have traveled

via the axoplasm of the cranial nerves. Virus continues to spread within the central nervous system; there is ganglioneuritis at many sites including those controlling vital functions.

The relative lack of gross lesions even in young swine is notable. Tonsillitis, pharyngitis, tracheitis, rhinitis, and esophagitis may be evident. Occasionally small necrotic foci may be found in the liver and spleen. Microscopically the principal findings in both swine and secondary hosts are in the central nervous system. There is a diffuse nonsuppurative meningoencephalitis and ganglioneuritis, marked perivascular cuffing, and focal gliosis associated with extensive necrosis of neuronal and glial cells. There is a correlation between the site and severity of clinical signs and the histological findings. Typical intranuclear herpesvirus inclusions are rarely found in the lesions in swine.

Some swine that have recovered from pseudorabies may shed virus continuously in their nasal secretions. Others from which virus cannot be isolated by conventional means may yield virus when explant cultures of tonsillar tissue are made, and pseudorabies virus DNA can be demonstrated in the trigeminal ganglia of recovered swine by *in situ* DNA hybridization.

Diagnosis. The history and clinical signs will often suggest the diagnosis. Virus isolation and serum neutralization tests are used for confirmation, but fluorescent-antibody staining of frozen tissue sections is more rapid than viral isolation and is often used. More rapid serological tests such as ELISA and passive hemagglutination have been developed; ELISA has been approved as a standard test in several countries.

Epidemiology and Control. The pig is the primary host and reservoir for pseudorabies virus, and the virus causes a uniformly fatal disease when transmitted to a wide variety of secondary hosts. Virus is shed in the saliva and nasal discharges of swine, so that licking, biting, and aerosols could result in transmission. Virus is not shed in the urine or feces. Contamination of livestock feed or ingestion of infected carcasses by swine is common, and ingestion of virus-contaminated material including pork is probably the most common source of infection for secondary hosts. Rats may contribute to farm-to-farm transfer, and sick or dead rats and other feral animals are probably the source of infection for dogs and cats. In the United States raccoons, because of their scavenging habits, have received particular attention, but their role in contributing to the natural spread of pseudorabies virus has not been defined.

Management practices influence epidemiological patterns of infection and disease in swine. Losses from severe overt disease occur when

nonimmune pregnant sows or swine less than 3 months old, born to nonimmune sows, are infected. Such a pattern is likely to be seen when virus is newly introduced into a herd or unit within a farm. When breeding sows are immune with adequate antibody levels, overt disease in their progeny is not observed or is greatly reduced. Where breeding and growing–finishing operations are conducted separately, significant losses from pseudorabies occur when weaned swine from several sources are brought together in the growing–finishing unit, but the disease in these older swine is less severe than that in piglets. If care is taken to prevent the entry of pseudorabies, the move toward complete integration of swine husbandry, "farrow to finish" operations, provide an ideal situation by which to produce and maintain pseudorabies-free herds and thus avoid the costs of disease losses and the problems associated with vaccination.

Vaccination of swine in areas where the virus is endemic and spreading can reduce losses, both attenuated live-virus and inactivated vaccines being used. These do not prevent infection or the establishment of latent infection by the wild-type virus. Vaccination of secondary hosts is rarely undertaken because of the sporadic incidence of the disease.

There is a growing consensus that pseudorabies virus can be eradicated from industrialized countries or parts of them, and a national eradication program has been established in the United Kingdom. Regional programs operate in some states of the United States, where the interstate movement of swine is allowed only after a negative serological test is obtained, carried out following prescribed protocols in certified laboratories.

Equine Alphaherpesvirus Infections

Three alphaherpesviruses, designated 1, 3, and 4, have been identified as causes of diseases in horses. Until recently, equine herpesvirus 1 and equine herpesvirus 4 were thought to be one virus, equine herpesvirus 1, since both cause rhinopneumonitis and abortion. It is now clear that they are distinct viruses; their DNAs show less than 20% homology, but they are antigenically related, as demonstrated by a variety of serological procedures including neutralization tests.

Equine herpesvirus 1 is the major cause of equine abortion, which may frequently occur as an epizootic ("abortion storm"), but occasionally causes rhinopneumonitis. It is also recognized as a cause of perinatal foal mortality, and some strains produce encephalitis, either as a sporadic or an epizootic disease.

The acute upper respiratory disease usually observed in epizootic

form in foals during the first year or two of life is most often caused by equine herpesvirus 4, which has also been recovered from sporadic cases of abortion.

Equine Abortion Caused by Equine Herpesvirus 1

Abortion may occur as early as the fourth month of gestation, although most occur during the last 4 months. It occurs without premonitory signs and there are usually no complications. The fetus is born dead, although foals born close to term may survive for a few hours.

Perinatal infection may result in a fatal generalized disease in which respiratory distress due to interstitial pneumonia is the dominant clinical feature. Encephalitis occurs sporadically or as epizootics, usually in association with respiratory disease or abortion. Clinical signs vary from mild ataxia to complete recumbency with forelimb and hindlimb paralysis, leading to death.

Pathology. In fetuses aborted before 6 months there is diffusely scattered cell necrosis with inclusion bodies and a lack of an inflammatory cell response. Gross lesions are sometimes present in fetuses aborted after 6 months and may include small necrotic foci in the liver. Characteristic microscopic lesions include bronchiolitis, pneumonitis, severe necrosis of splenic white pulp, and focal hepatic necrosis, accompanied by a marked inflammatory cell response. Typical herpetic intranuclear inclusion bodies are readily demonstrated in these lesions.

Only certain strains of equine herpesvirus 1 cause encephalitis, which is characterized by vasculitis leading to thrombosis and hypoxic degeneration of adjacent neural tissue. In contrast to alphaherpesvirus encephalitis in other species, it is usually difficult or impossible to isolate virus from neural tissues; the vasculitis may be produced by virus–antibody complexes.

Epidemiology. Abortion, perinatal mortality, and, less commonly, encephalitis affecting up to 25% of horses in a herd may follow the occurrence of an index case, usually in a recently introduced horse. Equine herpesvirus 1 is the cause of most abortions, including all abortion storms, but equine herpesvirus 4 may cause sporadic abortions.

Equine Rhinopneumonitis

Acute respiratory disease of foals from about weaning time to yearling age is very common and occurs worldwide. Of the several viruses known to cause acute respiratory disease of foals, the most important in equine herpesvirus 4.

Clinical Features. Acute respiratory disease due to equine herpesvirus 4 occurs mainly in foals over 2 months old, weanlings, and yearlings. There is fever, anorexia, and a profuse serous nasal discharge which later beomes mucopurulent. Most affected foals recover completely, and mild or subclinical infections are common. More severe disease including bronchopneumonia and death may occur when there is crowding, stress, poor hygiene, and secondary infection.

Epidemiology and Control. The source of virus is thought to be from older horses in which inapparent virus shedding occurs, following reactivation of the latent virus.

Both inactivated and attenuated live equine herpesvirus 1 vaccines are used to control both respiratory disease and abortion, but their efficacy is doubtful, particularly in relation to abortion.

Equine Coital Exanthema

A disease that was probably equine coital exanthema has long been known, but its causative agent was shown to be an alphaherpesvirus (equine herpesvirus 3) as recently as 1968. Equine herpesvirus 3 shows no serological cross-reactivity with other equine herpesviruses by neutralization tests, but shares antigens with equine herpesvirus 1. Equine herpesvirus 3 grows only in cells of equine origin and produces large plaques. Though rapidly cytopathogenic, the virus tends to remain cell-associated.

Equine coital exanthema is an acute, usually mild disease characterized by the formation of pustular and ulcerative lesions on the vaginal and vestibular mucosa, on the skin of the penis, prepuce, and the perineal region, especially of the mare, and occasionally on the teats, lips, and the respiratory mucosa. The incidence of antibody in sexually active horses is much higher (>50%) than the reported incidence of clinical disease. The incubation period may be as short as 2 days and in uncomplicated cases healing is usually complete by 14 days. Where the skin of the vulva, penis, and prepuce is black, white depigmented spots mark for life the site of earlier lesions and identify potential carriers.

Although genital lesions may be extensive, there are no systemic signs and, unless the affected areas are carefully examined, cases are readily missed. Abortion or infertility are not associated with equine herpesvirus 3 infection; indeed mares usually conceive to the service in which they acquire the disease, although abortion occurs following experimental *in utero* inoculation.

Affected stallions show decreased libido and the presence of the disease may seriously disrupt breeding schedules. Recurrent disease is more likely to occur when stallions are in frequent use. Management of

the disease consists of the removal of stallions from service and symptomatic treatment.

Equine herpesvirus 3 can cause subclinical respiratory infection in yearling horses and has been isolated from vesicular lesions on the muzzles of foals in contact with infected mares.

Hemorrhagic Disease of Pups

Canine herpesvirus 1 was first recognized in the United States in 1965 as the cause of a highly fatal, generalized hemorrhagic disease of pups under 4 weeks of age. The highly fatal disease in pups is rare, and the prevalence of the virus, based on antibody surveys, is low (<20%). It probably occurs worldwide. In sexually mature dogs canine herpesvirus 1 causes genital disease, although this is rarely diagnosed clinically.

Clinical Features. The incubation period varies from 3 to 8 days and in fatal disease the course is brief, 1–2 days. Signs include painful crying, abdominal pain, anorexia, and dyspnea. In older dogs there may be vaginal or prepucial discharge, and on careful examination a focal nodular lesion of the vaginal and penile and prepucial epithelium. The virus may also cause respiratory disease and may be part of the "kennel cough" syndrome.

Pathogenesis and Pathology. Pups born to presumably seronegative bitches are infected oronasally either from their dam's vagina, or from other infected dogs. Pups less than 4 weeks old which become hypothermic develop the generalized, often fatal disease. There is a cell-associated viremia followed by viral replication in blood vessel walls. The optimal temperature for viral replication is about 33°C, i.e., the temperature of the genital and upper respiratory tracts. At birth the pup is relatively immature and the hypothalamic thermoregulatory centers are not fully operative until about 4 weeks of age. Accordingly, in the context of canine herpesvirus 1 infection, the pup is critically dependent on ambient temperature and maternal contact for the maintenance of its normal body temperature. The more severe the hypothermia the more severe and rapid is the course of the disease.

The gross findings in pups are frequently dramatic. Large ecchymotic hemorrhages are particularly obvious in the kidney, adrenal, and gastrointestinal tract. Microscopically they are seen as necrotic foci; however, not all such necrotic foci are marked by gross hemorrhage. Typical herpesvirus inclusions are found.

Diagnosis. This sporadic disease is rarely diagnosed during life. The gross postmortem findings, particularly the ecchymotic hemorrhages of the kidney and gastrointestinal tract, are characteristic. Inclusion bodies

may be present in liver cells, and the causative virus can be readily isolated in canine cell cultures.

Control. The low incidence of severe disease in pups and the mild nature of infections in older dogs have not warranted the development of vaccines. Losses may be prevented or arrested if the ambient temperature minimizes the risk of hypothermia. Raising the body temperature early in the course of infection may have therapeutic value.

Feline Herpesvirus Diseases

First isolated in 1957, feline herpesvirus 1 is one of the two common causes of acute respiratory disease of kittens. About half of the cats with respiratory disease will have feline herpesvirus infection, about half calicivirus infection (see Chapter 24), and a few *Chlamydia psittaci* infection. The incidence of feline herpesvirus antibody in colony cats is over 70%, while for household cats the figure is less than 50%. All species of the family Felidae are believed to be susceptible.

Feline herpesvirus 1 causes acute disease of the upper respiratory tract in the first year or so of life. After an incubation period of 24 to 48 hours there is a sudden onset of bouts of sneezing, coughing, profuse serous nasal and ocular discharges, frothy salivation, dyspnea, anorexia, weight loss, and fever. Occasionally there may be ulcers on the tongue. Keratitis associated with punctate corneal ulcers are common. In fully susceptible kittens up to 4 weeks old the extensive rhinotracheitis and an associated bronchopneumonia may be fatal.

Pregnant queens may abort, although there is no evidence that the virus crosses the placenta and fatally infects fetuses, and virus has not been isolated from aborted placenta or fetuses; abortion is thought to be secondary to fever and toxemia. Infection of cats over 6 months of age is likely to result in mild or subclinical disease.

Clinically, the acute disease is very similar to that caused by caliciviruses. Profuse frothy salivation and corneal ulcers suggest feline herpesvirus infection, while ulcers of the tongue, palate, and pharynx are more frequently encountered in calicivirus infections.

There is necrosis of epithelia of the nasal cavity, pharynx, epiglottis, tonsils, larynx, trachea, and, in extreme cases in young kittens, a bronchopneumonia. Typical intranuclear inclusion bodies may be detected if death occurs within 7 to 9 days after infection.

Inactivated and attenuated live-virus vaccines are used for the control of infections due to feline herpesvirus 1; they reduce disease but do not prevent infection.

Avian Infectious Laryngotracheitis

Identified as a specific viral disease of chickens in the United States in 1926, infectious laryngotracheitis occurs among chickens worldwide. Rarely, the etiological agent, avian herpesvirus 1, is recognized as a cause of disease in other avian species. Strains of the virus vary considerably in virulence.

Clinical Features. Chickens of all ages are susceptible, but disease is most common in those aged 4–18 months. After an incubation period of 2 to 8 days, mild coughing and sneezing are followed by nasal and ocular discharge, dyspnea, loud gasping and coughing, and depression. In severe cases the neck is raised and the head extended during inspiration—"pump handle respiration." Head shaking with coughing is characteristic and may be associated with expectoration of bloody mucus and frank blood which appear on the beak, face, and feathers. Morbidity approaches 100% and for virulent strains the mortality may be 50–70%—for strains of low virulence, about 20%. Strains of low virulence are associated with conjunctivitis, ocular discharge, swollen infraorbital and nasal sinuses, and lowered egg production.

Pathology and Pathogenesis. There is severe laryngotracheitis characterized by necrosis, hemorrhage, ulceration, and the formation of diphtheritic membranes. The latter may form a second tube for the length of the trachea, greatly restricting, and in some cases totally occluding, air flow. The extensive diphtheritic membrane formation and death from asphyxia prompted the designation "fowl diphtheria."

The virus probably persists as a latent infection, and has been recovered from tracheal explant cultures over 3 months after infection.

Diagnosis. Clinical and postmortem findings are characteristic. Fluorescent-antibody staining of smears and tissues and isolation of the virus either by inoculation on the chorioallantoic membrane of embryonated eggs or cell cultures are also used. Neutralizing antibody may be detected by pock or plaque reduction assays.

Epidemiology and Control. Infectious laryngotracheitis virus is usually introduced into a flock via carrier birds and is transmitted by aerosol and inhalation, less commonly by ingestion.

Although infectious laryngotracheitis spreads rapidly through a flock, new clinical cases may occur over a period of 2 to 8 weeks; this rate of spread is somewhat slower than that for other acute respiratory diseases of chickens, such as Newcastle disease, influenza, and infectious bronchitis.

It is feasible to establish and maintain flocks free of infectious laryngotracheitis, and, where management systems allow, this practice is increasingly adopted, particularly in the broiler industry where birds are harvested at 9 weeks of age and where "all-in, all-out" management is possible. However, vaccination is still widely practiced for breeding and egg production flocks.

Infectious laryngotracheitis is the first herpesvirus disease for which practical methods of vaccination were developed and historically provides an interesting case study. The first approach, adopted about 1930, was to mix young susceptible birds with known recovered birds, before egg production began. The second approach, introduced in about 1934, was to inoculate birds with wild-type, virulent virus via the cloaca, which produced immunity without serious respiratory signs. This method was very successful in reducing losses and remained in vogue for 30 years. Since birds missed during this "vaccination" procedure were likely to develop respiratory disease within a few days, it was important to do all birds in a single unit on the same day. Spread of the virulent virus used for "vaccination" to nonvaccinated birds in adjoining units or even adjoining farms sometimes occurred, and it was obviously important not to introduce vaccinated birds into a susceptible flock.

In the 1950s it was recognized that strains of low virulence occurred and could be identified by pock morphology on the chorioallantoic membrane, and these viruses were then introduced for cloacal vaccination. The virus was prepared as a homogenate of infected chorioallantoic membranes and allantoic fluids and administered via the cloaca as a drop or with a stiff brush that slightly abraded the epithelium. The labor intensity of cloacal vaccination resulted in alternative procedures including feather follicle vaccination, whereby a few feathers were plucked and virus was brushed into the empty feather follicles, and wingweb inoculation, in which virus was "stabbed" through the wingweb with a special inoculating needle. As more attenuated vaccine strains grown in cell cultures became available, inoculation via the infraorbital sinus or by intranasal and eye drop procedures were adopted. Mass vaccination procedures based on aerosol and drinking water administration have also been developed, but technical problems which would guarantee 100% successful vaccination have not been overcome and these procedures have not been generally adopted.

Immunization with attenuated live-virus vaccine protects birds against clinical disease, but does not protect against infection with virulent virus or the development of a latent carrier status for either the virulent or the vaccine viruses. Control by vaccination therefore implies an acceptance of the fact that virulent virus persists in the flock and that

some losses due to infectious laryngotracheitis virus, either alone or in concert with other pathogens, will continue.

Duck Plague (Duck Enteritis)

Duck plague was first recognized in the Netherlands in 1923, where it was initially diagnosed as influenza. Subsequently, it was recognized as a major disease throughout Europe, North America, China, and India. In addition to domestic ducks, wild ducks, geese, swans, and other waterfowl are equally susceptible. Migratory waterfowl may contribute to spread within and between continents. A major epizootic occurred in South Dakota in the spring of 1973, in which 48,000 waterfowl, mostly ducks, died; such epizootics are still common. The virus (anatid herpesvirus 1) caused catastrophic losses when first introduced into the intensive Pekin duck farms on Long Island, New York, in 1967.

Strains of virus vary in virulence, although only a single antigenic type has been recognized. The virus grows readily on the chorioallantoic membrane of embryonated duck eggs and in duck embryo fibroblast cell cultures, but only poorly or not at all in similar substrates of chicken origin, although it may be adapted to grow in chicken cells.

Clinical Features. Attention is drawn to the disease by the occurrence of a sudden and persistent increased mortality within flocks of ducks. The incubation period is 3–7 days. There is anorexia, depression, nasal discharge, ruffled, dull feathers, adherent eyelids, photophobia, extreme thirst, ataxia leading to recumbency with outstretched wings with head extended forward, tremors, watery diarrhea, and soiled vents. Egg production drops 25 to 40%. Morbidity and mortality vary from 5 to 100%. Most ducks that develop clinical signs die. Sick wild ducks conceal themselves and die in vegetation at the water's edge.

Pathology. Ingested virus causes enteritis and viremic spread leads to vasculitis and widespread focal necrosis. Blood is present in the body cavities including gizzard and intestinal lumens, and petechial hemorrhages are present in many tissues. There may be elevated crusty plaques of diphtheritic membrane in the esophagus, cecum, rectum, cloaca, and bursa. Herpesvirus inclusions are most readily demonstrated in hepatocytes, intestinal epithelium, and lymphoid tissues.

Diagnosis. Clinical and gross postmortem findings may be confirmed by the finding of herpesvirus inclusion bodies or positive immunofluorescence. Duck plague needs to be differentiated from duck hepatitis (due to a picornavirus), and from Newcastle disease and influenza.

Epidemiology and Control. Ingestion of contaminated water is thought to be the major mode of transmission, although the virus may also be transmitted by contact. Virus has been isolated from wild ducks up to a year after infection.

A chick embryo-adapted attenuated live-virus vaccine has been used in the United States. However, despite the continued threat of reintroduction from wild birds, the disease has been eliminated from the duck farms of Long Island and vaccination is not now routinely practiced.

B Virus Disease of Monkeys

Monkeys suffer from a herpesvirus infection caused by B virus (cercopithecid herpesvirus 1), the natural history of which is very like that of herpes simplex type 1 infection in humans. It is mentioned here because a number of fatal cases of ascending paralysis and encephalitis in animal handlers have occurred, infection being transmitted by monkey bite. It is a continuing risk to personnel working with primates.

DISEASES CAUSED BY BETAHERPESVIRUSES

The betaherpesviruses replicate more slowly than alphaherpesviruses and often produce greatly enlarged cells, hence the designation "cytomegalovirus." Their host range is narrow and in latent infections virus or viral DNA is believed to be sequestered in secretory glands, lymphoreticular tissues, and kidney. Rather than being subject to periodic reactivation, betaherpesviruses are often excreted continuously. They have been associated with diseases of economic importance in cattle, horses, and swine (Table 19-3).

Bovine Cytomegalovirus Infections

Betaherpesviruses that are slowly cytopathogenic have been isolated throughout the world from cattle suffering from a variety of diseases including conjunctivitis, respiratory disease, vaginitis, metritis, skin nodules, and lymphosarcoma. However, there is no proven etiological association between the disease and the cytomegaloviruses (e.g., bovine herpesvirus 3) occasionally isolated from cases. When experimentally inoculated into susceptible cattle these viruses produce no disease. Strains of cytomegalovirus have been isolated when cell cultures are prepared from tissues of apparently normal cattle; they have also been isolated from semen of normal bulls. Few have been adequately studied.

TABLE 19-3
Herpesviruses of the Subfamily Betaherpesvirinae *That Cause Disease in Domestic Animals*

Virus	Disease
Bovine herpesvirus 3	Bovine cytomegalovirus infection
Equine herpesvirus 2	Equine cytomegalovirus infection
Porcine herpesvirus 2	Inclusion body rhinitis; generalized cytomegalovirus infection

Porcine Cytomegalovirus Infections

First recognized in the United Kingdom in 1955, porcine herpesvirus 2 is enzootic in many swine herds worldwide. In the United Kingdom some 50% of herds are infected, while a survey in Iowa indicated infection in 12% of herds. Within a herd up to 90% of swine may carry the virus. Often disease is not seen in herds in which the virus is enzootic; it is more likely to be associated with recent introduction of the virus or with environmental factors such as poor nutrition and intercurrent disease. Virus-free herds have been established.

Clinical Features. Rhinitis occurs in swine up to 10 weeks of age, beyond which infection is subclinical, and it is most severe in swine less than 2 weeks old. There is sneezing, coughing, serous nasal and ocular discharge, and depression. The discharge becomes mucopurulent and may block the nasal passages, which interferes with suckling; such piglets lose weight rapidly and die within a few days. Survivors are stunted. A generalized disease following viremic spread is also recognized in young swine. Porcine herpesvirus 2 crosses the placenta and may cause fetal death or result in generalized disease in the first 2 weeks after birth, or there may be runting and poor weight gains.

Pathology. Large basophilic intranuclear inclusions are found in enlarged cells of the mucous glands of the turbinate mucosa (hence the synonym "inclusion body rhinitis").

Epidemiology and Control. When newly introduced into a susceptible herd, virus is transmitted both transplacentally and horizontally. In herds in which the virus is enzootic, transmission is predominantly horizontal, but since young swine are infected when maternal antibody is present, the infection is subclinical. Disease occurs when the virus is

introduced into susceptible herds or if susceptible swine are mixed with carrier swine. Virus-free swine can be produced by hysterotomy, although because the virus crosses the placenta swine produced in this way must be monitored carefully for porcine herpesvirus 2 antibody for at least 70 days after delivery.

Equine Cytomegalovirus Infections

Equine cytomegalovirus (equine herpesvirus 2) may be isolated from nasal swab filtrates or from buffy coat cells of some 70% of all horses. Horses are infected in the first weeks of life even in the presence of maternal antibody. Neutralization tests suggest that several antigenic types exist; more than one antigenic type may be recovered at different times from the same horse.

Equine cytomegalovirus has been recovered from horses with respiratory disease sometimes characterized by coughing, swollen submaxillary and parotid lymph nodes, and pharyngeal ulceration, from conjunctivitis, and from the genital tract. The role of the virus in these and other diseases is uncertain.

DISEASES CAUSED BY GAMMAHERPESVIRUSES

Gammaherpesviruses are characterized by their replication in lymphoblastoid cells, different members of the subfamily being specific for either B or T lymphocytes. In the lymphocyte, infection is frequently arrested at the prelytic stage, with persistence and minimum expression of the viral genome, or at the lytic stage, causing cell death without production of virions. Latent virus can be demonstrated in lymphoid tissue.

Bovine malignant catarrhal fever virus has been tentatively classified as a gammaherpesvirus. A very important disease of chickens, Marek's disease, is due to a gammaherpesvirus, Marek's disease virus. Gammaherpesviruses of primates produce lymphoid tumors which have been used experimentally to study tumorigenesis; human infection with a gammaherpesvirus causes infectious mononucleosis and is associated with Burkitt's lymphoma and nasopharyngeal carcinoma.

Bovine Malignant Catarrhal Fever

Malignant catarrhal fever is an invariably fatal, generalized disease of cattle and some wild ruminants (deer, buffalo, antelope), primarily affecting lymphoid tissues and epithelial cells of the respiratory and gas-

trointestinal tracts. Three distinct epidemiological patterns are recognized, and from only one of these has a herpesvirus been isolated. In Africa (and zoos), epizootics of the disease occur in cattle (and captive, susceptible wild ruminants) following transmission of the virus from wildebeest (*Connochaetes gnu* and *C. taurinus*), and to a less extent from hartbeest (*Alcephus buselaphus*) and topi (*Damaliscus korrigum*), particularly at calving time. A herpesvirus (alcephaline herpesvirus 1) has been isolated from this *African form* of malignant catarrhal fever and shown experimentally to reproduce the disease; it has been tentatively classified as a gammaherpesvirus.

Outside Africa and zoos, a disease described as malignant catarrhal fever in cattle and deer occurs when these species are kept in close contact with sheep, especially during lambing time. The *sheep-associated form* can be transmitted by inoculation of cattle or deer with blood from known carrier sheep. It has been shown that sheep have antibody to the alcephaline herpesvirus 1; but, the virus responsible for the ovine form of the disease has not been isolated.

A third epidemiological form of the syndrome described as malignant catarrhal fever is recognized in feedlot cattle in North America, in the absence of contact with sheep. It occurs as minor epizootics; the identity and source of virus in this third form is unknown. Both alcephaline herpesvirus 1 and the putative sheep-associated herpesviruses produce a disease resembling malignant catarrhal fever in rabbits. The description which follows refers to the African form of the disease.

Clinical Features. After an incubation period of about 3 weeks malignant catarrhal fever is characterized by fever, depression, leukopenia, profuse nasal and ocular discharges, bilateral ophthalmia, generalized lymphadenopathy, extensive mucosal erosions, and central nervous system signs. The ophthalmia is associated with corneal opacity which begins peripherally and progresses centripetally, often leading to blindness. Erosions of the gastrointestinal mucosa lead to diarrhea. Death occurs about a week after the onset of clinical signs.

Pathology and Pathogenesis. Postmortem findings vary according to the duration of the disease. There are usually extensive erosions, edema, and hemorrhage through the gastrointestinal tract. There is a generalized lymphadenopathy; all lymph nodes are enlarged, edematous, sometimes hemorrhagic. Frequently there are multiple raised necrotic lesions accompanied by ecchymotic hemorrhages in the kidney, and erosions of the mucosa of the turbinates, larynx, and trachea. Histologically there is widespread lymphoid cell proliferation and multifocal areas of necrosis, centered on small blood vessels.

Diagnosis. The history and clinical signs, particularly the presence of bilateral ophthalmia, suggest the diagnosis of malignant catarrhal fever. The virus can be isolated when washed, peripheral blood leukocytes are inoculated in calf thyroid cells. Cell-free inocula do not yield virus. The cytopathic changes require at least 3 days to appear, and often several passages in cell culture are necessary. They are characterized by syncytia and the presence of typical herpesvirus intranuclear inclusion bodies.

Epidemiology and Control. The virus does not appear to be pathogenic for wildebeest, and in this species it appears to be transmitted from mother to offspring in the immediate postcalving period, via nasal secretions. Cattle are believed to be infected via the relatively large amounts of virus present in the nasal secretions of wildebeest calves. The virus is not transmitted between cattle, which appear to be "dead-end" hosts.

Attempts to develop a vaccine have been unsuccessful.

Marek's Disease

In 1907 the Hungarian physician-pathologist, Marek, described paralysis associated with a polyneuritis affecting some roosters kept in his backyard. Marek's disease seemed to be a distinct disease, although for about 50 years it was considered part of a large group of diseases referred to as the avian leukosis complex. The specific herpesvirus etiology of Marek's disease was established in 1967. The avian leukoses are caused by retroviruses (see Chapter 31).

Marek's disease occurs worldwide, and few large chicken flocks are free. In the United States it was first described in 1914, and was often seen when young chickens, turned onto pasture, developed paralysis ("range paralysis"). The adoption of intensive systems of poultry management after 1949 saw a dramatic increase in its incidence. Prior to the introduction of vaccination in 1970, it was the most common lymphoproliferative disease of chickens, causing annual losses in the United States of $150 million and in the United Kingdom of $40 million. Vaccination has dramatically reduced the incidence of disease but has not reduced the incidence of infection. Because of continuing losses from disease and the costs of vaccination, it remains a most important disease of chickens.

Marek's disease has been an important model for the study of the tumorigenic potential of herpesviruses. The virus is slowly cytopathic and remains highly cell-associated, so that cell-free infectious virus is virtually impossible to obtain, except in dander from feather follicles.

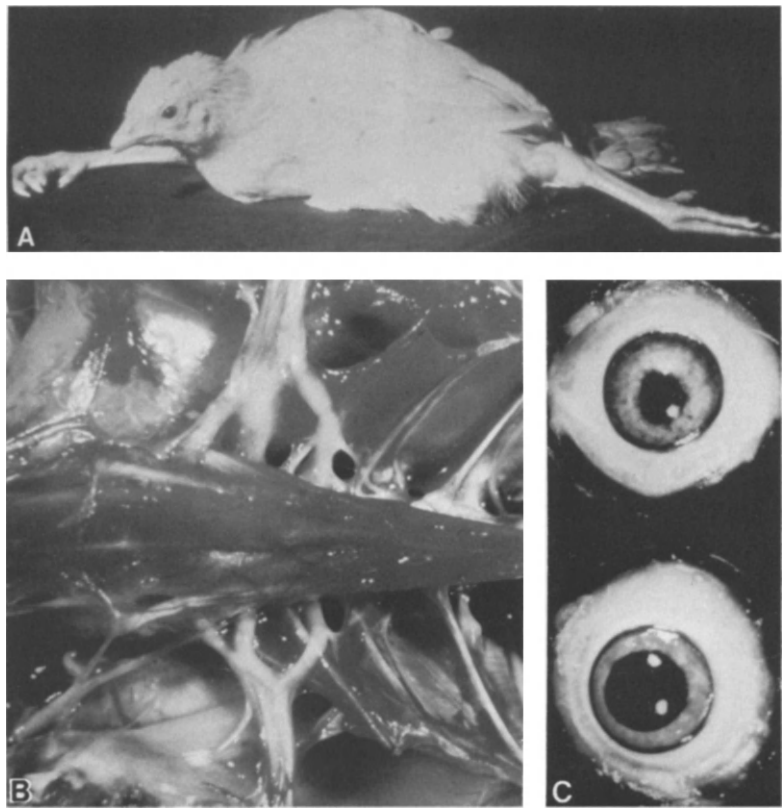

PLATE 19-5. *Marek's disease of chickens. (A) Paralysis. (B) Enlargement of sciatic nerves. (C) Ocular lesions. Lower eye is normal, with dilated pupil; pupil of upper eye failed to dilate and has irregular outline due to infiltration of transformed lymphocytes.*

Clinical Features. Marek's disease is a progressive disease with variable signs; four overlapping syndromes are described. In *neurolymphomatosis* or so-called classical Marek's disease there is an asymmetric paralysis of one or both legs or wings (Plate 19-5). Incoordination is a common early sign; one leg is held forward and the other backward when stationary because of unilateral paresis or paralysis. Wing dropping and lowering of the head and neck are common. If the vagus nerve is involved there may be dilation of the crop and gasping. *Acute Marek's disease* occurs in explosive outbreaks in which a large proportion of birds in a flock show depression followed after a few days by ataxia and

paralysis of some birds. Significant mortality occurs without localizing neurological signs. In *ocular lymphomatosis* the iris of one or both eyes is gray in color because of lymphoblastoid cell infiltration; the pupil is irregular and eccentric and there is partial or total blindness. *Cutaneous Marek's disease* is readily recognized after plucking, when round, nodular lesions up to 1 cm in diameter are seen, particularly at feather follicles.

Pathology. Enlargement of one or more peripheral nerve trunks is the most constant gross finding. In the vast majority of cases a diagnosis can be made if the celiac, cranial, intercostal, mesenteric, brachial, sciatic, and greater splanchnic nerves are examined. In a diseased bird, the nerves are up to three times their normal diameter, show loss of striations, and are edematous, gray or yellowish, and somewhat translucent in appearance. Because enlargement is frequently unilateral it is especially helpful to compare contralateral nerves.

Lymphomatous lesions, indistinguishable from those of avian leukosis, are usually small, diffuse, grayish, and translucent. They are most common in acute Marek's disease, and occur in the gonads, particularly the ovary, and other tissues.

Pathogenesis. The outcome of infection of chickens by Marek's disease virus is influenced by the virus strain, dose, and route of infection, and by the age, sex, immune status, and genetic susceptibility of the chickens. Subclinical infection with virus shedding is common. Infection is acquired by inhalation of dander. Epithelial cells of the respiratory tract are productively infected, and contribute to a cell-associated viremia involving macrophages. By the sixth day there is productive infection of lymphoid cells in a variety of organs, including the thymus, bursa of Fabricius, bone marrow, and spleen, resulting in immune suppression. During the second week after infection there is a persistent cell-associated viremia followed by a proliferation of T lymphoblastoid cells, and a week later deaths begin to occur, although regression may also occur from this time.

T lymphocytes are transformed by the virus, and up to 90 genome equivalents of Marek's disease virus DNA can be demonstrated in transformed cells. Viral DNA is present in both plasmid and integrated forms; it is uncertain which is critical in the process of transformation.

The lesions of Marek's disease result from the infiltration and *in situ* proliferation of T lymphocytes, which may result in leukemia, but in addition there is often a significant inflammatory cell response to the lysis of nonlymphoid cells by the virus. Lesions of the feather follicle are invariably a mixture of lymphoblastoid and inflammatory cells. Epi-

thelial cells at the base of feather follicles are exceptional in that productive infection of these cells is also associated with the release of cell-free infectious virus.

The basis for genetic resistance is not fully defined, but resistance has been correlated with the B21 alloantigen of the B red blood cell group. Maternal antibody may persist in newly hatched chicks for up to 3 weeks, and infection of such chicks with virulent Marek's disease virus may not produce disease, but lead to an active immune response. Chickens that are bursectomized and then actively immunized also survive challenge infection.

Many apparently healthy birds are lifelong carriers and shedders of virus, but the virus is not transmitted *in ovo*. When fully susceptible day-old chicks are infected with virulent virus, the minimum time for detection of microscopic lesions is 1–2 weeks, and gross lesions are present by 3 to 4 weeks. Maximal virus shedding occurs at 5 to 6 weeks after infection.

Diagnosis. Where sufficient numbers of birds are examined, history, age, clinical signs, and gross postmortem findings are adequate for the diagnosis, which can be confirmed by histopthological examination. Detection of viral antigen by immunofluorescence is the simplest reliable laboratory diagnostic procedure. Gel diffusion, indirect immunofluorescence, or virus neutralization are used for detection of viral antibody.

A variety of methods can be used for viral isolation: inoculation of cell cultures, the chorioallantoic membrane, or the yolk sac of 4-day-old embryonated eggs with suspensions of buffy coat or spleen cells. The presence of virus can be demonstrated by immunofluorescence or electron microscopy.

Marek's disease and avian leukosis are usually present in the same flock, and both diseases may occur in the same bird. The two diseases were long confused (see Chapter 31), but can be differentiated by clinical and pathological features (Table 19-4), or by specific tests for virus, viral antigens, or viral antibody.

Epidemiology and Control. Most birds have antibody to Marek's disease virus by the time they are mature; infection persists and virus is released in dander from the feather follicles. Congenital infection does not occur, and chicks are protected by maternal antibody for the first few weeks of life. They then become infected by the inhalation of virus in the dust. Epizootics of Marek's disease usually involve sexually immature birds 2–5 months old; there is a high mortality (about 80%).

Isolates of Marek's disease virus vary considerably in virulence and in the types of lesions produced. Avirulent strains are recognized and used

TABLE 19-4
Clinical and Histological Differentiation of Marek's Disease and Avian Leukosis

Disease parameter	Marek's disease	Avian leukosis
Etiology	Herpesvirus	Retrovirus
Target cells	T lymphocytes	Various hematopoietic cells
Age of onset for signs	4 weeks	16 weeks
Paralysis	+	–
Gross lesions		
Liver, spleen, kidney	+	+
Gonads, lungs, heart	+	Rare
Nerve trunks	+ (neural form)	Rare
Iris	+ (ocular form)	Rare
Skin	+ (cutaneous form)	Rare
Bursa	Rare	+ (nodular)
Histology		
Size of affected lymphoblasts	Varied	Uniform
Intranuclear inclusion bodies	+	–

for vaccine, although the antigenically related turkey gammaherpesvirus is preferred as a vaccine strain, primarily because it infects cells productively. Marek's disease virus and turkey herpesvirus are about 95% homologous by DNA–DNA hybridization.

Vaccination is the principal method of control. Day-old chicks are vaccinated parenterally, the vaccine being available as either a lyophilized cell-free preparation or a cell-associated preparation. The cell-free vaccine does not take in chicks with maternal antibody whereas the cell-associated vaccines do. Protective immunity develops within about 2 weeks. Vaccination decreases the incidence of disease, particularly of lymphomatous lesions in visceral organs, and has been most successful in the control of acute Marek's disease. Neurological disease continues to occur in vaccinated flocks, but at reduced incidence.

A further level of control can be achieved if flocks are built up with birds carrying the B21 alloantigen. It is possible to establish flocks free of Marek's disease, but commercially it is extremely difficult to maintain the disease-free status. The production of chickens on the "all-in, all-out" principle, whereby they are hatched, started, raised, and dispersed as a unit, would improve the efficacy of vaccination as a control measure.

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CHAPTER 20

Iridoviridae and African Swine Fever Virus

Large icosahedral cytoplasmic DNA viruses that were grouped together as the family *Iridoviridae* occur in a wide range of animal species, both vertebrate and invertebrate (Table 20-1). In 1984 the species of greatest veterinary importance, African swine fever virus, was removed from the family because of major differences in the structure of its DNA and its mode of replication, but it was not immediately allocated to any other family.

African swine fever is probably the most serious viral disease threatening the swine industries of developing and developed countries. Since its recognition in 1921 in East Africa, the disease has spread to Europe and the Western Hemisphere, causing the death of many hundreds of thousands of domestic swine. African swine fever virus is transmitted by soft ticks of the genus *Ornithodoros;* it is the only DNA virus that can be designated as an arbovirus.

Lymphocystis in fish is not comparable in importance to African swine fever in swine, but it is attracting increasing attention as marine and freshwater pisciculture develop. The virus causes tumorlike lesions on the skin which make affected fish unmarketable. Since lymphocystis virus has been reported to cause disease in more than 90 different spe-

TABLE 20-1
Diseases Caused by Iridoviruses and African Swine Fever Virus

Genus	Viruses	Animal species affected	Disease
Iridovirus *Chloriridovirus*	Two genera which include a large number of viruses	Many species of insects	Affects larvae; large masses of virions produce iridescence
Ranavirus	Large number, principally from frogs	Amphibians	Death in tadpoles but no disease in adult frogs
Lymphocystivirus	Lymphocystis disease	Many species of fish	Tumorlike growths in skin
African swine fever virus group	African swine fever virus	Wild pigs and domestic swine	No disease in warthog (natural host) but severe disease in domestic swine, sometimes with persistent infection in survivors

cies of marine, brackish, and freshwater fish, it is potentially an important pathogen.

PROPERTIES OF THE VIRUSES

The virions of all iridoviruses of vertebrates and of African swine fever virus are morphologically similar (Plate 20-1). An irregular lipoprotein envelope derived from the plasma membrane gives the virion an irregular spherical shape, about 200 to 220 nm in diameter. Within the envelope there is an icosahedral capsid which contains a very large number of capsomers, estimated to be either 1892 or 2172. The capsid in turn encloses a second lipoprotein membrane which surrounds the DNA-containing core.

The genome is a single molecule of dsDNA, 95–150 kbp (Table 20-2), which in viruses of the genera *Lymphocystivirus* and *Ranavirus* is methylated, circularly permuted, and contains terminal repetitions, but in African swine fever virus has covalently closed ends and terminal inverted repetitions.

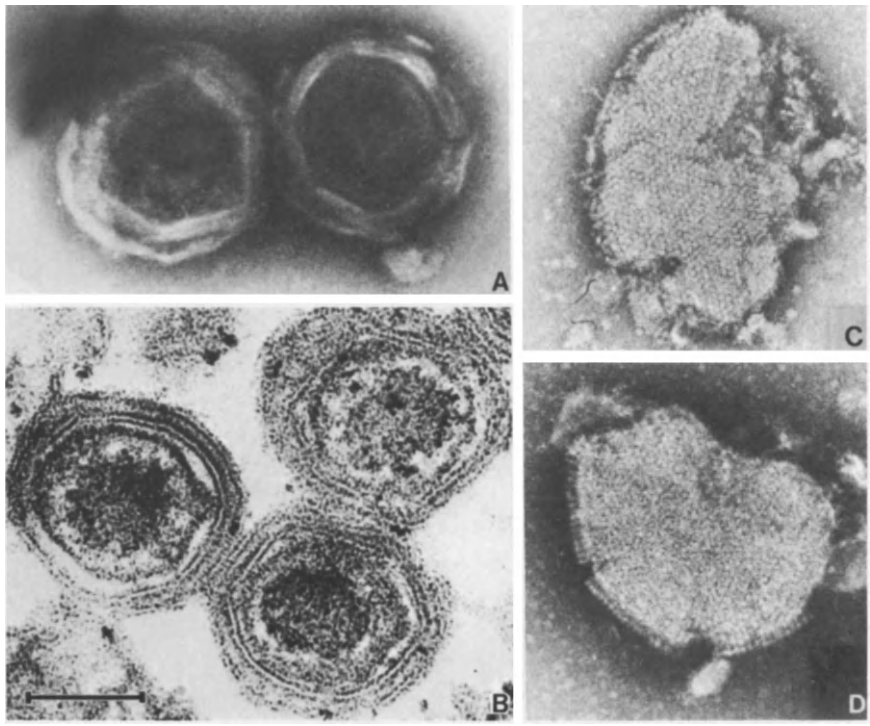

PLATE 20-1. *Virions of African swine fever virus. (A) Negatively stained virions, showing icosahedral capsid and envelope. (B) Thin sections of virions, showing multiple membranes surrounding the core. (C and D) Negatively stained capsids, showing the ordered arrangement of a very large number of capsomers (bar = 100 nm). [From J. L. Carrascosa* et al., Virology **132,** *160 (1984). Courtesy Dr. J. L. Carrascosa.]*

TABLE 20-2
Properties of Iridoviruses of Vertebrates and African Swine Fever Virus

Spherical enveloped virion, 200–220 nm diameter
Icosahedral capsid (1892 or 2172 capsomers) surrounds an internal lipid-containing membrane which encloses the DNA-containing core
Linear dsDNA. African swine fever virus: 150 kbp, covalently closed ends, and terminal inverted repetitions. *Lymphocystivirus:* 95 kbp; *Ranavirus:* 150 kbp; methylated, circularly permuted, and terminally redundant
Nucleus involved in DNA replication
Assembly of capsids in cytoplasm; virions mature by budding from plasma membrane

The presence in African swine fever virions of a DNA-dependent RNA polymerase and several other enzymes similar to those present in poxviruses suggests that the early stages of gene expression may be similar to those of poxviruses. However, the vertebrate iridoviruses lack the enzymes required for mRNA synthesis and depend on host cell RNA polymerase II.

The iridoviruses of vertebrates and African swine fever virus are sensitive to ether, chloroform, and deoxycholate. Infectivity is also sensitive to heat; African swine fever virus is inactivated in 30 minutes at 56°C but survives well—for months and even years—in refrigerated meat. African swine fever virus is also resistant to a wide range of pH, some infectivity surviving for several hours at pH 4 or pH 13; hence, it is not surprising that many common disinfectants are ineffective.

Serotypes have not yet been recognized within either African swine fever virus or lymphocystis virus isolates. However, there are differences in antigenicity and hemadsorption patterns among different African swine fever virus isolates.

VIRAL REPLICATION

African swine fever virus and lymphocystis virus are most easily isolated in cells derived from their respective host species. African swine fever virus replicates in primary cultures of swine bone marrow and peripheral blood leukocytes, in purified cultures of lymphocytes and macrophages, and can be adapted to grow in various cell lines such as PK-2a and Vero. A cytopathic effect is seen within a few days of inoculation.

After penetration, probably by endocytosis, African swine fever virus is uncoated and the DNA is transcribed in the cytoplasm by the virion-associated RNA polymerase. New mRNA species are transcribed after DNA replication, again by the viral transcriptase. About 50 virus-induced polypeptides have been identified. Synthesis of viral DNA occurs in the cytoplasm, in association with inclusion bodies, and requires a virus-induced DNA polymerase and a functional nucleus in the infected cell. Late in infection, a virus-specific protein appears in the plasma membrane and can be recognized by hemadsorption (see Plate 20-2).

AFRICAN SWINE FEVER

African swine fever virus has probably caused subclinical enzootic infection in warthogs in eastern and southern African for centuries, but

its presence was not detected until the beginning of the twentieth century, when European settlers reported deaths in introduced domestic swine. In 1921 Montgomery described the disease in domestic swine in Kenya as a peracute disease clinically similar to hog cholera, that commonly caused up to 100% mortality in herds. He showed that the disease was caused by a virus serologically distinct from hog cholera virus (a *Pestivirus*, see Chapter 25) and demonstrated that warthogs (*Phacochoerus aethiopicus*) and to a lesser extent bush pigs (*Potamochoerus porcus*) were sources of the infection.

The potential for African swine fever to become a worldwide threat was recognized only when it spread to Portugal in 1957, causing heavy losses, and subsequently to Spain in 1960. African swine fever is now enzootic in Spain and Portugal. The numbers of new outbreaks each year has varied in cycles, with peaks of several thousand cases in 1963, 1967, 1971, and 1978 in Spain, and comparable numbers in Portugal. It is from these two countries that recent further spread of the disease has occurred (Table 20-3).

TABLE 20-3
New Outbreaks of African Swine Fever in Non-African Countries Other Than Spain and Portugal, 1964–1985

Country	Years with outbreaks	Number of outbreaks	Current status (1986)
Europe			
France	1964	5	Eradicated
	1967	1	Eradicated
	1974	1	Eradicated
Italy	1967–1969	12	Eradicated
	1980	1	Eradicated
Madeira	1965; 1974	?	Eradicated
	1976	1	Eradicated
Malta	1978–1981	304	Eradicated
Sardinia	1978–1981	92	Endemic
Belgium	1985	9	Eradicated
South American and Caribbean			
Brazil	1978–1979	226	Eradicated
Cuba	1971	36	Eradicated
	1980	56	Eradicated
Dominican Republic	1978–1980	374	Eradicated
Haiti	1979	?	Probably eradicated

Clinical Features

Swine infected with African swine fever virus develop fever (40.5°–42°C) which occurs 5–15 days after exposure and persists for about 4 days. Other clinical signs, usually starting 1–2 days after the onset of fever, include inappetance, incoordination, and recumbency. Swine may die at this stage without other clinical signs being noticed. In some swine cyanotic areas may be observed on ears, limbs, and extremities. Other clinical signs include dyspnea, vomiting, nasal and conjunctival discharge, and hemorrhage from the nose or anus. Pregnant sows often abort. In East and South Africa, mortality is often 100% and swine die 4–7 days after onset of fever. Since becoming enzootic in domestic swine outside Africa, the virulence of the virus has decreased, the mortality in some herds being no higher than 30%, with most animals suffering from subacute or chronic forms of the disease. Some of the swine that survive are persistently infected and constitute a source of infection for healthy animals.

Pathology and Pathogenesis

In acute, fatal cases, gross lesions are most prominent in the lymphatic and vascular systems. Hemorrhages occur widely, and the visceral lymph nodes may resemble blood clots. The spleen is often large and friable and there are petechial hemorrhages in the cortex of the kidney. The chronic disease is characterized by cutaneous ulcers, pneumonia, pericarditis, pleuritis, and arthritis.

African swine fever virus is usually transmitted between domestic swine by the respiratory route, but only when there is direct contact between animals. Infection can also occur by ingestion of infected meat and meat products, and by the bite of soft ticks belonging to the genus *Ornithodoros*, which act as biological vectors.

If infection is acquired via the respiratory tract, the virus replicates first in the pharyngeal tonsils and lymph nodes draining the nasal mucosa before being rapidly disseminated throughout the body by a primary viremia, in which virions are associated with both erythrocytes and leukocytes. A generalized infection follows with titers up to 10^9 ID_{50} per milliliter of blood or per gram of tissue. Consequently, all secretions and excretions contain infectious virus.

Experimental studies have shown that African swine fever virus replicates in several cell types within the reticuloendothelial system and causes a severe leukopenia. Infected swine probably die through the indirect effects of viral replication on platelets and complement functions, rather than by the direct cytolytic effect of the virus.

Swine that survive may appear healthy or chronically diseased, but both groups may remain persistently infected. Indeed, swine may become persistently infected without ever showing clinical signs. The duration of the persistent infection is not known, but low levels of virus have been detected in tissues over a year after exposure. Viremia has been induced in such animals by injection of corticosteroids 6 months after initial infection.

Immunity

The immunological response of swine to African swine fever virus is puzzling. Virus infection induces antibody detectable by complement fixation, precipitation, immunofluorescence, hemadsorption inhibition, and ELISA tests; often in chronic infections so much antibody is formed that there is a hypergammaglobulinemia. However, neutralizing antibody has never been demonstrated. For this reason, efforts to produce a vaccine for African swine fever have so far been fruitless.

Diagnosis

The clinical signs of African swine fever are similar to those of several diseases such as erysipelas and acute salmonellosis, but the major diagnostic problem is in distinguishing it from hog cholera.

Any febrile disease in swine associated with hemorrhage and death should raise suspicion of African swine fever. Laboratory confirmation is essential, and samples of blood, spleen, and visceral lymph nodes should be collected for virus isolation and for detection of antigen and antibody. Virus isolation is done in swine bone marrow or blood leukocyte cultures, in which hemadsorption to infected cells can be demonstrated (Plate 20-2). Antigen can be detected by immunofluorescent staining of tissue smears or frozen sections, or by immunodiffusion using tissue suspensions.

Epidemiology

Two distinct epidemiological patterns occur: a sylvatic cycle in warthogs in Africa, and epizootic and enzootic cycles in domestic swine.

Sylvatic Cycle. In its original ecological niche in southern and eastern Africa, African swine fever virus is maintained in a sylvatic cycle involving asymptomatic infection in wild pigs (warthogs—*Phacochoerus aethiopicus,* and to a lesser extent bush pigs—*Potamochoerus porcus*) and argasid ticks (soft ticks) which occur in the burrows used by these animals. The soft ticks belong to the genus *Ornithodoros.* The tick is a biological vector of the virus (Fig. 20-1). Most tick populations in southern and

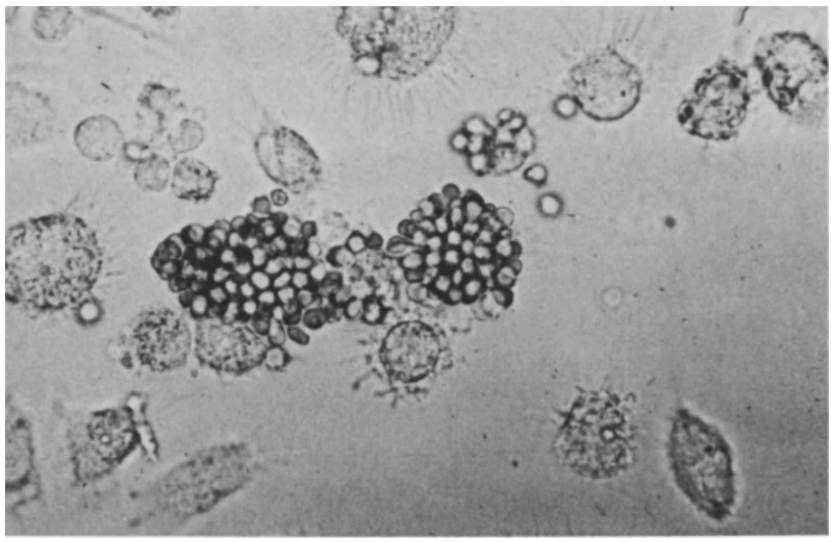

PLATE 20-2. *Hemadsorption of swine erythrocytes to swine leukocytes infected with African swine fever virus (phase contrast). (Courtesy Dr. P. J. Wilkinson.)*

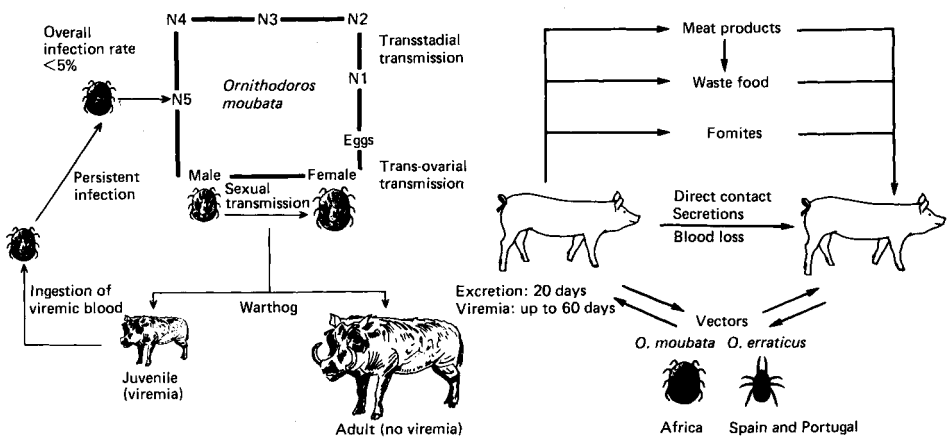

FIG. 20-1. *(A) Transmission cycles of African swine fever virus in warthogs and* Ornithodoros moubata. *(B) Transmission of African swine fever virus in domestic swine and* Ornithodoros *spp. [From P. J. Wilkinson,* Prev. Vet. Med. **2,** *71 (1984).]*

eastern Africa (Fig. 20-1A) are infected, with infection rates sometimes as high as 5%. After ingestion during feeding on viremic swine, the virus replicates in the gut of the tick and subsequently infects its reproductive system. This leads to transovarial and venereal transmission of the virus between ticks. The virus is also transmitted between developmental stages of the tick—transstadial transmission—and is excreted in tick saliva, coxal fluid, and Malpighian excrement. Infected ticks may live for several years and be capable of transmitting disease to swine at each feeding.

Serological studies indicate that many warthog populations in southern and eastern Africa are infected. After primary infection, young warthogs develop viremia sufficient to infect at least some of the ticks feeding upon them. Older warthogs are persistently infected but are seldom viremic. It is therefore likely that virus is maintained in a cycle involving young warthogs and ticks.

Domestic Cycle. Primary outbreaks of African swine fever in domestic swine in Africa probably result from the bite of an infected tick, although tissues of acutely infected warthogs, if eaten by domestic swine, can also cause infection.

Introduction of the virus into a previously noninfected country may result in indigenous ticks becoming infected and acting as biological vectors and reservoirs of disease—a feature of great epidemiological significance. Several species of soft tick found in association with domestic and feral swine in the Western Hemisphere (the United States and the Caribbean islands) have been shown in experimental studies to be capable of biological transmission of African swine fever virus, although there is no evidence that they have become naturally infected during the recent epizootics in the Caribbean islands and South America.

Once the virus has been introduced to domestic swine, either by the bite of infected ticks or through infected meat, infected animals form the most important source of virus for susceptible swine (Fig. 20-1B). Disease spreads rapidly by contact and within buildings by aerosol. The mechanical spread of African swine fever virus by people, vehicles, and fomites is possible because of the stability of the virus in blood, feces, and tissues.

The international spread of African swine fever virus has invariably been linked to feeding swine waste food containing scraps of uncooked meat from infected swine. When the virus appeared in Portugal in 1957 and in Brazil in 1978, it was first reported in the vicinity of international airports, among swine fed on food scraps. Virus spread to the Caribbean and Mediterranean islands in 1978 may have arisen from the unloading of infected food scraps from ships.

Prevention and Control

The prevention and control of African swine fever is difficult because of several features; the lack of an effective vaccine, the transmission of virus in fresh meat and some cured pork products, the existence of persistent infection in some swine, the clinical similarity to hog cholera, and the recognition that in some parts of the world soft ticks of the genus *Ornithodoros* are involved in the biological transmission of the disease.

The presence of the virus in ticks and warthogs in many countries of sub-Saharan Africa makes it impossible to break the sylvatic cycle of the virus. However, domestic swine can be reared in Africa if the management system avoids feeding uncooked waste food scraps and prevents access of ticks and warthogs, usually by double fencing with the wire mesh of the perimeter fence buried 2 m.

Elsewhere in the world, countries that are free of African swine fever maintain their position by (1) prohibiting importation of live swine and swine products from infected countries, and (2) monitoring the efficient destruction of all waste food scraps from ships and aircraft involved in international commerce.

If disease does occur in a previously noninfected country, control depends first on early recognition and rapid laboratory diagnosis. The virulent forms of African swine fever cause such dramatic mortality that episodes are quickly brought to the attention of veterinary authorities, but the disease caused by less virulent strains that are now found outside Africa can cause confusion with other diseases—especially hog cholera—and may not be recognized until the virus is well established in the swine population.

Once African swine fever is confirmed in a country that has hitherto been free of disease, its importance necessitates prompt action, first to control and then to eradicate it. All non-African countries that have become infected have elected to attempt eradication and, in many cases, have received financial assistance from international agencies such as the Food and Agricultural Organization of the United Nations. The strategy for eradication involves slaughter of infected swine and swine in contact with them, and disposal of carcasses, preferably by burning. Movement of swine between farms is controlled and feeding of waste food prohibited. Where soft ticks are known to occur, infested buildings are sprayed with acaricides. Restocking of the farm is allowed only if sentinel swine do not become infected.

Using this approach, eradication has been successful in some countries (e.g., France, Malta, and Cuba), but in others (e.g., Spain and

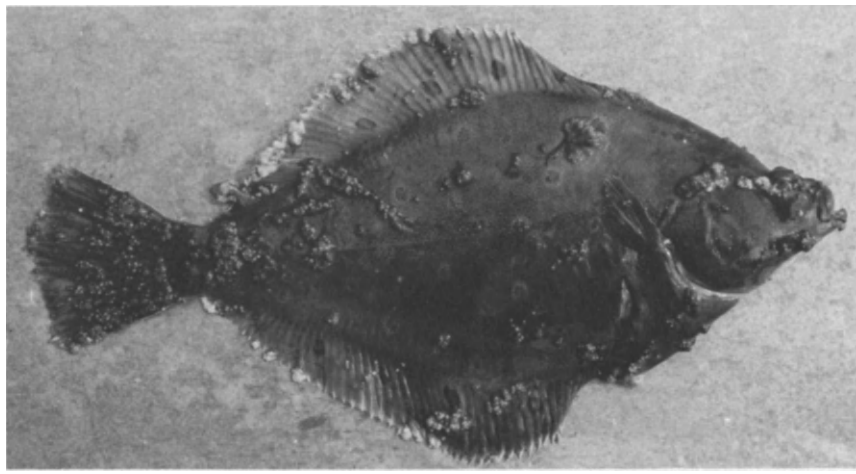

PLATE 20-3. *Lymphocystis in plaice. (Courtesy Dr. P. van Banning.)*

Portugal) eradication has proved difficult—almost certainly because ticks have become infected.

LYMPHOCYSTIS OF FISH

Lymphocystis is not a lethal disease and the lesions eventually heal. The disease is most noticeable on the skin, where papillomalike lesions are produced by enormously hypertrophied dermal cells, but internal organs and tissues also may be affected (Plate 20-3).

Lymphocystis occurs worldwide and is probably transmitted via abrasions. The disease is most commonly seen in brackish water or seawater with a slow circulation. Peak incidence is recorded in summer and local pollution may predispose fish to infection. While currently not an economically important disease, the fact that the virus can infect a wide range of fish species poses an economic threat to those involved in intensive fish farming, since affected fish are unmarketable.

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CHAPTER 21

Poxviridae

The family *Poxviridae* includes several viruses of veterinary and medical importance. Poxvirus diseases occur in most animal species and they are of considerable economic importance in some regions of the world. Diseases such as sheeppox are now eradicated in the developed countries, but are still a cause of major losses in some developing countries.

The history of the poxviruses has been dominated by smallpox. This disease, once a cosmopolitan and greatly feared infectious disease of humans, has now been eradicated by use of a simple live-virus vaccine that traces its ancestry to the cow sheds of Gloucestershire in England (see Chapter 16). With the eradication of smallpox, use of smallpox vaccine was discontinued throughout the world, but may soon be resurrected in a new guise. Vaccinia virus is now the subject of intense scientific study as a vector for recombinant DNA vaccines, for both veterinary and medical use (see Chapter 14).

TABLE 21-1
Classification of Poxviruses of Vertebrates[a]

Genera	Prototype virus
Orthopoxvirus	Vaccinia virus
Parapoxvirus	Pseudocowpox virus
Capripoxvirus	Sheeppox virus
Suipoxvirus	Swinepox virus
Leporipoxvirus	Myxoma virus
Avipoxvirus	Fowlpox virus

[a]Family, *Poxviridae;* subfamily, *Chordopoxvirinae.*

Classification

The family *Poxviridae* is subdivided into two subfamilies: *Chordopoxvirinae* (poxviruses of vertebrates) and *Entomopoxvirinae* (poxviruses of insects). The subfamily *Chordopoxvirinae* is subdivided into six named genera (Table 21-1), and several other chordopoxviruses that have not yet been classified. Each of the six genera include species that cause diseases in domestic animals.

PROPERTIES OF POXVIRUSES

The poxviruses are the largest and most complex of all viruses. Plate 21-1A, C, and D illustrates the structure of the virion of vaccinia. Virions of all other genera of the chordopoxviruses are similar, except those belonging to the genus *Parapoxvirus* (Plate 21-1B). There is no nucleocapsid conforming to either of the two types of symmetry found in most other viruses (see Chapter 1), hence it is called a "complex" virion. An outer membrane encloses a dumbbell-shaped core and two "lateral bodies" of unknown nature. The core contains the viral DNA together with protein. Especially in particles released naturally from cells, rather than by cellular disruption, there is an envelope (Plate 21-1D) which contains cellular lipids and several virus-specified polypeptides.

The nucleic acid is dsDNA, varying in size from 130 kbp (parapoxviruses to 280 kbp (fowlpox virus). Within the genus *Orthopoxvirus,* restriction endonuclease maps provide the definitive criteria for the allocation of strains of virus recovered from various sources (e.g., cowpox

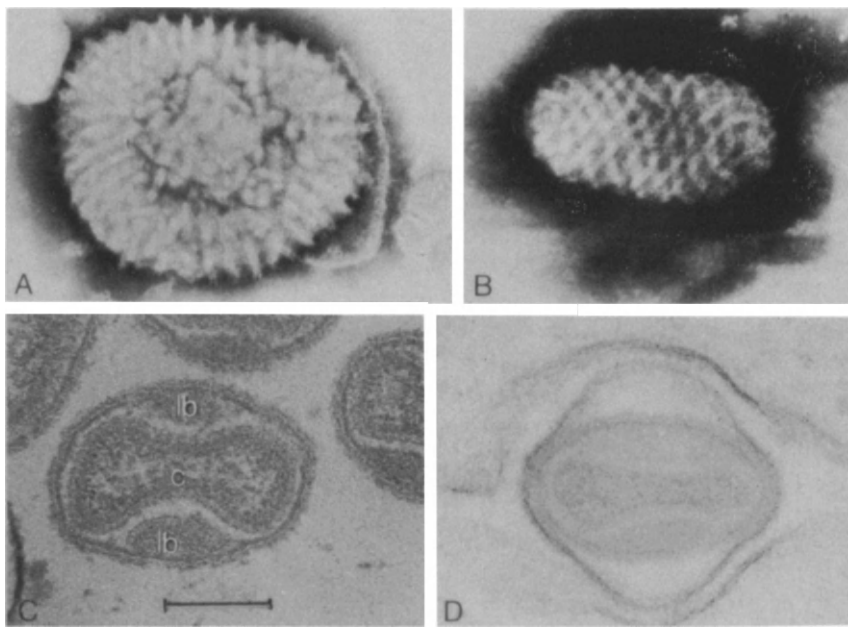

PLATE 21-1. Poxviridae *(bar = 100 nm). (A) Negatively stained vaccinia virion, showing surface structure of tubules characteristic of the outer membrane of all genera except* Parapoxvirus. *(B) Negatively stained orf virion, showing characteristic surface structure of the outer membrane of* Parapoxvirus. *(C) Thin section of vaccinia virion in its narrow aspect, showing the biconcave core (c) and the two lateral bodies (lb). (D) Thin section of mature enveloped extracellular virion lying between two cells. [(A and D, from S. Dales,* J. Cell Biol. **18,** *51 (1963); B, from J. Nagington* et al., Virology **23,** *461 (1964); C, from B. G. T. Pogo and S. Dales,* Proc. Natl. Acad. Sci. U.S.A. **63,** *820 (1969).]*

TABLE 21-2
Properties of Poxviruses

Most genera: brick-shaped virion, 300 × 240 × 100 nm, irregular arrangement of tubules on outer membrane.
Parapoxviruses: ovoid, 260 × 160 nm, with regular spiral arrangement of "tubule" on outer membrane.
Complex structure with core, lateral bodies, outer membrane, and sometimes envelope
Linear dsDNA, 130 kbp (*Parapoxvirus*); 165–210 kbp (*Orthopoxvirus*); 280 kbp (*Avipoxvirus*)
Transcriptase and several other enzymes in virion
Cytoplasmic replication

virus, see Table 21-3) to a particular species. Preliminary studies suggest that it may also be possible to group the three recognized species of *Parapoxvirus* (see Table 21-4) in this way.

There are over 100 polypeptides in the virion. The core proteins include a transcriptase and several other enzymes. The virion contains numerous antigens recognizable by immunodiffusion, most of which are common to all members of any one genus, although each species is characterized by certain specific polypeptides. A few others appear to be shared by all poxviruses of vertebrates. There is extensive cross-neutralization and cross-protection between viruses belonging to the same genus, but none between viruses of different genera. Genetic recombination occurs between viruses of the same genus, but not between those of different genera.

Poxviruses are resistant to ambient temperatures and may survive many years in dried scabs. Orthopoxviruses and most avipoxviruses are ether resistant, but parapoxviruses, capripoxviruses, and leporipoxviruses are ether sensitive.

VIRAL REPLICATION

Replication of poxviruses occurs in the cytoplasm and can be demonstrated in enucleate cells. After fusion of the virion with the plasma membrane or via endocytosis, the viral core is released into the cytoplasm. Transcription is initiated by the viral transcriptase and functional capped and polyadenylated mRNAs are produced within minutes after infection. The polypeptides produced by translation of these mRNAs complete the uncoating of the core and transcription of about 100 genes, distributed throughout the genome, occurs before viral DNA synthesis begins.

Early proteins include thymidine kinase, DNA polymerase, and several other enzymes. With the onset of DNA replication 1.5–6 hours after infection, there is a dramatic shift in gene expression and almost the entire genome is transcribed, but transcripts from the early genes (i.e., those transcribed before DNA replication begins) are not translated.

Virion formation occurs in circumscribed areas of the cytoplasm ("viral factories"). Spherical immature particles can be visualized by electron microscopy; their outer bilayer becomes the outer membrane of the virion and the core and lateral bodies differentiate within it. Some of these mature particles move to the vicinity of the Golgi complex, acquire an envelope, and are released from the cell. However, most particles are not enveloped and are released by cell disruption. Both enveloped and nonenveloped particles are infectious.

PATHOGENESIS AND IMMUNITY

All poxvirus infections are associated with lesions of the skin. These may affect only one part of the animal (e.g., the teats of milking cows with cowpox) or the disease may be generalized (e.g., lumpyskin disease in cattle). The lesions associated with these two diseases and many other pox diseases are essentially pustular; with other poxviruses the lesions may be more proliferative (e.g., the nodules of pseudocowpox), or they may be tumorlike, as in myxomatosis. Several poxviruses, e.g., sheeppox, cause generalized disease with lesions throughout the viscera, and are associated with a significant mortality.

Generalized poxvirus infections have a stage of cell-associated viremia, which leads to localization in the skin and to a lesser extent in internal organs. Immunity to such infections is prolonged. However, in some localized poxvirus infections, notably those produced by parapoxviruses, immunity is short-lived and reinfection is common.

LABORATORY DIAGNOSIS

Because of the large size and distinctive structure of poxvirus virions, electron microscopic examination of lesion material usually allows their ready identification and is the preferred method of laboratory diagnosis. Parapoxviruses have a distinctive structure (Plate 21-1B); other poxviruses are morphologically similar to each other, but their source (species of animal, type of lesion) should make specific diagnosis easy.

Most poxviruses grow in cell culture. Except for parapoxviruses, most also produce pocks on the chorioallantoic membrane of embryonated hen's eggs, the morphology of which is used to differentiate orthopoxvirus species from each other.

EPIDEMIOLOGY AND CONTROL

Poxviruses can be transmitted between animals by several routes: (1) by introduction of virus into small skin abrasions from other infected animals or from a contaminated environment (e.g., orf), (2) by aerosol infection of the respiratory tract (e.g., sheeppox), or (3) through mechanical transmission by biting arthropods (e.g., swinepox).

DISEASES CAUSED BY ORTHOPOXVIRUSES

Six of the nine recognized species of *Orthopoxvirus* cause infections in domestic animals, including laboratory animals (Table 21-3).

TABLE 21-3
Orthopoxviruses That Affect Domestic and Laboratory Animals: Host Ranges and Geographical Distribution

Virus	Animals found naturally infected	Host range in laboratory animals	Geographical range; natural infections
Vaccinia virus	Numerous: human, cow,[a] buffalo,[a] pig,[a] rabbit[a]	Broad	Worldwide
Cowpox virus	Numerous: cow, human, rats, cats, gerbils, large felines, elephant, rhinoceros, okapi	Broad	Europe
Camelpox virus	Camel	Narrow	Asia and Africa
Ectromelia virus	Mice, ? voles	Narrow	Europe
Monkeypox virus	Numerous: squirrels, monkeys, anteater, great apes, rabbits, human	Broad	Western and central Africa
Uasin Gishu disease virus	Horse	Broad	Eastern Africa

[a]Infected from human.

Vaccinia

Vaccinia virus has long been the "model" poxvirus for laboratory studies. In its biological properties and its restriction endonuclease map it is clearly different from cowpox virus, as originally used by Jenner. Because of its widespread use and its wide host range, vaccinia virus sometimes caused naturally spreading diseases in domestic animals (e.g., "buffalopox") and also in laboratory rabbits ("rabbitpox"). In Holland in 1963, 8 of 36 outbreaks of cowpox were found to be caused by vaccinia virus and the rest by authentic cowpox virus (see below).

Buffalopox. This disease occurred in water buffaloes (*Bubalis bubalis*) in Egypt, the Indian subcontinent, and Indonesia. The causative virus appears to be vaccinia virus, although differing from laboratory strains in some properties. The disease is characterized by pustular lesions on the teats and udders of milking buffaloes; occasionally, especially in calves, a generalized disease is seen. During 1984 and 1985 outbreaks were still being reported in India, sometimes producing lesions on the hands and face of milkers, who were no longer protected by vaccination against smallpox.

Cowpox

Cowpox virus can be differentiated from vaccinia virus by its biological properties in laboratory animals, and it has a larger genome (220 kbp compared with 185 kbp for vaccinia virus) and a different restriction endonuclease map. Usually seen as a lesion on the teats or udders of cows, it occasionally infects milkers and may be transferred by them from one animal to another.

Since the mid-1950s outbreaks of severe generalized poxvirus infection, in a variety of zoo and circus animals including elephants and large felines, have been shown to be caused by cowpox virus. Cowpox virus infection in domestic cats has also been reported. It appears to be maintained in nature as an infection of rodents. Since the virus has a wide host range, sporadic cases occur in many other species, presumably due to contact with infected rodents. Infections in elephants and large felines may be severe and often fatal.

The incubation period of cowpox in cattle and in humans is about 5 days. Local erythema and edema are followed by the development of a multiloculate vesicle, then a pustule, which ruptures and suppurates. A scab can then form, but in milking cows ulceration is common. The lesions, which are often found on all four teats as well as the skin of the udder, may take several weeks to heal. The diagnosis of cowpox cannot be made without laboratory assistance, since a parapoxvirus and a herpesvirus (bovine mammillitis virus, see Chapter 19) cause similar lesions on the teats of cows.

Camelpox

Camelpox causes a severe generalized disease in camels, with extensive skin lesions. It is an important disease, especially in countries of Africa and southwestern Asia where the camel is an important beast of burden and milk animal. The more severe cases usually occur in young animals, and in epizootics the case fatality rate may be as high as 25%.

The causative virus is a distinctive species, and its restriction endonuclease map differentiates it from other orthopoxviruses. It has a narrow host range, and in spite of the frequent exposure of unvaccinated humans to florid cases of camelpox, human infection has not been seen.

Ectromelia

Ectromelia virus causes mousepox, a disease known only in laboratory mice. Its veterinary importance derives entirely from the fact that it is enzootic in many mouse colonies in Europe, China, and Japan, and has

periodically been imported to the United States, where devastating outbreaks have occurred in mouse colonies—most recently in 1979 to 1980. Depending mainly on the genotype of the mouse, the infection may cause inapparent disease or acute death with extensive necrosis of the liver and spleen. Mice that survive the acute infection often develop widespread skin lesions.

Demonstration of poxvirus particles in lesion material is diagnostic, since no other poxvirus causes a naturally spreading disease in mice. If a positive serological result is found in a country normally free of the disease, like the United States, it should be confirmed by other tests, since infected laboratory colonies may have to be destroyed.

Vaccination with vaccinia virus provides some protection and can be used for particularly valuable mouse stocks, but spread of mousepox can occur in vaccinated mice. Hence, the usual recommendation is that infected mouse stocks should be destroyed and the mouse breeding quarters thoroughly disinfected before reestablishing the colony.

Monkeypox

First observed in captive Asian monkeys in a laboratory in Copenhagen in 1958, this generalized poxvirus infection was subsequently diagnosed in eight similar outbreaks in Europe and the United States between 1958 and 1968. Affected monkeys developed a generalized rash; the disease is especially severe in great apes. No infections occurred among animal attendants. The virus has a distinctive restriction endonuclease map, and its biological properties also distinguish it clearly from other orthopoxviruses. It has a wide host range.

In 1970 it was discovered that in western and central Africa it caused a disease in humans, with a generalized pustular rash indistinguishable from that of smallpox. The human disease is a rare zoonosis, occurring only among Africans living in small villages in tropical rainforests. Monkeys and squirrels may be sources of human infection. Rarely, human-to-human transmission may occur. Originally important to veterinarians as a sporadic generalized pox disease of captive monkeys, in laboratory or zoos, monkeypox now derives its significance as being the only smallpoxlike disease of humans.

Uasin Gishu Disease

An orthopoxvirus has been found to cause papular skin lesions in horses in Kenya and Zambia. It is a rare disease, and is presumably contracted from a wildlife source.

DISEASES CAUSED BY PARAPOXVIRUSES

Parapoxviruses (Table 21-4) infect a range of species, but are most important in cattle, sheep, goats, and camels. The viruses are zoonotic; farmers, shearers, veterinarians, butchers, and other others who handle infected livestock can develop a localized lesion, usually on the hands (see Plate 21-2). The lesion, which is identical irrespective of the source of the virus, begins as an inflammatory papule, then enlarges to form a granulomatous lesion before regressing. It may persist for several weeks. If the infection is acquired from milking cattle, the lesion is known as "milker's nodule"; if from sheep, as "orf."

Pseudocowpox

Pseudocowpox occurs as a common enzootic infection in cattle in most countries of the world. It is an unimportant, chronic infection in many milking herds, and occasionally occurs in beef herds.

The first clinical sign of pseudocowpox is a small papule on the teat;

TABLE 21-4
Other Poxviruses of Veterinary Importance: Host Ranges and Geographical Distribution

Genus	Virus	Animals found naturally infected	Host range in laboratory animals	Geographical range, natural infections
Parapoxvirus	Pseudocowpox virus	Cattle, human	Narrow	Worldwide
	Bovine papular stomatitis virus	Cattle, human	Narrow	Worldwide
	Orf virus	Sheep, goat, human	Narrow	Worldwide
Capripoxvirus	Sheeppox virus	Sheep, goat	Narrow	Africa, Asia
	Goatpox virus	Goat, sheep	Narrow	Africa, Asia
	Lumpyskin disease virus	Cattle, buffalo	Narrow	Africa
Suipoxvirus	Swinepox virus	Swine	Narrow	Worldwide
Leporipoxvirus	Myxoma virus	Rabbit (*Oryctolagus* and *Sylvilagus*)	Narrow	Americas, Australia, Europe
Avipoxvirus	Fowlpox virus	Chickens, turkey, other birds	Narrow	Worldwide

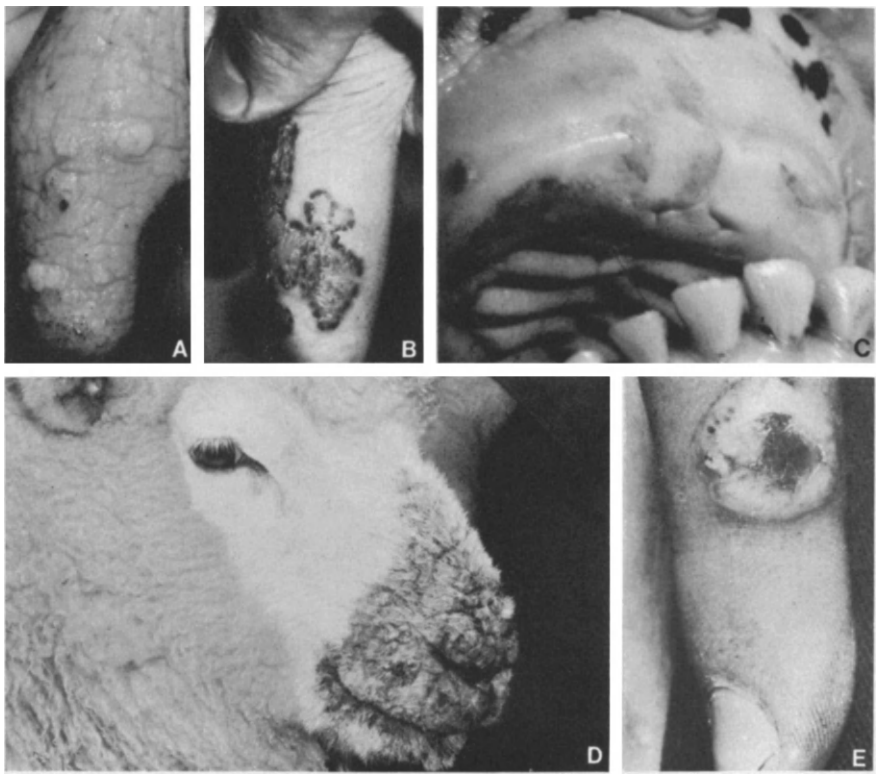

PLATE 21-2. *Parapoxvirus infections: pseudocowpox, bovine papular stomatitis, and orf in animals and humans. (A and B) Pseudocowpox lesions on teats of cow, at pustular and scab stages. (C) Bovine papular stomatitis. (D) Scabby mouth caused by orf virus, in a lamb. (E) Orf lesion on the hand of a man. (A and B, courtesy Dr. D. C. Blood; D, courtesy of Dr. A. Robinson; E, courtesy of Dr. J. Nagington.)*

this soon develops into a small, dark-red scab, the edges of which extend, while the center becomes umbilicated and then scabbed. The central part of the scab desquamates, leaving a "ring" or "horseshoe" scab that is pathognomonic for the disease (Plate 21-2B). Several such lesions may coalesce to form linear scabs. Ulceration is unusual, and the lesions usually heal within 6 weeks without scarring, although occasionally cattle develop chronic infection. Similar lesions can develop on the muzzles and within the mouths of nursing calves.

Infection is transmitted by cross-suckling of calves, improperly disinfected teat clusters of milking machines, and probably by the mechanical

transfer of virus by flies. Attention to hygiene in the milking shed and the use of teat dips reduces the risk of transmission.

Bovine Papular Stomatitis

This disease is usually of little clinical importance, but occurs worldwide, affecting cattle of both sexes and all ages, although the incidence is higher in animals less than 2 years old. The development of lesions on the muzzle, margins of the lips, and buccal mucosa is similar to that of pseudocowpox in calves (Plate 21-2C). Immunity is of short duration and cattle can become reinfected. Bovine papular stomatitis can be differentiated from other vesicular diseases by demonstration by electron microscopy of the characteristic virions in lesion scrapings.

Orf

Orf (contagious pustular dermatitis, scabby mouth) is a more important disease in sheep and goats than either pseudocowpox or bovine papular stomatitis in cattle, and is common throughout the world. Orf, which is old English for "rough," commonly involves only the muzzle and lips (Plate 21-2D), although lesions within the mouth affecting the gums and tongue can occur, especially in young lambs. The lesions can also affect the eyelids, feet, and the teats of ewes. Human infection can occur among persons occupationally exposed (Plate 21-2E).

Lesions of orf progress from papules to pustules, and then to thick crusts. The scabs are often friable, and mild trauma causes the lesions to bleed. Orf may prevent lambs from suckling. Severely affected animals may lose weight and be predisposed to secondary infections. Morbidity is high in young lambs, but mortality is usually low. Clinical differentiation of orf from other diseases seldom presents a problem, but if necessary electron microsopy can be used to confirm the diagnosis.

Sheep are susceptible to reinfection, and chronic infections can occur. These features, and the resistance of the virus to desiccation, explain how the virus, once introduced to a flock, can be difficult to eradicate. Spread of infection can be by direct contact or through exposure to contaminated feeding troughs and similar fomites, including thorny plants.

Ewes can be vaccinated several weeks before lambing, using commercial live-virus vaccines derived from infected scabs collected from sheep. These are applied to scarified skin, preferably in the axilla, where a localized lesion develops. A short-lived immunity is generated; ewes are thus less likely to develop orf at lambing time, thereby minimizing the risk of an epidemic of orf in the lambs.

DISEASES CAUSED BY CAPRIPOXVIRUSES

The genus *Capripoxvirus* (see Table 21-4) comprises sheep and goat poxvirus and lumpyskin disease virus, which affects cattle. Although the geographical distribution is different, indicating that they are distinct viruses, sheep and goatpox virus and lumpyskin disease virus are indistinguishable by conventional serology and sheeppox vaccine has been used to protect cattle from lumpyskin disease. Sheeppox and goatpox are often considered to be host-specific, but in parts of Africa where sheep and goats are herded together, strains occur which affect both species.

For presumptive diagnosis, negative-staining electron microscopy can be used to demonstrate virus particles in clinical material, the virions being indistinguishable from those of vaccinia virus. Capripoxviruses can be isolated in various cell cultures derived from sheep, cattle, or goats, in which cytoplasmic inclusions are formed.

Sheeppox and Goatpox

Sheeppox and goatpox are the most serious of all pox diseases of domestic animals, causing high mortality in young animals and significant economic loss. They occur as enzootic infections in southwestern Asia, the Indian subcontinent, and most parts of Africa except southern Africa.

Sheeppox has a documented history almost as old as that of smallpox. The disease was apparently present in Europe as early as the second century A.D., and its infectious nature was recognized in the mid-eighteenth century. Eradication was achieved in Britain in 1866, but in other areas of Europe eradication was more difficult, probably because of the extensive live-animal trade between countries.

The clinical signs vary in different hosts and in different geographical areas. Sheep and goats of all ages may be affected, but the disease is generally more severe in young animals. An epizootic in a susceptible flock of sheep can affect over 75% of the animals, with a mortality as high as 50%; case fatality rates in lambs may approach 100%.

After an incubation period of 4 to 8 days, there is a rise in temperature, an increase in respiratory rate, edema of the eyelids, and a mucous discharge from the nose. Affected sheep may lose their appetite and stand with an arched back. One to two days later, cutaneous nodules 0.5–1.5 cm in diameter develop, which may be widely distributed over the body. The nodules are most obvious in the areas of skin where

PLATE 21-3. *(A) Sheeppox and (B) goatpox in native breeds in Ghana. (Courtesy Dr. M. Bonniwell.)*

the wool or hair is shortest, such as the head, neck, ears, axillae, and the perineum (Plate 21-3). The nodules usually scab, and persist for 3 to 4 weeks, healing to leave a permanent depressed scar. Lesions within the mouth affect the tongue and gums, and ulcerate. Such lesions constitute an important source of virus for infection of other animals. In some sheep, lesions develop in the lungs, as multiple consolidated areas 0.5–2.0 cm in diameter. Goatpox is clinically similar to sheeppox (Plate 21-3B).

Sheeppox and goatpox are notifiable diseases in most countries of the world, and any clinical suspicion of disease should be reported to the appropriate authorities. Apart from occasional outbreaks in partly immune flocks—where the disease may be mild—or when the presence of orf complicates the diagnosis, sheeppox and goatpox present little difficulty in clinical diagnosis. When confirmation is required, the most rapid diagnosis is by electron microscopy.

In common with most poxviruses, environmental contamination can lead to introduction of virus into small skin wounds. Scabs that have been shed by infected sheep remain infective for several months. The common practice of herding sheep and goats into enclosures at night in the countries where the disease occurs provides adequate exposure to maintain enzootic infection. During an outbreak, the virus is probably transmitted between sheep by aerosol, and there is circumstantial evidence that mechanical transmission of virus by biting arthropods such as stable flies may also occur.

Attenuated live-virus and inactivated vaccines are available for use in countries where the diseases are enzootic.

Lumpyskin Disease

Lumpyskin disease affects cattle breeds derived from both *Bos taurus* and *Bos indicus*, and was first recognized in an extensive epizootic in Zambia in 1929. An epizootic in 1943–1944, which involved other countries including South Africa, emphasized the importance of this disease, which remained restricted to southern Africa until 1956, when it spread to central and eastern Africa. Since the 1950s, it has continued to spread progressively throughout Africa, first north to the Sudan and subsequently westward, to appear by the mid-1970s in most countries of western Africa. There are as yet no reports of lumpyskin disease in continents other than Africa.

Lumpyskin disease is characterized by fever, followed shortly by the development of nodular lesions in the skin which subsequently undergo necrosis. General lymphadenitis and edema of the limbs are common. During the early stages of the disease, affected cattle show lacrimation, nasal discharge, and loss of appetite. The skin nodules involve the dermis and epidermis; they are raised and later ulcerate and may become secondarily infected. Ulcerated lesions may be present in the mouth and nares; postmortem, circumscribed nodules may be found in lungs and alimentary tract. Healing is slow, and affected animals often remain debilitated for several months.

Morbidity in susceptible herds can be as high as 100%, but mortality is rarely more than 1 or 2%. The economic importance of the disease relates to the prolonged convalescence and, in this respect, lumpyskin disease is similar to foot-and-mouth disease; indeed it is regarded as economically more important in South Africa.

The clinical diagnosis presents few problems to clinicians familiar with it, although the early skin lesions can be confused with generalized skin infections of pseudolumpyskin disease, caused by bovine herpesvirus 2 (see Chapter 19).

It is likely that the virus is mechanically transmitted between cattle by biting insects, the virus being perpetuated in a wildlife reservoir host, possibly the African Cape buffalo. Control is by vaccination. Two vaccines are currently available; in South Africa an attenuated live-virus vaccine (Neethling), in Kenya a strain of sheep and goatpox virus propagated in tissue culture has been used successfully.

Lumpyskin disease has the potential to spread outside continental Africa. Since it is principally transmitted by insect vectors, the importation of wild ruminants to zoos in different continents could establish new foci of infection, if suitable vectors were available.

DISEASES CAUSED BY SUIPOXVIRUSES

Swinepox (see Table 21-4) is seen sporadically in swine-raising areas throughout the world. Many outbreaks of pox disease in swine have been caused by vaccinia virus, but swinepox virus, which belongs to a different genus, is now the primary cause of the disease. Swinepox is usually a mild disease with lesions restricted to the skin (Plate 21-4). Lesions may occur anywhere, but are most obvious on the belly. A transient low-grade fever may precede the development of papules which, within 1 to 2 days, become vesicles and then umbilicated pustules, 1–2 cm in diameter. The pocks crust over, and scab by 7 days; healing is usually complete by 3 weeks. The clinical signs are characteristic, and it is seldom necessary to seek laboratory confirmation.

Swinepox is transmitted between pigs by the bite of the hog louse, *Hematopinus suis,* which is common in many herds; the virus does not replicate in the hog louse. No vaccines are available for swinepox, which is most easily controlled by elimination of the hog louse from the affected herd.

DISEASES CAUSED BY LEPORIPOXVIRUSES

There are five species of virus in the genus *Leporipoxvirus* (see Table 21-4), but only myxoma virus is of veterinary importance. It constitutes a serious risk to breeders of European rabbits in California and Europe. It is also important as having provided the most successful example yet of the use of a virus to control a vertebrate pest (the wild European rabbit in Australia and Europe, see Chapter 5).

Myxomatosis

Myxoma virus causes a localized benign fibroma in wild rabbits (*Sylvilagus* species) in the Americas; in contrast, it causes a severe generalized disease in the European rabbit (*Oryctolagus cuniculus*), with a very high mortality (Plate 21-5). The characteristic early signs of myxomatosis in the European rabbit are blepharoconjunctivitis and swelling of the muzzle and anogenital region, giving the rabbit a leonine appearance. Affected rabbits are listless, febrile, and often die within 48 hours of onset of these early signs, an outcome seen especially commonly in infections due to the California strain of myxoma virus. In rabbits that survive longer, subcutaneous gelatinous swellings (hence the name myxomatosis) appear all over the body 2–3 days later. More than 99% of

PLATE 21-4. *Swinepox. (Courtesy Dr. R. Miller.)*

rabbits infected from a wild (*Sylvilagus*) source of myxoma virus die within 12 days of infection. Transmission can occur by aerosol, but is usually due to mechanical transfer of virus by biting arthropods.

Diagnosis of myxomatosis in European rabbits can be made by the clinical appearance, or virus isolation in rabbits, on the chorioallantoic membrane, or in cultured rabbit or chicken cells. Electron microscopy of

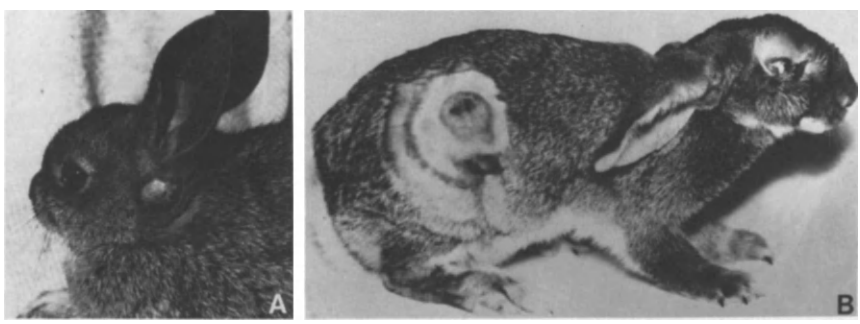

PLATE 21-5. *Myxomatosis. (A) Localized fibroma in* Sylvilagus bachmani. *(B) Severe generalized disease in* Oryctolagus cuniculus, *showing large tumor at site of intradermal inoculation on the flank, generalized lesions, and blepharoconjunctivitis. (A, courtesy Dr. D. Regnery.)*

the exudate or smear preparations of the lesions reveal virions indistinguishable from those of vaccinia virus.

Laboratory or hutch rabbits can be protected against myxomatosis by inoculation with the related rabbit fibroma virus, or with attenuated live-virus vaccines, developed in California and France.

DISEASES CAUSED BY AVIPOXVIRUSES

Poxviruses that are serologically related to each other and specifically infect birds have been recovered from lesions found in all species of poultry and many species of wild birds. Viruses recovered from various species of birds are given names related to their hosts: fowlpox, canarypox, turkeypox, pigeonpox, magpiepox, etc., viruses. As judged by their pathogenicity in various avian hosts, there seem to be a number of different species of avian poxvirus; however, no systematic analysis of restriction endonuclease patterns of their DNAs has yet been made. Mechanical transmission by arthropods, especially mosquitoes, provides a mechanism for transfer of the virus between a variety of different species of birds (see Table 21-4).

Fowlpox

Fowlpox is a serious disease of poultry that has occurred worldwide for centuries. Effective vaccines have now reduced the economic loss formerly associated with the disease. There are two forms, probably associated with different modes of infection. The most common, which probably results from infection by biting arthropods, is characterized by small papules on the comb, wattles, and around the beak (Plate 21-6); occasionally lesions develop on the legs and feet and around the cloaca. The nodules become yellowish and progress to a thick dark scab. Multiple lesions often coalesce. Involvement of the skin around the nares may cause nasal discharge, and lesions on the eyelids can cause excessive lacrimation and predispose poultry to secondary bacterial infections. In uncomplicated cases, healing occurs within 3 weeks.

The second form of fowlpox is probably due to aerosol infection and involves infection of the mucous membranes of the mouth, pharynx, larynx, and sometimes the trachea. This is often referred to as the "diphtheritic" form of fowlpox because the lesions, as they coalesce, result in a necrotic pseudomembrane which can cause death by asphyxiation. The prognosis for this form of fowlpox is poor.

Extensive infection in a flock may cause a slow decline in egg produc-

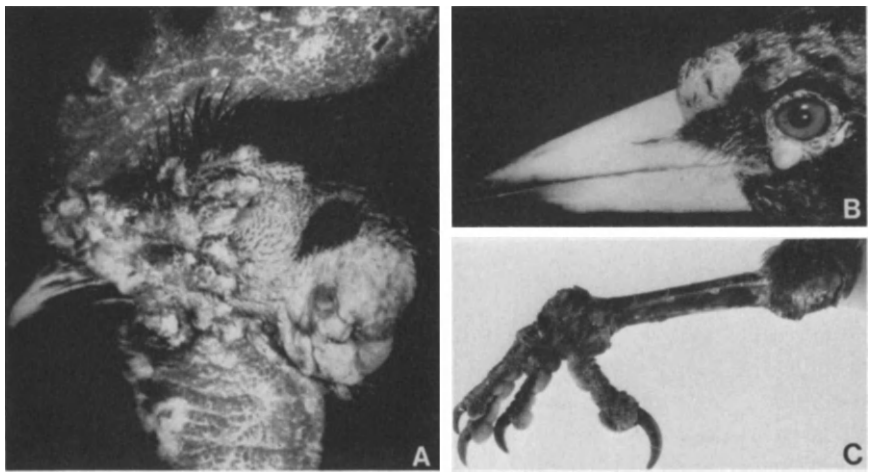

PLATE 21-6. *Avian poxvirus diseases. (A) Fowlpox. (B and C) Poxvirus infection in an Australian magpie. (B) Lesions at the base of the beak and under the eye. (C) Lesions on the foot. (B and C, courtesy K. E. Harrigan.)*

tion. Cutaneous infection causes little mortality, and these flocks return to normal production upon recovery. Recovered birds are immune.

The cutaneous form of fowlpox seldom presents a diagnostic problem. The diphtheritic form is more difficult to diagnose because it can occur in the absence of skin lesions, and it may be confused with vitamin A deficiency and several other respiratory diseases caused by viruses. Electron microscopy can be used to confirm the clinical diagnosis. The virus can be isolated by inoculation of avian cell cultures or the chorioallantoic membrane of the developing chick embryo.

Fowlpox is transmitted within a flock by direct contact with infected birds, by movement of birds into contaminated buildings, and by the mechanical transfer of virus on the mouthparts of mosquitoes, lice, and ticks. If not infected by arthropod vectors, many birds become infected through small abrasions in the skin and possibly by inhalation of virus.

Chickens can be protected by vaccination with pigeonpox virus (another avian poxvirus which is less virulent for chickens), which is applied by light scarification of the skin of the thigh. Attenuated fowlpox vaccines are also available, but produce a more severe reaction, and in Germany fowlpox vaccines have been developed which can be administered in drinking water. In enzootically infected flocks, poultry should be vaccinated during the first few weeks of life and again 8–12 weeks later.

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CHAPTER 22

Parvoviridae

Members of the family *Parvoviridae* have a small icosahedral virion and a genome of ssDNA. There are three genera: *Parvovirus*, members of which infect vertebrates and replicate autonomously; *Dependovirus*, which are defective and depend on a helper virus, usually an adenovirus, for their replication; and *Densovirus*, all members of which infect insects. Only the genus *Parvovirus* will be further discussed; these viruses replicate selectively in cycling cells and cause important diseases in swine, cats, dogs, mink, and geese (Table 22-1).

The parvoviruses responsible for infections in cats, dogs, and mink are closely related. Porcine parvovirus infection is usually subclinical but may affect the fetus; goose parvovirus causes a lethal disease in goslings. Rodent parvoviruses are used as models in studies of the pathogenesis of certain fetal abnormalities, and they are common contaminants of cultured rodent cells and tumors. A human parvovirus has recently been incriminated as the cause of an exanthematous disease, erythema infectiosum.

TABLE 22-1
Diseases of Domestic Animals Caused by Parvoviruses[a]

Virus	Disease
Porcine parvovirus	Stillbirth, abortion, fetal death, mummification, and infertility
Feline panleukopenia virus	Cerebral hypoplasia, panleukopenia, enteritis
Canine parvovirus	Generalized neonatal disease, enteritis, myocarditis, panleukopenia
Mink enteritis virus	Panleukopenia, enteritis
Aleutian mink disease virus	Chronic immune complex disease, encephalopathy
Goose parvovirus	Hepatitis

[a]Parvoviruses have also been recovered from cattle and from laboratory rodents, but under natural conditions are not known to cause disease in these species.

PROPERTIES OF PARVOVIRUSES

The virion of the parvoviruses (Table 22-2) is about 20 nm in diameter, and consists of an icosahedral capsid, probably of 32 capsomers, surrounding a (−) sense ssDNA genome, 5.2 kb in size (Plate 22-1). Parvoviruses of all genera may package either a (−) sense or a (+) sense strand, in proportions ranging from 50 : 50 to 99 : 1, but members of the genus *Parvovirus* preferentially package (−) sense DNA. The complete nucleotide sequences of several parvoviruses are now available.

Parvoviruses are remarkably stable to environmental conditions (extremes of heat and pH), hence, disinfection of contaminated premises is difficult.

VIRAL REPLICATION

Members of the genus *Parvovirus* replicate in the nucleus of cycling cells. They require cell functions which are generated during late S or early G2 phases of the cell cycle (Fig. 22-1A). Because of their small size, parvoviruses has been intensively studied as a model for understanding DNA replication and gene expression. Two main overlapping transcription units have been identified, from which three major mRNA species are transcribed. The mRNAs have a common 3′ terminus, and are polyadenylated and spliced. The most abundant mRNA codes for structural proteins late in infection, the relevant information mapping in the right half of the genome (Fig. 22-1B), whereas the information for a nonstructural protein involved in DNA replication resides in the left half of the DNA. There are three structural proteins—VP1, 2, and 3—all derived

TABLE 22-2
Properties of Parvoviruses

Icosahedral virion, 18–26 nm diameter, probably 32 capsomers
(−) sense ssDNA genome, 5.2 kb
Replicate in nucleus of cycling cells; large eosinophilic inclusion bodies
Resist 60°C for 60 minutes and pH 3–pH 9
Genus *Parvovirus*, replication-competent; *Dependovirus*, defective, requires helper *Adenovirus*; *Densovirus*, infects insects
Most hemagglutinate

from a common sequence, which is read in the same frame. VP3, the most abundant (80% of the protein mass), is derived from VP2 by post-translational cleavage.

DNA replication occurs via a double-stranded replicative form, initiated by a self-priming mechanism via the 3′-terminal palindromic sequence (Fig. 22-1B). Following DNA replication, viral capsids assemble, into which progeny ssDNA is packaged.

Parvoviruses produce large intranuclear inclusion bodies (Plate 22-1C).

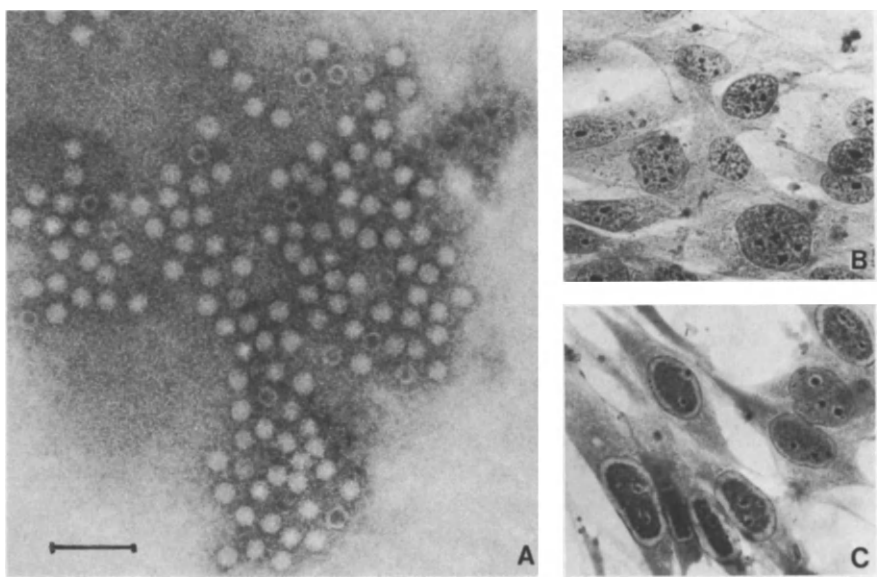

PLATE 22-1. Parvoviridae. *(A) Negatively stained virions of canine parvovirus (bar = 100 nm). (B) Normal feline embryo cells. (C) Feline embryo cells infected with feline panleukopenia virus. All nuclei show inclusion bodies in various stages of development (H and E stain; ×525). [B and C, from M. J. Studdert* et al., Vet. Rec. **93,** *156 (1973).]*

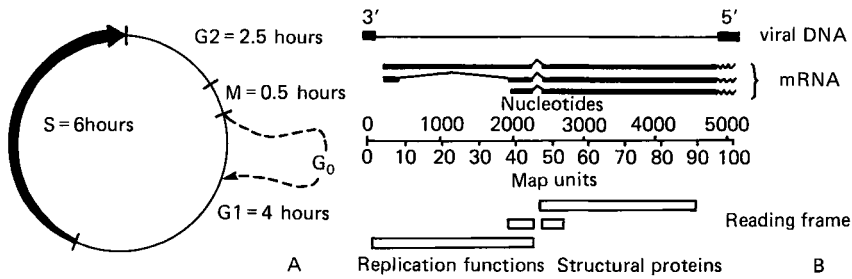

FIG. 22-1. *(A) Phases of the cell cycle of feline embryo cells. The total cell cycle time is 13 hours. Autonomous parvovirus replication depends on events that occur in the S phase. (B) Diagram illustrating the organization of the genome of a* Parvovirus. *The palindromic termini (thick lines) occupy approximately 2 map units at the left and 4 map units at the right-hand end of the viral DNA. The three major cytoplasmic transcripts are represented by thick black lines; the thin lines indicate the introns spliced out of the mature mRNA. The open reading frames are shown in open blocks. [From S. F. Cotmore* et al., Virology **129,** *333 (1983).]*

PATHOGENESIS AND IMMUNITY

The pathogenesis of parvoviruses is determined by the requirement for cycling cells for viral replication. Following infection of the fetus (pig or cat) or newborn (dog or cat), the virus may be pantropic, infecting a wide range of cells in various tissues and organs. In older animals a narrower range of cells is affected; the degree of cell cycling in a particular organ or tissue and the presence of appropriate virus receptors on the cells are probably the important factors determining susceptibility. Both are influenced by the age of the animal. Thus the cerebellum is selectively destroyed in feline fetuses or kittens infected in the perinatal period, and the myocardium is highly susceptible in pups 3–8 weeks of age and goslings 0–2 weeks of age. At all ages, the continuous division of cells of the lymphoid tissue and intestinal epithelium renders them highly susceptible, hence the common occurrence of panleukopenia, with concomitant immunosuppression, and enteritis.

Following natural infection there is a rapid immune response. Different antigenic determinants appear to be involved in neutralization and hemagglutination inhibition. Neutralizing antibody can be detected within 3 to 5 days of infection and may rise to very high titers before there is clinical recovery. The presence of high-titer antibody is correlated with protection, and immunity after natural infection appears to be lifelong. In cats and dogs some maternal antibody may be transferred transplacentally, but most is transferred with colostrum. The titer of

natural passive antibody in kittens or pups parallels the maternal antibody titer and is therefore quite variable, providing protection for only a few weeks or for as long as 22 weeks. Cytotoxic T cells are generated after both infection and vaccination.

PORCINE PARVOVIRUS INFECTIONS

Porcine parvovirus was originally identified as a contaminant in cell cultures prepared from apparently healthy piglets. It was also recognized as a contaminant of cell cultures of nonporcine origin; the source of such contamination was traced to trypsin prepared from swine pancreases (widely used in the preparation of cell cultures), in which it survived because of the great stability of parvoviruses. There is only a single serotype of porcine parvovirus.

Disease in Swine

Porcine parvovirus infection is an important disease of swine, and the virus has been isolated in association with reproductive failure in swine, which includes *s*tillbirth, *m*ummification, *e*mbryonic *d*eath, *i*nfertility (Plate 22-2), (in respect of which the acronym SMEDI has been applied), abortion, neonatal death, and low fertility of boars. Virus has been recovered from semen and vaginal mucus, and from diseased piglets as well as from the tissues of healthy piglets.

Serological surveys show that the incidence of infection is far higher than the incidence of reported disease. SMEDI is most noticeable when the virus is first introduced into a herd, and is then an important component of fetal loss. For the many herds in which the virus remains enzootic, losses are less serious or obvious. Inactivated vaccines are used in selected herds to control reproductive losses.

FELINE, CANINE, AND MINK PARVOVIRUS INFECTIONS

Feline panleukopenia has been recognized for about 100 years, mink enteritis was first described in 1949, and canine parvovirus emerged as a new pathogen in 1978. The three parvoviruses that cause these diseases are closely related; the mink and canine viruses appear to have arisen as host range mutants of feline parvovirus. The diseases in cats, mink, and dogs are remarkably similar, particularly in respect of panleukopenia and enteritis. In animals infected in the perinatal period, the feline virus produces cerebellar hypoplasia while the canine virus produces myocarditis.

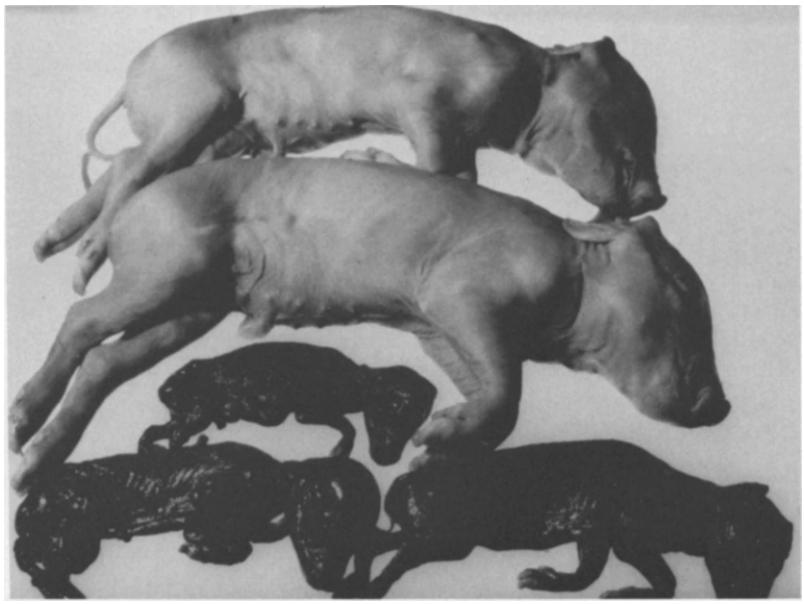

PLATE 22-2. *Porcine parvovirus infection. Infected fetuses in various stages of mummification, compared with normal fetuses. (Courtesy Dr. R. H. Johnston and Dr. H. S. Joo.)*

Feline and mink parvoviruses are indistinguishable antigenically by hemagglutination inhibition and serum neutralization tests and by DNA analysis with restriction endonucleases; though closely related, canine parvovirus can be distinguished from the other viruses by these criteria. Most strains of feline and mink parvovirus hemagglutinate pig and rhesus monkey red blood cells to low titer at 4°C at pH 6.5, the hemagglutination patterns being unstable at room temperature. On the other hand, under the same conditions canine parvovirus agglutinates these cells to high titer and the hemagglutination patterns are stable at room temperature.

Feline Panleukopenia

It is thought that all Felidae are susceptible to infection with feline panleukopenia virus, which is probably the most important of all feline viral infections and occurs worldwide.

Clinical Signs. Feline panleukopenia is most common in kittens about the time of weaning, but cats of all ages are susceptible. Infection usually occurs via the oral route but may also be acquired by inhalation. The incubation period averages 5 days (range 2–10 days). Beginning 2–5

days after infection, leukopenia develops and is most marked at 5 to 6 days after infection, when there may be fewer than 100 circulating white blood cells per cubic millimeter. The severity of clinical disease and the mortality rate parallel the severity of the leukopenia. Clinical signs include fever (>40°C) which persists for about 24 hours and during which, in the peracute form of the disease, death occurs. The temperature returns to normal and rises again on the third or fourth day, at which time clinical illness is apparent, with lassitude, inappetance, a rough coat, and repeated vomiting. A profuse, persistent, frequently bloody diarrhea develops 2–4 days after the initial fever. Dehydration is a major contributing factor to death. The prognosis is grave if total white blood cell counts fall below 1000 cells per cubic millimeter. For reasons that are not well understood, the severe disease often observed after natural infection is not seen following experimental infection.

Kittens which have been infected from 2 weeks before to about 2 weeks after birth develop cerebellar hypoplasia (see Plate 22-3B). Affected kittens are noticeably ataxic when they become ambulatory at about 3 weeks of age; they have a wide-based stance and move with exaggerated steps, tending to overshoot the mark and to pause and oscillate about an intended goal.

Pathogenesis. Following initial proliferation in pharyngeal lymphoid tissue, virus is distributed to all organs and tissues via the bloodstream, with infection and lysis of cells which have appropriate receptors and are cycling, since virus replicates in cells in the S phase of the cell cycle. Following viral replication, these cells are blocked from entering mitosis. There may be profound panleukopenia in which all white blood cell elements—lymphocytes, granulocytes, monocytes, and platelets—are destroyed, both those present in the circulation and those in the lymphoid organs, including thymus, bone marrow, lymph nodes, spleen, and Peyer's patches. Paradoxically, not all white blood cells are dividing at any particular point in time, yet all are affected by the virus. For example, mature polymorphonuclear cells are nondividing end cells, and mature small lymphocytes normally divide only after stimulation by a specific antigen. It is possible that the virus acts as a universal mitogen for lymphocytes. Alternatively, the presence of virus bound to the surface of cells may render them a target for cytotoxic lysis by T_c, NK, and K cells. The majority of cells in the primary lymphoid tissues, notably in the thymus, are cycling; they are presumably destroyed by lysis following viral replication. Polymorphonuclear leukocytes may be lost across the gut wall.

The rapidly dividing intestinal epithelial cells in the crypts of Lieberkuhn are also very susceptible. Epithelial cells at the tips of the intestinal

villi are continuously lost into the lumen of the gut and are normally replaced by the division of the cells in the crypts, the cell cycle time for which is 8–12 hours. After feline parvovirus infection the normal loss of cells from the villus tips and the failure of replacement with cells from the crypts leads to greatly shortened, nonabsorptive villi, and hence to the rapid accumulation of fluid and ingesta in the gut lumen and thus to diarrhea. Where the clinical course is more prolonged, dehydration is a major factor contributing to death; most cats given adequate fluid therapy will recover.

At necropsy, lesions in the small intestine are usually patchy in their distribution; the entire small intestine needs to be carefully examined for evidence of slight congestion and thickening, which is visible from the serosal as well as the luminal surface. When enteritis has been present for several days before death the intestinal lesions are usually obvious and consist of segments in which the gut wall is greatly thickened into a rigid, hoselike structure. The lymph nodes may be enlarged and edematous. The bone marrow, for example at the proximal end of the femur, normally a jelly-firm red cylinder, may be pale and fluid, so that it can be poured from the marrow cavity.

Histologically, the lesions in affected pieces of gut are characteristic. The villi are greatly shortened and blunted; necrotic but still adherent cells may be present at the tips of the villi. The crypts are dilated and distended with mucus and cell debris. Rarely, intranuclear inclusions may be found in cells near the base of the crypts. There is evidence of widespread destruction of lymphoid cells and massive infiltration with polymorphonuclear cells.

In fetuses infected during the last 2 weeks of pregnancy or the first 2 weeks of life, lesions are found only in cells of the external granular layer of the cerebellum. During this period of development, these cells undergo rapid cell division and migrate to form the internal granular and Purkinje cell layers of the cerebellum, which control motor functions. Like the young of most animal species, the newborn kitten is somewhat ataxic for at least a week after birth. Motor neurons that are destroyed by feline parvovirus are not replaced, hence affected kittens remain permanently ataxic. However, the precision with which feline parvovirus destroys cells of the cerebellum may be apparent rather than real, for there is evidence that perinatal infection of kittens may indeed produce a generalized infection. Infected cells other than those of the cerebellum are apparently replaced without permanent damage. The high immunogenicity of the virus leads to a rapid immune response, which may be significant in limiting the extent of cell damage associated with these generalized infections.

Diagnosis. The clinical disease and postmortem findings are characteristic and usually suggest the diagnosis. Confirmatory procedures include hematological examination to demonstrate leukopenia, the direct hemagglutination of pig or rhesus monkey red blood cells by suitably prepared fecal samples, or isolation of the virus in cell culture. Fluorescent-antibody staining may be used for detection of antigen, and ELISA for detection of either antigen or antibody.

Epidemiology. Feline panleukopenia is highly contagious. The virus may be acquired by direct contact with other cats or via fomites (bedding, food dishes); fleas and humans may act as mechanical vectors. Virus is shed in the feces, vomit, urine, and saliva. The incidence of specific antibody in the cat population is much higher than the combined reported incidence of clinical disease and vaccination, indicating that subclinical infection is common. Recovered cats may excrete small amounts of virus for many months. Neither the exact duration of such excretion nor the underlying basis for persistent infection has been determined.

The stability of the virus, which survives exposure to 60°C for as long as 60 minutes, and the very high rates of viral excretion (up to 10^9 virus particles per gram of feces) result in persistent high levels of environmental contamination; hence, it may be extremely difficult to disinfect contaminated premises. Because of this, close contact between cats is not essential for transmission. The virus may be acquired from premises following the introduction of susceptible cats months, even up to a year, after previously affected cats have been removed. There is also evidence that the virus may carry a considerable distance (>200 m) on windblown fomites.

Control. Vaccination is universally practiced, both attenuated live-virus and inactivated vaccines being used.

In large catteries strict hygiene and quarantine of incoming animals are essential. Sick cats should be isolated and cats introduced into the colony should be held in isolation for several weeks before introduction to the main cattery. For disinfection 1% sodium hypochlorite applied to clean areas will destroy residual contaminating virus, but it is ineffective if the area is dirty or if the viral concentration is high. Organic iodine and phenolic disinfectants are also used.

Canine Parvovirus Disease

Canine parvovirus disease, caused by canine parvovirus 2, was first recognized in 1978, when it caused a worldwide panzootic. Subse-

TABLE 22-3
Relationship between Age of Host and Occurrence of Various Disease Syndromes after Feline Panleukopenia Virus or Canine Parvovirus Infection

Syndrome	Animal species	Age
Generalized neonatal disease	Cat and dog	2–12 days
Enteritis	Cat and dog	
Panleukopenia/enteritis		2–4 months
Enteritis		4–12 months
Cerebella hypoplasia	Cat	2 weeks before birth–4 weeks
Myocarditis	Dog	
Acute		3–8 weeks
Chronic		8 weeks

quently it has become enzootic in dogs throughout the world. Canine parvovirus 1 (also called minute canine virus) was identified in the feces of dogs in 1967 but has not been confirmed as a major cause of disease, although mild diarrhea may occur. It is antigenically and genetically quite distinct from the virus that emerged as a major new pathogen of dogs in 1978.

Clinical Signs. Three distinct age-related syndromes have been recognized in dogs (Table 22-3). Generalized neonatal disease is rare. The panleukopenia–enteritis syndromes precisely parallel the same syndromes in cats, but the cerebellar hypoplasia found in cats has not been recognized in dogs. Myocarditis due to canine parvovirus infection is usually recognized as an acute disease of pups characterized by sudden death, usually without any clinical signs. However, even though the lesions are extensive, the pup may survive with a scarred myocardium (Plate 22-3C). Although not necessarily producing abnormal electrocardiograms, it might be expected that such disease would limit the capacity for the dog for high performance and prolonged life.

Pathogenesis. The pathogenesis of canine parvovirus infections in the dog is similar to that of feline parvovirus infections of the cat, but the absence of cerebral hypoplasia and the occurrence of myocarditis is somewhat surprising, since myocardial cells are not normally considered as rapidly cycling. Like cerebellar hypoplasia in the cat, myocarditis in the dog is probably the result of a generalized infection.

Diagnosis. The simplest procedure for the laboratory diagnosis of canine parvovirus infection is hemagglutination of pig or rhesus monkey red blood cells (pH 6.5, 4°C) by fecal extracts, titrated in parallel in the presence of normal and immune dog serum. Fecal samples from

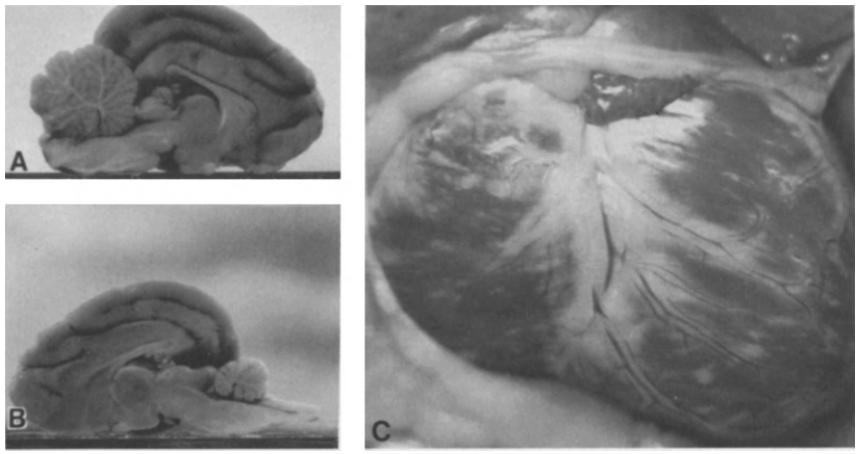

PLATE 22-3. *Cerebellar hypoplasia caused by feline parvovirus infection in a 3-month-old kitten (B) and matched control (A). (C) Canine parvovirus myocarditis, showing scar tissue throughout the myocardium. [From C. Lenghaus and M. J. Studdert,* Am. J. Pathol. **115,** *316 (1984).]*

dogs with acute enteritis may contain up to 20,000 HA units of virus per milliliter, which is the equivalent of about 10^9 virions per gram of feces. Electron microscopy, virus isolation, and ELISA procedures can also be used for laboratory confirmation of the clinical diagnosis.

Epidemiology. When canine parvovirus disease was first recognized in 1978, the canine population all over the world was completely susceptible. Generalized neonatal disease was then rarely recognized, but myocarditis and panleukopenia–enteritis were common. Like cerebellar hypoplasia in cats caused by feline parvovirus, myocarditis is now rare. With few exceptions, passively derived maternal antibody protect pups beyond the 2-week and the 8-week periods which appear to be the age limits for the development of generalized neonatal disease and myocarditis, respectively. Canine parvovirus disease, occurring predominantly as panleukopenia–enteritis, is now an enzootic worldwide disease of dogs, its epidemiology being similar to that of feline panleukopenia.

Control. Major control problems are encountered in large, crowded breeding colonies where hygiene is difficult to implement and maintain. Subclinical infections are common particularly in single, well-cared-for pups, emphasizing the importance of hygiene and general good health in limiting the occurrence of clinical disease. While vaccination with either attenuated live-virus or inactivated vaccines is effective, considerable problems are encountered in devising effective vaccination sched-

ules because of the variable levels of maternal antibodies transferred to pups (see Chapter 10).

Mink Enteritis

Mink enteritis and Aleutian disease of mink are caused by two antigenically different parvoviruses. The virus causing mink enteritis is closely related to the feline panleukopenia virus and produces in mink syndromes similar to panleukemia in cats, except that cerebellar hypoplasia has not been recognized.

Origins of Mink and Canine Parvoviruses

The restriction endonuclease maps of mink enteritis virus and feline panleukopenia virus are virtually identical, and it has been suggested that the feline parvovirus was introduced into mink in Ontario, Canada, about 1946. The origin of canine parvovirus is more intriguing but less clear, in part because of its apparently simultaneous appearance in five continents in 1978. Restriction endonuclease mapping reveals that strains of canine parvovirus recovered from all over the world in 1978 are identical, but differ in about 15% of restriction sites from wild-type and most vaccine strains of feline parvovirus. However, one feline vaccine strain shows a closer resemblance to canine parvovirus. Since the disease appeared in dogs in Australia in 1978 and there is a 6 months quarantine for the entry of dogs into that country, the mutant feline virus probably arrived with an international traveller, perhaps as a contaminant on shoes or other fomites.

Aleutian Disease of Mink

This is a chronic disease characterized by plasmacytosis, hypergammaglobulinemia, splenomegaly, lymphadenopathy, glomerulonephritis, arteritis, focal hepatitis, anemia, and death. The lesions are the result of the continued production of virus and a failure to eliminate virus–antibody complexes; the persistence of virus induces a virus-specific plasmacytosis, antibody-specific hypergammaglobulinemia, and immune complex-mediated disease. It occurs primarily in mink that are homozygous for the recessive pale ("Aleutian") coat color. This commercially desirable light-pelt color gene is linked to a gene associated with a lysosomal abnormality of the Chediak–Higashi type, whereby following phagocytosis, immune complexes are not destroyed. This defect is the key to the pathogenesis of Aleutian disease. The level of the hypergammaglobulinemia is cyclic, death occurring during a peak re-

sponse, between 2 and 5 months after infection. Many of the pathological findings at postmortem are mediated by immune complexes, with segmental periarteritis, often associated with hemorrhages, and renal failure due to immune complex glomerulonephritis, which is the usual cause of death.

Despite the extremely high levels of virus-specific antibody, this does not neutralize infectivity and infectious virus can be recovered from immune complexes. Immunization of mink carrying the Aleutian gene with killed-virus vaccine increases, and immunosuppression diminishes, the severity of the disease.

GOOSE PARVOVIRUS INFECTIONS

Goose parvovirus causes a highly lethal disease of goslings 8–30 days old, which is characterized by focal or diffuse hepatitis and widespread acute degeneration of striated and smooth muscle, including myocardium. Inclusion bodies are found mainly in the liver, but also in spleen, myocardium, thymus, thyroid, and intestines. Control is achieved by the vaccination of laying geese with attenuated live-virus vaccine; after which antibody persists in goslings for at least 4 weeks.

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CHAPTER 23

Picornaviridae

Four genera are included in the family *Picornaviridae*, each of which contains viruses causing diseases in domestic animals (see Table 23-2). The sigla "picornavirus" is derived from *p*oliovirus, *i*nsensitivity to ether, *c*oxsackievirus, *o*rphan virus, *r*hinovirus, and *ri*bo*n*ucleic *a*cid (the two *r*'s are contracted to one). Moreover, since the term "pico" is used to denote small measurements, the word *picornavirus* is an appropriate description for a group of small RNA viruses. Hidden within this prosaic nomenclature are milestones in the history of virology. In 1897, Loeffler and Frosch showed that foot-and-mouth disease was caused by an agent that passed through filters that held back bacteria; this was the first demonstration that a disease of animals could be produced by a filterable agent. The introduction of cell culture techniques in the 1950s and the development of genetically engineered and synthetic vaccines in the 1980s were closely linked to the study of picornaviruses, and very recently they became the first animal viruses for which the full three-dimensional atomic structure of the virion was determined.

Many developed nations have controlled several of the serious picornaviral diseases of humans and animals, such as poliomyelitis and foot-

and-mouth disease. Worldwide, however, picornaviruses still create major problems in human and veterinary medicine.

PROPERTIES OF PICORNAVIRUSES

The picornavirion is a nonenveloped icosahedron 25–30 nm in diameter (Plate 23-1). The capsid, appearing smooth and round in outline, is constructed from 60 capsomers, each comprising a single molecule of each of four polypeptides: VP1, 2, and 3, each of about 30K, and VP4, of about 10K (see Fig. 4-9). Though less immunogenic than intact inactivated virions, preparations of purified VP1 elicit neutralizing antibody, a discovery that has provided a major impetus for the development of recombinant DNA and synthetic peptide vaccines for foot-and-mouth disease (see Chapter 14). Minor polypeptides of unknown function have been reported to occur in the virions of many picornaviruses. That of foot-and-mouth disease virus contains small amounts of a virus-coded component of the RNA-dependent RNA polymerase. Some properties of the picornaviruses are set out in Table 23-1.

The family *Picornaviridae* is divided into four genera: *Enterovirus, Cardiovirus, Rhinovirus,* and *Aphthovirus* (Table 23-2). The most important differences between the physicochemical properties of the virions of viruses of the four genera are in their pH susceptibility. The aphthoviruses are unstable below pH 7, the rhinoviruses lose activity below pH 5, and the enteroviruses and cardioviruses are stable at pH 3. The cardioviruses can be distinguished from the enteroviruses by their biphasic

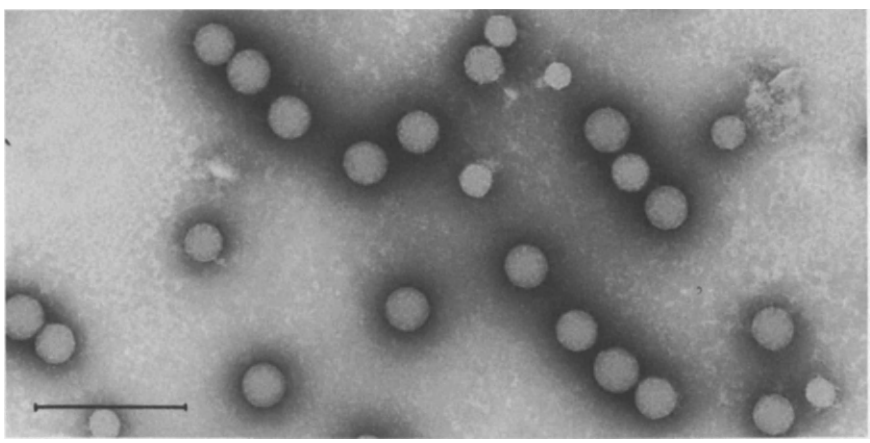

PLATE 23-1. Picornaviridae. *Electron micrographs of negatively stained virions of foot-and-mouth disease virus (bar = 100 nm). (Courtesy Dr. S. H. Wool.)*

TABLE 23-1
Properties of Picornaviruses

Nonenveloped icosahedral capsid, 25–30 nm in diameter, 60 capsomers
Linear, (+) sense ssRNA genome, 7.5–8.5 kb, polyadenylated at 3′ end, protein VPg at 5′ end, infectious
Cytoplasmic replication
Virion RNA acts as mRNA and is translated into a polyprotein which is then cleaved

pH stability in the presence of 0.1 *M* halide ions. Though stable at pH 3, cardioviruses are labile between pH 5 and 6. Another important difference is the presence of a polycytidylic acid tract, of unknown function, in the genome of the aphthoviruses and cardioviruses, but not in that of either enteroviruses or rhinoviruses.

There are a very large number of picornaviruses, especially in the genera *Enterovirus* and *Rhinovirus*, each antigenically distinct, as determined by neutralization tests.

The stability of picornaviruses to environmental conditions is impor-

TABLE 23-2
Diseases of Domestic Animals Caused by Picornaviruses

Genus	Virus	Principal species affected	Disease
Enterovirus	Bovine enteroviruses, types 1–7	Cattle	Mostly subclinical infections
	Porcine enteroviruses, types 1–11	Swine	Infection frequently subclinical but type 1 causes polioencephalomyelitis
	Swine vesicular disease virus	Swine	Swine vesicular disease
	Avian enteroviruses	Chickens	Avian encephalomyelitis
		Ducks	Hepatitis
		Turkeys	Hepatitis
Cardiovirus	Encephalomyocarditis virus	Mammals in contact with rodents	Rarely, encephalomyocarditis
Rhinovirus	Bovine rhinoviruses types 1–3	Cattle	Rhinitis
	Equine rhinoviruses types 1–3	Horses	Mild rhinitis; viremia occurs
Aphthovirus	Foot-and-mouth disease viruses: 7 types, 80 subtypes	Cattle, sheep, goats and pigs; ruminant wildlife	Foot-and-mouth disease

tant in the epidemiology of the diseases they cause and in the selection of methods of disinfection. If protected by mucus and shielded from strong sunlight, picornaviruses are relatively heat stable at normal ambient temperatures. Enteroviruses may survive several days and often weeks in feces. Aerosols of rhinoviruses and aphthoviruses are less stable, but under conditions of high humidity and low levels of UV light the viruses may remain viable for several hours. Because of differences in their pH stability, sodium carbonate (washing soda) is effective against the aphthoviruses of foot-and-mouth disease, but it is not a suitable disinfectant against the enterovirus of swine vesicular disease.

VIRAL REPLICATION

Early in the study of viral replication, poliovirus became the preferred model for the analysis of the replication of RNA viruses. The work of Baltimore in particular has provided us with a detailed description of the poliovirus genome, and of the mechanism of RNA replication, post-translational proteolytic processing of polyproteins, and the morphogenesis of simple icosahedral virions. Study of foot-and-mouth disease virus parallels that of poliovirus. These processes are described fully in Chapter 4, but are briefly summarized here.

Following adsorption, penetration, and uncoating, VPg is removed from the virion RNA by cellular enzymes. The virion RNA, acting as mRNA, is translated without interruption into a polyprotein. This is then cleaved into four primary products, which are further cleaved into smaller proteins (Fig. 23-1). One of the primary cleavage products is

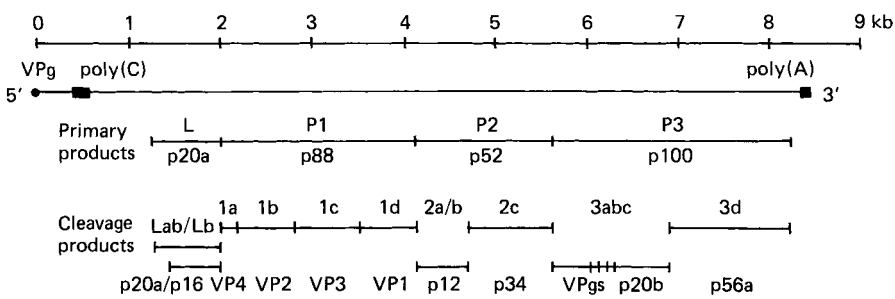

FIG. 23-1. *Organization of the genome of foot-and-mouth disease virus, showing the 5' terminal VPg, the sites of polycytidylic and polyadenylic acid sequences, the primary products of translation, and the cleavage products. The lettering above the lines referring to polypeptides shows the new nomenclature (post-1983), and the lettering below, the old nomenclature. [Modified from A. R. Carroll* et al., Nucleic Acids Res. **12,** *2461 (1984).]*

cleaved into the four structural proteins, and another is the precursor of the viral moiety of the RNA polymerase. The function of the other two primary polypeptides is unknown. The RNA polymerase transcribes a complementary [(−) sense] strand that in turn serves as a template for the synthesis of new (+) sense strands. These (+) sense strands then act as additional mRNAs for the further synthesis of viral proteins or are incorporated into progeny virions.

Picornaviruses are unique among the RNA viruses in that when a cell is doubly infected with two distinguishable strains of the same species of virus, their genomes may undergo intramolecular recombination, a feature that has been demonstrated with both foot-and-mouth disease virus and poliovirus.

APHTHOVIRUSES: FOOT-AND-MOUTH DISEASE

Viruses of the genus *Aphthovirus* cause one disease, foot-and-mouth disease, which is a major world problem. At one time or another, foot-and-mouth disease has occurred in most parts of the world, often causing extensive epidemics in domestic cattle, sheep, and swine (Table 23-3). Many species of wildlife are also susceptible. Mortality is low but morbidity is high; convalescence of affected animals is protracted and it is this feature that makes foot-and-mouth disease so important, especially when the virus is introduced into countries previously free of disease.

During the nineteenth century foot-and-mouth disease was widely reported in Europe, Asia, Africa, South and North America, and occurred on one occasion in Australia. From 1880 onward, the control of rinderpest (see Chapter 27) and the improved husbandry in the livestock industries in Europe focused attention on foot-and-mouth disease. Its sequelae were found to be more important than the acute illness. In dairy herds the febrile disease resulted in the loss of milk production for the rest of the lactation period, and mastitis often resulted in a permanent loss of more than 25% of milk production; the growth of beef cattle was retarded. Today, several countries have either eradicated foot-and-mouth disease by compulsory slaughter of infected animals or have greatly reduced its incidence by extensive vaccination programs (see Fig. 16-1).

Seven serotypes of foot-and-mouth disease virus have been identified by cross-protection tests and serological tests such as complement fixation, virus neutralization, and, more recently, ELISA. In order of identification, they were designated O (Oise) and A (Allemagne) in 1922; C (in anticipation that O and A would be renamed to allow recognition of

TABLE 23-3
Geographical Distribution of Foot-and-Mouth Disease

Region	Types	Status
South America	O, A, C	Endemic in most countries but vaccination practiced; not currently seen in Surinam and Guyana, French Guyana, and parts of Chile, Argentina, and Colombia
Europe	O, A, C	Incidence low in continental Europe where comprehensive vaccination program exists; United Kingdom, Eire, Sweden, Norway, and Iceland free of infection and no vaccination practiced
Africa	O, A, C SAT 1, 2, 3	Endemic in most countries; predominance of European types (O, A, C) in north and SAT 1, 2, 3 south of the Sahara; vaccination programs in a few countries only
Asia	O, A, C Asia 1	Endemic in countries of Near East, Far East, and Central Asia; vaccination programs in some countries; Japan free of infection
North and Central America	—	Disease-free; last outbreaks: United States, 1929; Canada, 1951; Mexico, 1954
Caribbean	—	Disease-free; last outbreaks: Curacao, 1961; Guadeloupe 1964
Oceania	—	Australia and New Zealand free of infection

further types A, B, C, etc.) in 1926; SAT 1, SAT 2, SAT 3 (South African territories) from 1952–1958, and Asia 1 in 1957. RNA hybridization tests clearly divide the types into two groups: (1) O, A, C, and Asia 1, and (2) SAT 1, 2, and 3, with 60 to 70% homology within each group and only 25–40% homology between groups.

Historically, each type has been further subtyped on the basis of quantitative differences in cross-protection and serological tests. Antigenic variation within a type occurs as a continuous process of antigenic drift without clear-cut demarcations between subtypes. This antigenic heterogeneity facilitates tracing of the epizootic spread of foot-and-mouth disease viruses, and has important economic implications for vaccine development and selection; immunity acquired through infection or vaccination is strictly type specific and, to a lesser degree, subtype specific. Difficulty in defining the threshold at which a new isolate should be given subtype status has always been a problem, and current attitudes reflect a pragmatic approach. New strains are now compared with the established vaccine strains of commercial producers, thereby avoiding the complexity of classifying new isolates within an ever-in-

creasing catalog of subtypes, many of which have little relevance to current problems in the field.

Clinical Features

Aphthoviruses infect a wide variety of cloven-hoofed domestic and wild animal species. Although the horse is refractory to infection, cattle, water buffalo, sheep, goats, llamas, camels, and swine are susceptible and develop clinical signs. In addition to domestic farm animals, more than 70 species of wild mammals belonging to more than 20 families are known to be susceptible to either natural or experimental infection.

An outbreak of foot-and-mouth disease on a farm usually involves all susceptible species, but occasionally the virus becomes highly adapted to one species. In general, the clinical signs are most severe in cattle and swine, but outbreaks have been reported in swine while cattle in close contact with them did not develop clinical disease. Sheep and goats usually develop subclinical infections. Wild animals show a spectrum of responses ranging from inapparent infection to severe disease and even death.

Cattle. After an incubation period of 2 to 8 days, there is fever, loss of appetite, depression, and a marked drop in milk production. Within 24 hours, drooling of saliva commences (Plate 23-2A) and vesicles develop on the tongue and gums (Plate 23-2B). The animal may open and close its mouth with a characteristic smacking sound. Vesicles may also be found in the interdigital skin and coronary band of the feet and on the

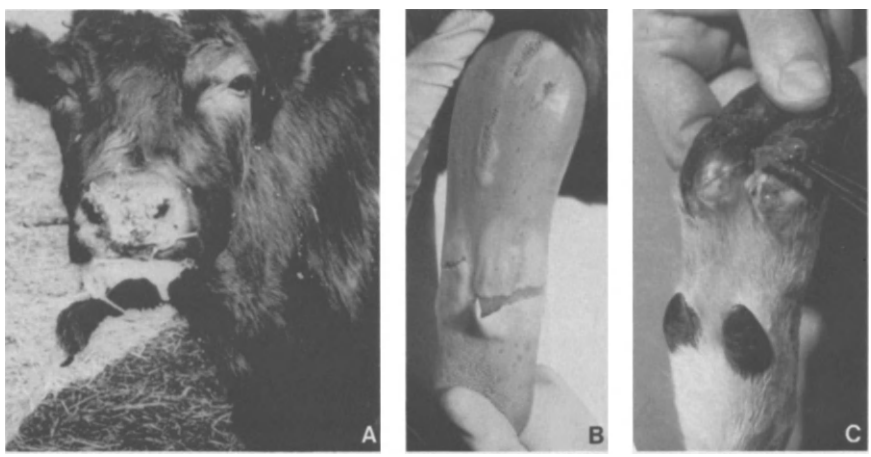

PLATE 23-2. *Foot-and-mouth disease. (A) Drooling by diseased cow. (B) Ruptured vesicles on the tongue of a steer. (C) Vesicular lesions on foot of deer.*

teats. The vesicles soon rupture, producing large denuded ulcerative lesions. Those on the tongue often heal within a few days, but those on the feet and within the nasal cavities often become secondarily infected with bacteria, resulting in prolonged lameness and a mucopurulent nasal discharge. In calves up to 6 months of age, foot-and-mouth disease virus can cause death through myocarditis. The mortality in adult cattle is very low, but although the virus does not cross the placenta, cattle may abort, presumably as a consequence of fever. Also, affected cattle become nonproductive or poorly productive for long periods. They may eat little for a week after the onset of clinical signs and are often very lame, and mastitis and abortion further lower milk production. In endemic areas, where cattle may have partial immunity, the disease may be mild or subclinical.

Swine. In swine, lameness is often the first sign. Foot lesions can be severe and may be sufficiently painful to prevent the pig from standing. Denuded areas between the claws usually become infected with bacteria; this causes suppuration and in some cases loss of the claw and prolonged lameness. Vesicles within the mouth are usually less prominent than in cattle, although large vesicles, which quickly rupture, often develop on the snout.

Other Animals. The clinical disease in sheep, goats, and wild ruminants is usually milder than in cattle and is characterized by foot lesions accompanied by lameness (Plate 23-2C).

Pathogenesis

The main route of infection in ruminants is through inhalation of aerosols, but ingestion of infected food, inoculation with contaminated vaccines, insemination with contaminated semen, and contact with contaminated clothing, veterinary instruments, etc. can all produce infection. In animals infected via the respiratory tract, initial viral replication occurs in the pharynx, followed by viremic spread to other tissues and organs before the onset of clinical disease. Viral excretion commences about 24 hours prior to the onset of clinical disease and continues for several days. Aerosols produced by infected animals contain large amounts of virus, particularly those produced by swine. Large amounts of virus are also excreted in the milk. The excretion of virus in high titer in aerosols and in milk has epidemiological significance and is also important for the control of disease (see below).

Foot-and-mouth disease virus may persist in the pharynx of some animals for a prolonged period after recovery. In cattle, virus may be

detectable for periods up to 2 years after exposure to infection, in sheep for about 6 months. Viral persistence does not occur in swine. This carrier state has also been observed in wild animals, particularly the African Cape buffalo (*Syncerus caffer*), which is commonly found to be infected with more than one of the SAT virus types even in areas where foot-and-mouth disease does not occur in cattle.

The mechanisms by which the virus produces a persistent infection in ruminants are unknown. The virus is present in the pharynx in an infectious form, for if pharyngeal fluids are inoculated into susceptible animals they develop foot-and-mouth disease. Attempts to demonstrate that carrier cattle can transmit disease by placing them in contact with susceptible animals have given equivocal results, but transmission of virus from persistently infected African buffalo to cattle has been observed.

Immunity

Recovery from clinical foot-and-mouth disease is correlated with the development of antibody. The early IgM antibodies neutralize the homologous type of virus and may also be effective against heterologous types. In contrast, the IgG produced during convalescence is type specific and, to varying degrees, subtype specific. Little information is available on the role of cell-mediated immunity in recovery from foot-and-mouth disease, but as in other picornavirus infections, it has been assumed to be of minor importance.

The duration of immunity following natural infection is difficult to assess; cattle that have recovered from foot-and-mouth disease are usually immune to infection with the same virus type for a year or more, but immunity is not considered to be lifelong. Recovered animals, however, can be immediately infected with one of the other types of foot-and-mouth disease virus and develop clinical disease.

Laboratory Diagnosis

Rapid diagnosis of foot-and-mouth disease is of paramount importance, especially in countries that are usually free of infection, so that eradication can proceed as quickly as possible. Since three other viruses can produce clinically indistinguishable lesions in domestic animals, confirmation by laboratory diagnosis is essential, although the history of the disease and the involvement of different species can be valuable pointers to the diagnosis (Table 23-4). Foot-and-mouth disease is a notifiable disease in most countries; thus, whenever a vesicular disease of domestic animals is seen, it should be reported immediately to the ap-

TABLE 23-4
Differential Diagnosis of Vesicular Diseases Based on Naturally Occurring Disease in Different Species

Disease	Viral family	Species[a]			
		Cattle	Sheep	Swine	Horse
Foot-and mouth disease	*Picornaviridae*	S	S	S	R
Swine vesicular disease	*Picornaviridae*	R	R	S	R
Vesicular stomatitis	*Rhabdoviridae*	S	S	S	S
Vesicular exanthema	*Caliciviridae*	R	R	S	R

[a]S, Susceptible by natural exposure; R, resistant by natural exposure.

propriate government authority so that prompt action can be taken to obtain a definitive laboratory diagnosis.

Specimens for diagnosis should be collected from those animals that developed clinical signs most recently. Samples from at least two animals should include the following: vesicular fluid, epithelial tissue from the edge of ruptured vesicles, blood in anticoagulant, serum, and esophageal and pharyngeal fluids collected with a *cup-probang*. These samples are diluted immediately with an equal volume of tissue culture medium containing 10% fetal calf serum. From dead animals, additional tissue samples may be collected from lymph nodes, thyroid, and heart. Samples should be frozen (preferably at −70°C) and sent immediately to the laboratory in the frozen state. In places where maintenance of the "cold chain" is difficult, duplicate samples should be collected and transported in glycerol buffer at pH 7.6.

A range of diagnostic tests is available for the differentiation of the vesicular diseases, but the complement fixation test has been developed and standardized whereby, if vesicular fluid or tissues contain adequate amounts of antigen, a differential diagnosis is available within a few hours. This test can also be used to identify which of the seven types of foot-and-mouth disease virus or seven types of vesicular stomatitis virus is the cause of the disease.

Cell cultures, suckling mice, and, on occasion, cattle are used to isolate virus when the concentration of virus in the vesicular epithelium or fluid is low. Cell cultures are generally used to isolate virus from other tissues, blood, and esophageal or pharyngeal fluids. The isolated virus is identified by the complement fixation or neutralization test.

Epidemiology

The existence of enzootic disease on four continents, the multiplicity of types of virus, the wide range of domestic and wild animal species

that can become naturally infected, the variety of methods and the rapidity with which it can spread between animals, herds, countries, and continents, and the development of persistent infection in some species, are all factors contributing to an epidemiology of considerable complexity.

The recognition of foot-and-mouth disease as the most important viral disease constraining efficient animal production in many parts of the world has resulted in intensive study of its epidemiology. Control measures are unlikely to be successful unless the nuances of its epidemiology under different geographical and social conditions are understood.

Countries Free of Enzootic Foot-and-Mouth Disease. In countries where foot-and-mouth disease either has not previously existed or has been eradicated, a "virgin-soil" epizootic can rapidly develop from introduction of disease to one farm. Within a short period, often measured in days rather than weeks, the outbreak can extend to so many farms that veterinary authorities have difficulty in controlling its spread (see Fig. 15-1). The reasons for the rapidity of spread are the highly infectious nature of foot-and-mouth disease virus, including the production of aerosols of virus by infected animals, and the short incubation period.

Foot-and-mouth disease is rapidly spread within a locality by movement of infected animals to market and, on occasion, by the mechanical transmission of virus to susceptible animals on fomites such as clothing, shoes, and veterinary instruments. The excretion of virus for up to 24 hours prior to onset of clinical signs means that virus dissemination may have occurred from a farm before any suspicion of disease is raised.

It was not until the dramatic epidemic of 1967–1968 in England, in which approximately 634,000 animals were slaughtered before the disease was successfully eradicated, that the importance of long-distance airborne transmission was realized. The long-distance airborne spread of the virus is dependent on wind direction and speed, and is favored by factors which reduce the rate of viral inactivation, such as low temperature, high humidity, and overcast skies. Long-distance spread is therefore more likely to occur in temperate rather than tropical climates. So detailed is the knowledge of the characteristics of aerosols of foot-and-mouth disease virus that computer modeling was used in 1981 to predict the likelihood of spread of disease from France across the English Channel to England (Fig. 23-2).

In contrast to humans, who are generally free to move from country to country without extensive health checks, the international movement of domestic food animals and their products is carefully controlled (see Chapter 16). Nowadays, most introductions of foot-and-mouth disease

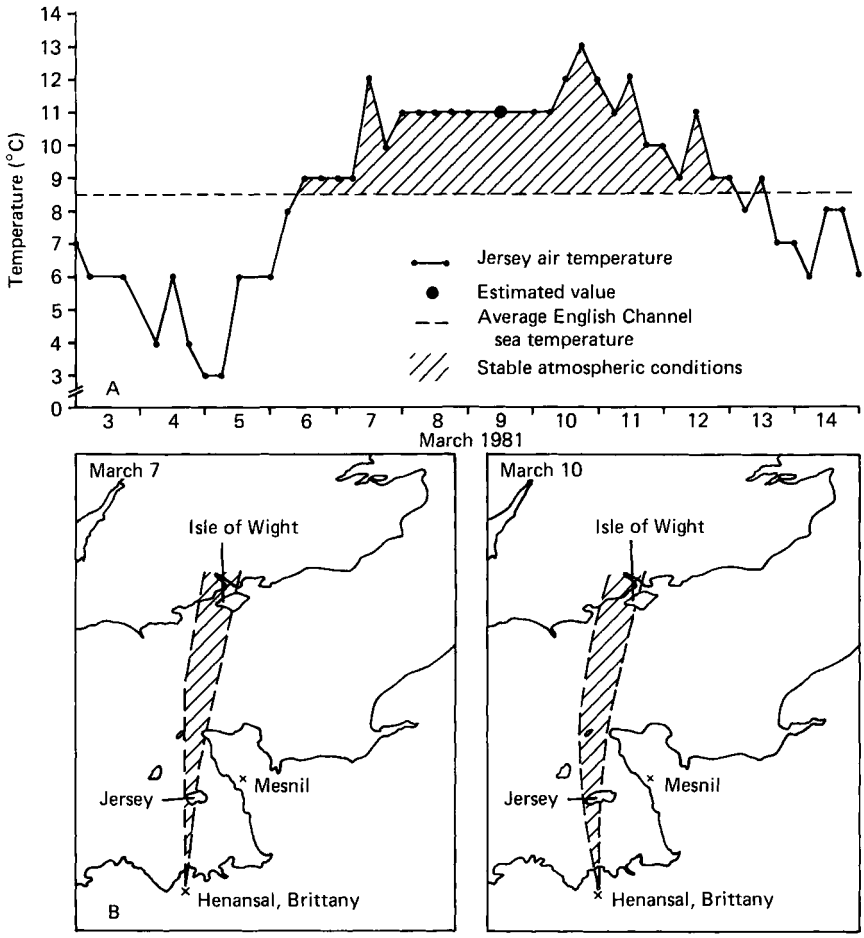

FIG. 23-2. *Airborne spread of foot-and-mouth disease. Between March 4 and March 26, 1981, the French veterinary authorities reported 13 outbreaks of foot-and-mouth disease virus type O in Brittany. On March 6, a team of meteorologists and virologists in the United Kingdom began analyses to determine whether weather conditions might be favorable for the airborne spread of foot-and-mouth disease virus from France to England. From this analysis it was considered that the risk for the Channel Islands was high, but that it was low in southern England, since the furthest distance previously reported for airborne spread of foot-and-mouth disease virus was approximately 100 km, from Denmark to Sweden in 1966. Single outbreaks were detected in both the areas predicted, on Jersey and on the Isle of Wight. [From A. I. Donaldson* et al., Vet. Rec. **110,** *53 (1982).] (A) The difference between the air and sea temperatures during the defined period. From March 6 to March 13 the lower atmosphere was extremely stable, the sea temperature being up to 4.5°C colder than the air temperature (hatched area). With a wind speed of approximately 7 m/second, virus emitted during these periods would have been confined*

virus to nonendemic countries can be traced either to scraps of infected meat in garbage being fed to swine or to long-distance spread by aerosols of virus.

The use of oligonucleotide "fingerprinting" of viral RNA and electrofocusing of viral polypeptides, to compare an isolate causing disease in one country with a possible source of infection in another country, can provide strong evidence to link the two events. Using this approach, scientists investigating the 1981 outbreak in England showed that the outbreaks in England and France were caused by viruses closely related to each other, and to a strain of serotype "O" used in the preparation of "inactivated" vaccines. It was concluded that the outbreaks must have been caused by the reintroduction into the field of the vaccine strain, possibly by escape from a laboratory, by the use of a vaccine contaminated with the virus, or by an incompletely inactivated vaccine.

Enzootic Countries. The introduction of a type not previously present in a country may cause a virgin-soil epizootic, because livestock will not have acquired immunity either through natural infection or through vaccination. For example, in 1961, the spread of SAT 1 from Africa through the countries of the Near East—where different types of foot-and-mouth disease virus are enzootic—was more dramatic than any recorded spread in Africa.

Anyone attempting to understand the epidemiology of enzootic foot-and-mouth disease will immediately be struck by the difference in the characteristics of the disease compared with the classical descriptions of outbreaks that have occurred in North America, the United Kingdom, and other areas normally free of disease. There may be long periods when disease is not reported, and when a search is made over a wide area, only a small number of animals with mild lesions may be found. There are several explanations. In some countries—particularly those in the temperate zones with European breeds of cattle—the severity of disease is modified by vaccination. In subtropical and tropical countries, with predominantly local breeds of cattle, it would appear that the virus has evolved with its host, probably over thousands of years, in such a way that the enzootic strains produce only mild disease in indigenous cattle, but cause severe disease in introduced European breeds. Thus it

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to a height of less than a few hundred meters. (B) Analysis of wind direction based on data obtained in Jersey revealed that there were two periods, each of 24 hours, on March 7 and 10, which were ideal for transmission of virus from Brittany to Jersey and the Isle of Wight. The distance between Henansal, Brittany and the Isle of Wight is approximately 250 km.

is only when such countries begin to improve cattle production by importing cattle from other countries that the economic problems associated with foot-and-mouth disease are fully appreciated.

The developing countries of Africa and Asia also have to contend with a greater complexity of antigenic types of foot-and-mouth disease virus than in Europe and South America and, in Africa particularly, there is a large wildlife population that can become involved in the epidemiology. The African Cape buffalo (*Syncerus caffer*) is believed to be the natural host for the perpetuation of SAT 1, 2, and 3 types of foot-and-mouth disease virus. Transmission of virus occurs between buffalo, but clinical disease has not been recorded. The African buffalo does not seem to transmit disease readily to domestic cattle, despite the fact that many buffalo are carriers of the virus. There are many reports of other species of wild cloven-hoofed animals transmitting foot-and-mouth disease to domestic cattle in Africa, but it is believed that control of the disease in domestic livestock results in the disappearance of foot-and-mouth disease from wild ruminants, with the exception of buffalo.

Control

Foot-and-mouth disease, more than any other disease, has influenced the development of international codes designed to minimize the risk of introducing infectious animal diseases into a country. Some countries have successfully avoided the introduction of disease by prohibiting the importation from countries where disease exists of all domestic animals susceptible to foot-and-mouth disease, all fresh meat products, and many animal by-products. The United States adopted this policy from 1929 to 1980, and only recently—in the light of improved diagnostic procedures—has it relaxed this embargo to allow small numbers of cattle to be imported for breeding purposes.

For many countries such as Australia, Canada, the United Kingdom, and the United States that have a recent history of freedom from foot-and-mouth disease, cost–benefit analyses justify a "stamping out" policy whenever disease occurs or is suspected. This is based on slaughter of affected animals and all animals exposed through direct and indirect contact, and rigid enforcement of quarantine and restrictions on movement. Vaccination is not used. To support such policies, detailed legislation is available, the most important provision being that foot-and-mouth disease is a notifiable disease. Any suspicion of disease must be brought to the attention of national or state veterinary authorities.

Immunization. In many countries of the world a "stamping out" policy is not pursued until the prevalence of disease has been reduced

by national vaccination programs. The importance of foot-and-mouth disease has always ensured that research on vaccination has been of high priority. Many different types of vaccines have been evaluated since work in Europe in the 1920s demonstrated that formolized suspensions of tongue epithelium from experimentally infected cattle could be used to protect calves. After trials with attenuated vaccines produced in cell cultures in the 1950s revealed that cattle occasionally developed vaccine-induced clinical disease, attention was concentrated on inactivated vaccines. Inactivated vaccines, often produced by growing virus in suspension cultures of a baby hamster kidney cell line, inactivated with *N*-acetylethyleneimine and with aluminum hydroxide as an adjuvant, are now the only vaccines in general use. By systematic use of inactivated vaccines, many countries in Europe have now sufficiently controlled foot-and-mouth disease to adopt a "stamping out" policy whenever clinical disease occurs (see Fig. 15-1). In time, such countries anticipate they will be able to discontinue vaccination.

At present the inactivated vaccines that are commercially available have several disadvantages, the most important of which is the difficulty in producing a vaccine of consistent potency. In 1981 copy DNA representing the gene for VP1 was cloned in *E. coli,* and the resulting protein was shown to protect cattle against challenge. In 1982, synthetic peptides prepared using sequence information from copy DNA of VP1 were reported to induce neutralizing antibodies. The peptides synthesized were chosen by comparing antigenic variants of a series of isolates and reasoning that regions of hypervariability would correspond to antigenic determinants that would stimulate the production of neutralizing antibody. Current studies also include attempts to incorporate copy DNA of the gene coding for VP1 into viral vectors such as vaccinia virus. None of these experimental vaccines is yet in field use (see Chapter 14).

PORCINE ENTEROVIRUSES

Enteroviruses are ubiquitous and probably occur in all vertebrate species. However, only in swine and poultry do they cause diseases of economic significance.

A number of enteroviruses have been recovered from swine but only two cause diseases of any importance: an enterovirus that causes swine vesicular disease, the major importance of which is its clinical resemblance to foot-and-mouth disease, and porcine enterovirus 1, which causes porcine polioencephalomyelitis (Teschen–Talfan disease).

The natural history of the porcine enterovirus 1 and of 10 other identi-

fied porcine enteroviruses (porcine enteroviruses 2–11) is very similar to that of human enteroviruses: i.e., porcine enteroviruses are very widely distributed, they are transmitted via a fecal–oral cycle, and in most instances, infections remain confined to the intestinal tract and are subclinical.

Swine Vesicular Disease

As the name indicates, swine vesicular disease is characterized by the development of vesicles around the coronary bands of the feet and to a lesser extent on the snout, lips, and tongue. It was first recognized in Italy in 1966, but was not reported again until 1971 when it was diagnosed in Hong Kong. In 1972, the disease occurred in the United Kingdom and, within a short period, was reported in many other European countries. It has not been recognized in the Americas, Australia, or New Zealand.

Although swine vesicular disease virus does not cause disease in ruminants, and in swine usually results in only minor setbacks in production schedules, its clinical similarity to foot-and-mouth disease, vesicular stomatitis, and swine vesicular exanthema can produce an important problem in differential diagnosis. For this reason, veterinary authorities in European countries have elected to eradicate the disease by slaughtering infected herds.

Properties of the Virus. Swine vesicular disease virus occasionally causes an "influenzalike" illness in humans and is closely related serologically and by RNA hybridization tests to coxsackievirus B-5 of humans. Considerable variation exists, however, in the extent of homology even between different human coxsackievirus B-5 isolates. The restricted geographical distribution of swine vesicular disease virus suggests the possibility that it is a coxsackievirus B-5 which originated from humans.

Swine vesicular disease virus is remarkably stable over a wide pH range, its pH stability being influenced by temperature and time. At neutrality and held on ice, virus has been reported to survive for over 160 days without loss of titer. The conditions found on many swine farms are therefore conducive to gross and persistent contamination of the environment, a feature of major importance in the epidemiology of the disease.

Clinical Features. Disease is often detected by the sudden appearance of lameness in several swine in a herd. Affected swine limp, have an arched back, become recumbent, and are reluctant to rise, or

even to eat. A transient fever occurs. In typical cases, the vesicles appear first on one or more feet at the junction between the heel and the coronary band, and spread to encircle the digit. The horn and sole may subsequently separate and the claw(s) may slough. In severe cases, the swine are very lame and recovery is protracted. In about 10% of cases, lesions are found on the snout, lips, and tongue. Swine kept on soft straw often show only mild lesions, whereas those on rough concrete suffer more severe disease. Subclinical infections also occur. Occasionally, some infected swine develop signs of encephalomyelitis: ataxia, circling, and convulsions.

Pathogenesis. Initial infection with swine vesicular disease virus probably occurs through damaged skin, particularly abrasions around the feet. Infection can also occur if swine eat infected garbage, but the titer of virus required to establish infection is higher. Following infection, there is viremia and large quantities of virus are excreted, but persistent infection does not occur. Swine that have recovered from disease develop antibody which protects them from reinfection.

Laboratory Diagnosis. Swine vesicular disease cannot be differentiated clinically from the other vesicular diseases of swine, including foot-and-mouth disease, so that laboratory diagnosis is essential. A variety of rapid laboratory tests is available to distinguish the vesicular diseases. If sufficient vesicular fluid or epithelium is available, the complement fixation test or ELISA can be used to detect antigen and establish a diagnosis within 4 to 24 hours. The virus grows well in cultures of swine kidney cells, producing a cytopathic effect, sometimes as early as 6 hours after inoculation. The virus can also be isolated by intracerebral inoculation of newborn mice, which develop paralysis and die.

Epidemiology. There is no evidence that swine vesicular disease virus exists in any country without clinical disease being reported. Because of its resistance to low pH and ambient temperatures, it is easily transmitted between countries in infected meat. Various pork products which are prepared without heat treatment, such as salami, can harbor virus for several months. Fresh pork infected with swine vesicular disease virus can be an additional hazard within a country and delay eradication of disease, since infected carcasses may be placed unknowingly in cold storage for months or years and, when released, such infected meat can give rise to new outbreaks.

Because the virus is so stable, it is extremely difficult to decontaminate infected premises, particularly where swine have been housed on soil. The virus has been isolated from the surface and gut of earthworms

collected from soil above burial pits containing carcasses of swine slaughtered because of swine vesicular disease.

Control. It is important to emphasize again that swine vesicular disease is not of great economic importance in itself, but must be controlled so that diagnostic confusion with foot-and-mouth disease and other vesicular diseases can be avoided. For this reason, swine vesicular disease is a notifiable disease and most countries have elected to eradicate the virus by a slaughter program similar to that used for foot-and-mouth disease. However, it can be a difficult disease to eradicate, because clinical lesions may be trivial and go unnoticed by the farmer. Thus, all swine on the farm may be affected and spread to many other farms may have occurred through animal sales before the disease is reported.

Porcine Enterovirus 1

Porcine polioencephalomyelitis was first recognized in the town of Teschen in Czechoslovakia in 1930. The disease was described as a particularly virulent, highly fatal, nonsuppurative encephalomyelitis in which lesions were present throughout the central nervous system. This severe and classic form of the disease is still recognized, although less severe forms of disease, referred to originally as Talfan disease in the United Kingdom and as enzootic posterior paresis in Denmark, are more common and occur worldwide.

Clinical Features. After an incubation period of 4 to 28 days, the initial signs include fever, anorexia, and depression, followed by tremors and incoordination usually beginning with the hindlimbs. Initially the limbs may be stiff; then paralysis occurs, leading to prostration followed by convulsions, coma, and death. There may be enhanced responses to touch and sound, paralysis of facial muscles, and loss of voice. In severe outbreaks the mortality may reach 75%. In milder forms of disease the clinical signs are limited to ataxia associated with hindlimb paresis from which swine often recover completely in a few days.

Pathogenesis. The pathogenicity of strains of porcine enterovirus 1 varies and the severity of the disease is also influenced by age, being most severe in young swine. Virus replicates initially in the alimentary tract and associated lymphoid tissues, followed by viremia and invasion of the central nervous system. Histologically the lesions resemble those of other viral encephalomyelitides, with perivascular cuffing, neuronal degeneration, and gliosis. The extent of the lesions parallels the severity of clinical disease and in extreme cases involves the entire spinal cord, brain, and meninges.

Laboratory Diagnosis. Polioencephalomyelitis due to porcine enterovirus 1 must be differentiated from other viral encephalomyelitides including African swine fever, pseudorabies, hemagglutinating encephalomyelitis, rabies, and hog cholera. Virus is readily isolated in porcine cell cultures, neutralization assays being used for typing. Immunofluorescent staining of the infected cell culture is preferred for rapid, definitive diagnosis.

Epidemiology and Control. Infection is acquired by ingestion. Inactivated and attenuated live-virus vaccines, comparable to the Salk and Sabin vaccines for human poliomyelitis, have been developed. Universal vaccination is not practiced, since control in intensive swine units is often satisfactorily achieved by quarantine and hygiene.

Porcine Enteroviruses 2–11

These viruses are frequently isolated from the feces of normal swine, and also from swine with diarrhea or pericarditis and from aborted and stillborn fetuses. In the latter context the viruses were proposed as a cause for a range of reproduction problems to which the acronym SMEDI (for *s*tillbirth, *m*ummification, *e*mbryonic *d*eath, and *i*nfertility) was applied. However, their role in SMEDI and other diseases remains uncertain; porcine parvoviruses are now considered more significant causes of SMEDI (see Chapter 22). Several isolates of porcine enteroviruses 2–11 have been shown to cause encephalomyelitis following experimental infection of swine.

AVIAN ENTEROVIRUSES

Diseases due to enteroviruses occur in chickens, ducks, and turkeys, one species causing encephalomyelitis in chickens and other birds and two others causing hepatitis in ducks and turkeys, respectively.

Avian Encephalomyelitis

Avian encephalomyelitis was first described in the New England states of the United States in 1932 and is now recognized worldwide. Its natural history closely parallels that of human poliomyelitis and swine polioencephalomyelitis. Avian encephalomyelitis is an important disease of chickens 1–21 days of age, but the virus is not pathogenic in older chickens. When the virus is newly introduced into a flock the mortality rate may exceed 50%. There is only a single antigenic type, but strains vary in virulence. Avian encephalomyelitis virus produces rela-

tively mild encephalomyelitis in quail, turkeys, and pheasants; other avian species are susceptible following experimental infection.

Clinical Features. After an incubation period of 1 to 7 days, disease occurs which is characterized by dullness, progressive ataxia, tremors, particularly of the head and neck, weight loss, blindness, paralysis, and in severe cases prostration, coma, and death. Birds allowed to recover have deficits of the central nervous system and are usually destroyed.

Pathology. No obvious macroscopic lesions are seen at postmortem. Histological lesions typical of viral encephalitis, but not diagnostic of avian encephalomyelitis, are found throughout the central nervous system, with perivascular cuffing, neuronal degeneration, and gliosis.

Laboratory Diagnosis. Clinical signs and histopathological findings will strongly suggest a diagnosis of avian encephalomyelitis. Immunofluorescence is widely used for definitive diagnosis. The virus may be isolated either in cell culture or by inoculating 5- to 7-day-old embryonated hen eggs obtained from antibody-free hens by the yolk sac route; chicks are allowed to hatch and observed for 7 days for signs of encephalomyelitis. The disease needs to be differentiated from Newcastle disease as well as from a range of nonviral causes of central nervous system disease in chickens.

Epidemiology. High morbidity and mortality occur when avian encephalomyelitis virus is first introduced into a flock. The major mode of transmission is by a fecal–oral route, although transmission via the egg may occur in association with the brief viremic phase of the disease in laying hens. Once established in a flock, losses continue at a greatly reduced incidence, because maternal antibody provides protection for chicks during the critical first 21 days after hatching.

Control. The choice for control is either depopulation or vaccination. Attenuated live-virus vaccines administered in the drinking water are available. The vaccines are administered after chickens reach 10 weeks of age and are designed to provide protection for chicks during the first 21 days after hatching by ensuring that adequate levels of specific antibody are transferred from hens to progeny chicks. They are not administered to chicks because they are not sufficiently attenuated, nor is there sufficient time to provide protection for chicks hatched into a heavily contaminated environment. Inactivated vaccines are also available and are preferred when immunized birds are housed in close proximity to nonimmunized chickens. Vaccines are also used to control avian encephalomyelitis in quail and turkeys.

Duck Hepatitis

Duck hepatitis was first recognized in 1945, among ducks reared on Long Island, New York. There is only one serotype, and the natural history of the virus is similar to that of avian encephalomyelitis virus.

Clinical Features. Disease occurs in ducks less than 21 days of age, after an incubation period of 1 to 5 days. The course of the disease in a clutch of ducks is often dramatically swift, occurring over a 3-day period with a mortality rate approaching 100%. Affected ducks tend to stand still with partially closed eyes, fall to one side, paddle spasmodically, and die. There may be some diarrhea. At postmortem, the liver is enlarged, edematous, and mottled with hemorrhages. Histologically there is extensive hepatic necrosis, inflammatory cell infiltration, and proliferation of the bile duct epithelium.

Laboratory Diagnosis. The history, clinical signs, and characteristic postmortem findings are suggestive; immunofluorescence provides rapid, definitive diagnosis. The virus may be isolated in cell culture or by allantoic inoculation of 10-day-old embryonated hen eggs. Infected eggs, when subsequently candled, often show characteristic greenish discoloration of the embryonic fluids. Duck hepatitis needs to be differentiated from duck plague (a herpesvirus infection), influenza, and Newcastle disease.

Control. Recovered ducks are immune. Hyperimmune serum has been successfully used to reduce losses during outbreaks. Attenuated egg-adapted live-virus vaccines have been developed and are used following the same principles as already outlined for avian encephalomyelitis vaccines.

Turkey Hepatitis

Turkey hepatitis was first recognized in 1959 in Canada and the United States. The virus is antigenically related to duck hepatitis virus and the natural history of the disease resembles that of duck hepatitis.

CARDIOVIRUSES

In contrast to the very large genus *Enterovirus*, the genus *Cardiovirus* contains only one species, encephalomyocarditis virus. The natural hosts of the virus are rodents, including the water rat *Hydromys chrysogaster*. The virus is transmitted from rodents to humans, monkeys, horses, cattle, and swine. In these hosts, as well as in rodents, it occa-

sionally causes encephalomyelitis and myocarditis, although most infections are subclinical.

Severe epizootics of myocarditis, with fatalities, have occasionally been reported in swine and other species, such as elephants—notably in Florida and Australia. These outbreaks are usually associated with severe mouse, or less commonly rat, infestations.

RHINOVIRUSES

Among domestic animals, rhinoviruses are recognized only in horses and cattle, in which species only a few serotypes are known, compared with over 150 serotypes of human rhinovirus. The equine and bovine rhinoviruses are antigenically unrelated to each other or to the human rhinoviruses. They cause mild respiratory disease similar to the common cold in humans, but they may predispose to more severe forms of respiratory disease. Each is highly host-specific, but humans can undergo asymptomatic infection with equine rhinovirus 1.

Three equine rhinovirus serotypes have been identified. In addition to causing respiratory disease, the equine viruses are unusual in that they regularly produce viremia and can be isolated from feces. Three serotypes of bovine rhinovirus have been isolated from cattle with mild respiratory disease.

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CHAPTER 24

Caliciviridae

Although once listed as a genus within the family *Picornaviridae,* the caliciviruses have now been classified as a separate family, *Caliciviridae,* which contains one genus, *Calicivirus.* Two species have been cultivated and are well characterized: vesicular exanthema of swine virus, and feline calicivirus.

Vesicular exanthema of swine was first recognized in southern California in 1932 and caused concern because of its similarity to foot-and-mouth disease. It was apparently contracted from marine mammals, and was eradicated from swine in the United States in 1956. It has not been reported in swine since then. Feline calicivirus is one of the two major causes of viral upper respiratory tract disease in cats, and may cause glossitis in dogs.

PROPERTIES OF CALICIVIRUSES

The calicivirus virion is slightly larger than that of picornaviruses and its icosahedral capsid is constructed from a single polypeptide, rather than four different polypeptides, as in picornaviruses. The family derives its name from the 32 cup-shaped (*calix* = cup) surface depressions that give the virion its unique appearance (Plate 24-1). The virion is

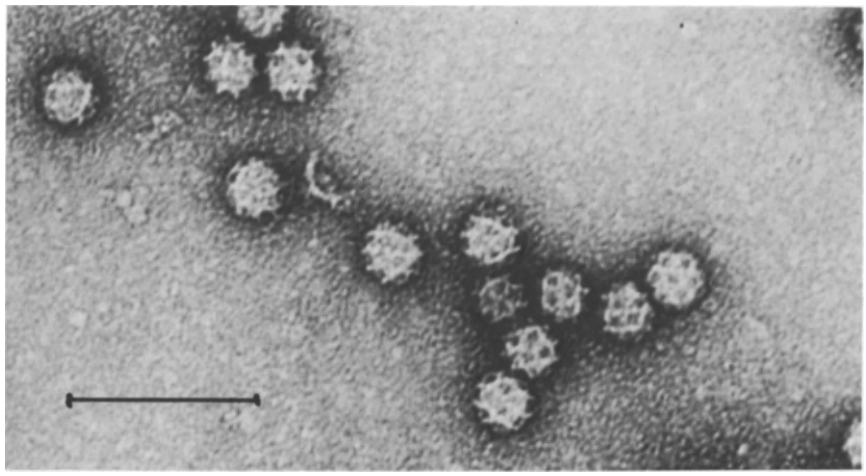

PLATE 24-1. Caliciviridae. *Electron micrograph of negatively stained virions of vesicular exanthema of swine virus (bar = 100 nm). (Courtesy Dr. S. S. Breese.)*

relatively resistant to heat and acid. Its linear (+) sense ssRNA genome resembles that of picornaviruses but its strategy of expression is different. Other properties are shown in Table 24-1.

Based on cross-protection experiments in swine and subsequently on serum neutralization assays in cultured cells, vesicular exanthema of swine virus shows a remarkable degree of antigenic heterogeneity; at least 13 distinct antigenic types have been identified. Even when recovered from within a single herd, any two isolates were rarely antigenically identical.

When antisera raised in rabbits were used for neutralization assays, feline caliciviruses seemed to show a similar antigenic heterogeneity. However, when specific pathogen-free cat sera were used and a reasonably large collection of viruses analyzed, a pattern of extensive cross-reactions was found, a finding which paved the way for the develop-

TABLE 24-1
Properties of Caliciviruses

Nonenveloped spherical virion, 35–40 nm diameter
Icosahedral capsid with 32 cup-shaped depressions; one capsid protein (60–70K)
Linear (+) sense ssRNA genome, 7.9 kb, polyadenylated at 3′ terminus, protein at 5′ terminus essential for infectivity of RNA
Cytoplasmic replication
Genomic RNA and two subgenomic mRNA transcripts produced during replication

ment of monotypic vaccines for the control of diseases caused by feline caliciviruses.

VIRAL REPLICATION

Caliciviruses replicate in the cytoplasm and are rapidly cytopathogenic. They grow well in cell cultures derived from their respective hosts; vesicular exanthema of swine virus also grows in Vero cells. Virions are found in the cytoplasm either as single scattered particles or as characteristic linear arrays, either along microfibrils or within membranous structures, or as paracrystalline arrays.

The isolated virion RNA is infectious. During replication at least three RNA species are transcribed from a (−) sense RNA template. One of these is of genomic size (7.9 kb) and two are subgenomic, the larger (3.3 kb) coding for the single large capsid polypeptide. Other polypeptides of various sizes are found in the cytoplasm of infected cells, but extensive posttranslational cleavage of a polyprotein, as found in picornaviruses, does not occur.

VESICULAR EXANTHEMA OF SWINE

Vesicular exanthema of swine is an acute, febrile contagious disease of swine characterized by the formation of vesicles on the snout, within the oral cavity and on the feet. The incubation period is 18–72 hours. Morbidity may be high but mortality is low, and in uncomplicated cases recovery occurs after 1 to 2 weeks. Its importance in veterinary medicine derives from the fact that clinically it is indistinguishable from the three other vesicular diseases of swine: foot-and-mouth disease, swine vesicular disease, and vesicular stomatitis (see Chapters 23 and 30).

Epidemiology

Vesicular exanthema of swine virus was first recognized in swine in southern California in 1932. It was originally diagnosed as foot-and-mouth disease and a slaughter policy was implemented, in spite of which sporadic outbreaks continued to occur. When it was established that the virus responsible was not foot-and-mouth disease virus, efforts for eradication diminished. Although there was a clear link between garbage (swill) feeding and outbreaks of the disease, ordinances requiring that all garbage fed to swine should be cooked were not rigorously enforced.

In 1952 vesicular exanthema of swine was diagnosed outside California for the first time, initially in Nebraska. The outbreak was traced to pork scraps taken from a Los Angeles–Chicago train and fed to swine which had become sick and were sent to market in Nebraska. By September 1953 the disease had occurred in swine in 42 states. These experiences led to the rigorous enforcement of garbage-cooking laws and a slaughter program which resulted in a rapid decline in the incidence of disease. The last outbreak of vesicular exanthema of swine was recorded in New Jersey in 1956, and later that year the disease was declared to be eradicated from the United States. It has not recurred there or been recognized anywhere else.

San Miguel Sea Lion Virus

Although sometimes listed as a separate virus, it is now clear that this virus is the same as vesicular exanthema of swine virus and was indeed the source of the disease in swine. It was recovered in 1972, when a calicivirus was isolated from material obtained from California sea lions inhabiting San Miguel Island, which showed several signs of disease including abortion and vesicular lesions of the flippers. Although serologically distinguishable from the 14 known vesicular exanthema of swine virus serotypes, this virus produced lesions when inoculated into swine. In California, carcasses of seals and sea lions washed up on mainland beaches were frequently fed to swine, thus providing the opportunity for infection. Retrospective evidence suggested that the multiple antigenic types of vesicular exanthema of swine virus were generated in the natural hosts of the virus, sea lions, rather than in swine. Seven antigenic types have been identified from the small number of San Miguel sea lion virus isolates that have been made since 1972.

FELINE CALICIVIRUS INFECTIONS

Feline calicivirus is one of the two major causes of respiratory disease in cats and causes an acute or subacute disease characterized by conjunctivitis, rhinitis, tracheitis, pneumonia, and vesiculation and ulceration of the oral epithelium (Plate 24-2). Other common signs are fever, anorexia, lethargy, stiff gait, and sometimes nasal and ocular discharge. Morbidity is high, mortality may reach 30%, and recovery is followed by a prolonged carrier state. Feline calicivirus occurs worldwide and al-

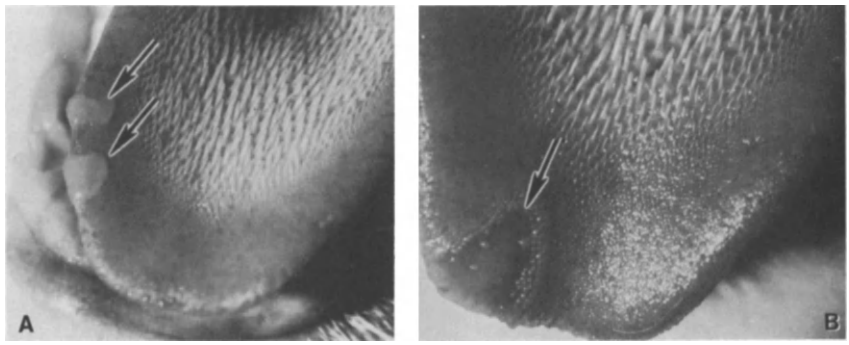

PLATE 24-2. *Signs of feline calicivirus infection. (A) Lingual vesicles. (B) Lingual ulcers following rupture of vesicles. [From E. A. Hoover and D. E. Kahn,* J. Am. Vet. Med. Assoc. **116,** *463 (1975).]*

though all Felidae are probably susceptible, infection has been reported only in domestic cats and cheetahs. Economic loss caused by the death of valuable kittens and the costs of providing supportive treatment for sick cats are substantial.

Strains of feline calicivirus vary in virulence. Some strains are associated mainly with subclinical infection or upper respiratory disease; highly virulent strains regularly produce pneumonia, especially in young kittens.

Laboratory Diagnosis

Clinically the disease cannot be differentiated from feline rhinotracheitis caused by feline herpesvirus 1 (see Chapter 19); diagnosis depends on the laboratory tests. The virus may be readily isolated in cultures of feline cells.

Epidemiology and Control

Feline calicivirus is recovered from about 50% of cats presenting with clinical signs of acute upper respiratory disease, but by the age of 1 year virtually all cats have antibodies to it. Clinical disease is rare in cats over 1 year of age. A high percentage of recovered cats remain persistently infected and shed virus from the oropharynx for several years, possibly for life. For control, attenuated live-virus and inactivated feline calicivirus vaccines are widely used, usually in combination with feline herpesvirus 1 vaccine.

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CHAPTER 25

Togaviridae and *Flaviviridae*

As outlined in Chapter 15, the term arbovirus (*ar*thropod-*bo*rne virus) is used without taxonomic implication to describe any virus which is perpetuated in nature by replication cycles involving hematophagous (blood-feeding) arthropod hosts and vertebrate hosts. The earliest attempts to classify the large number of viruses found to be transmitted by arthropods utilized serological tests. Later, as physicochemical and morphological data confirmed the fundamental basis of serological relationships, two major serogroups, which had been called the group A and group B arboviruses, were designated as the genera *Alphavirus* and *Flavivirus*, respectively, within the family *Togaviridae*. Other serogroups and serologically unrelated arboviruses were allocated to the families *Bunyaviridae, Rhabdoviridae,* and *Reoviridae* (genus *Orbivirus*) (see Chapter 2).

Some non-arthropod-borne viruses were then found to have physicochemical characteristics similar to those of the arthropod-borne members of the family *Togaviridae* and so were included in this family within the genera *Rubivirus, Pestivirus,* and *Arterivirus* (see Table 25-1). Recently, as substantial differences in the strategy of replication of members of the various genera became better appreciated, the genus *Flavivirus* was split off to become a separate family, *Flaviviridae.*

TABLE 25-1

Togaviruses That Cause Disease in Domestic Animals[a,b]

Genus	Virus	Mode of transmission	Animal host	Disease	Geographical distribution
Alphavirus	Eastern equine encephalitis virus	Mosquito	Horse (human)	Encephalitis	Americas
	Western equine encephalitis virus	Mosquito	Horse (human)	Encephalitis	Americas
	Venezuelan equine encephalitis virus	Mosquito	Horse (human)	Febrile disease, encephalitis	Americas
	Getah virus	Mosquito	Horse	Febrile disease	Southeast Asia
Pestivirus	Bovine virus diarrhea virus	Respiratory, contact, congenital	Cattle	Generalized infection: respiratory and reproductive signs, diarrhea	Worldwide
		Respiratory, contact, congenital	Sheep	Border disease, congenital	Worldwide
	Hog cholera virus	Respiratory, contact, congenital	Swine	Generalized infection	Worldwide, but eradicated in some countries
Arterivirus	Equine arteritis virus	Respiratory, contact, congenital	Horse	Generalized infection, abortion	Worldwide

[a]The alphaviruses Chikungunya, O'nyong-nyong, Ross River, Mayaro, and Sindbis cause human disease, often with arthritis.

[b]The other togavirus genus, *Rubivirus*, contains one virus—rubella virus—which infects only humans.

Since the great majority of togaviruses and flaviviruses are arthropod-borne, it is necessary to recall some of the essential features of the life cycles of arboviruses, in respect of their disease potential in domestic animals. Most have localized natural habitats in which specific arthropod and vertebrate hosts allow them to fulfill their life cycle. The vertebrate reservoir hosts are usually wild mammals or birds; domestic animals and humans are usually not involved in primary transmission cycles, although there are important exceptions to this generalization, e.g., Venezuelan equine encephalitis virus in horses and yellow fever and dengue in humans. Domestic animal species and humans are in most cases infected accidentally, for example by the geographical extension of a reservoir vertebrate host and/or a vector arthropod. Many ecological changes disturb natural viral life cycles and have been incriminated in the geographical spread or increased prevalence of arbovirus diseases (see Chapter 15).

Twenty-six viral species are recognized within the genus *Alphavirus*. Four of them cause diseases in horses; these viruses and another four species sometimes cause disease in humans. The nonarbovirus genera of the family *Togaviridae* have few members; but most members of all genera except *Rubivirus* (which contains only the specifically human rubella virus) cause serious diseases in domestic animals (Table 25-1).

The family *Flaviviridae* contains only one genus, *Flavivirus*, with some 60 members. Only 3 of these are of veterinary importance, although the genus includes many human pathogens (Table 25-2).

TABLE 25-2
Flaviviruses That Cause Disease in Domestic Animals[a]

Virus	Mode of transmission	Animal host	Disease	Geographical distribution
Louping ill virus	Tick	Sheep (human)	Encephalitis	United Kingdom
Wesselsbron disease virus	Mosquito	Sheep (human)	Generalized infection, abortion	Southern Africa
Japanese encephalitis virus	Mosquito	Swine (human)	Encephalitis, abortion	Japan, Southeast Asia

[a]Many other flaviviruses cause important human diseases, including yellow fever, dengue, Murray Valley, West Nile, and St. Louis encephalitis, Omsk hemorrhagic fever, Central European tick-borne encephalitis, Kyasanur virus disease.

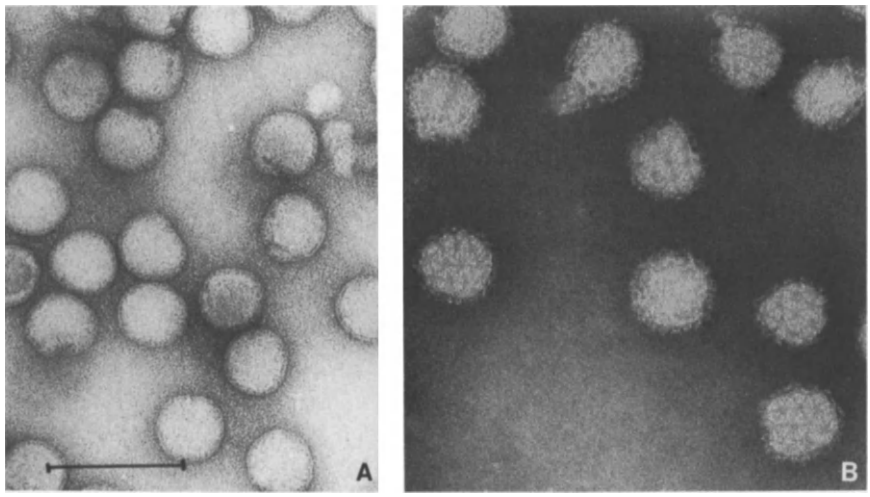

PLATE 25-1. *Negatively stained virions of (A) the flavivirus, tick-borne encephalitis virus (family* Flaviviridae*); (B) the alphavirus, Semliki Forest virus (family* Togaviridae*) (bar = 100 nm). (A, courtesy Drs. W. Tuma, F. X. Heinz, and C. Kunz; B, courtesy Dr. C. -H. von Bonsdorff.)*

PROPERTIES OF TOGAVIRUSES AND FLAVIVIRUSES

Togavirus and flavivirus virions are rather similar in morphology but each has a distinct genome organization and strategy of replication. Virions are spherical, 60–70 nm (alphaviruses and arteriviruses) or 40–50 nm (flaviviruses and pestiviruses) in diameter, and consist of a tightly adherent lipid bilayer envelope covered with glycoprotein peplomers surrounding an icosahedral capsid (Plate 25-1). The properties of togaviruses are summarized in Table 25-3, those of flaviviruses in Table 25-4.

TABLE 25-3
Properties of Togaviruses

Spherical virion, enveloped, diameter 60–70 nm
Icosahedral capsid, 28–35 nm
Two envelope glycoproteins: E_1 (45–53K) and E_2 (53–59K) contain epitopes for neutralizing antibodies and alphavirus serogroup and subgroup specificity; one capsid protein C (29–36K)
Linear, (+) sense ssRNA genome, 12 kb, 5′ end capped, 3′ end polyadenylated, infectious; genes for nonstructural proteins located at 5′ end of genome
Cytoplasmic replication, budding from plasma membrane
Full-length and subgenomic RNA transcripts; posttranslational cleavage of polyproteins
Four genera: *Alphavirus*, arthropod-borne; *Rubivirus*, *Pestivirus*, and *Arterivirus* are not

TABLE 25-4
Properties of Flaviviruses

Spherical virion, enveloped, diameter 40–50 nm
Probable icosahedral capsid, 25–30 nm
One envelope glycoprotein E (50–60K) contains epitopes for neutralizing antibodies; one nonglycosylated envelope protein M (8K); one capsid protein C (14K)
Linear (+) sense ssRNA genome (12 kb), 5′ end capped, but 3′ end not polyadenylated, infectious; genes for structural proteins located at 5′ end of genome
Cytoplasmic replication, maturation within cytoplasmic vesicles
Only one genus, *Flavivirus*, most members of which are arthropod-borne

Togaviruses and flaviviruses are not very stable in the environment and are easily inactivated by disinfectants.

VIRAL REPLICATION

Alphaviruses and flaviviruses replicate well and cause cytopathic effects in many kinds of cell cultures, e.g., Vero (African green monkey kidney) cells, BHK-21 (baby hamster kidney) cells, primary chick and duck embryo fibroblasts, and mosquito cells, such as cell lines derived from *Aedes albopictus*. Experimentally, these viruses kill newborn mice, in which most species of both genera were first isolated.

The pestiviruses and arteriviruses replicate in primary and continuous cell cultures derived from the principal host species: (1) bovine virus diarrhea virus in bovine embryonic kidney cells, (2) hog cholera virus in porcine lymphoid or kidney cells, and (3) equine arteritis in equine kidney cells. In most cases these viruses do not cause disease in these cells, so their growth must be detected indirectly, e.g., by immunofluorescence.

Alphavirus Replication

Alphavirus replication has been intensively investigated and may be taken as a model for the family *Togaviridae*. Virions are taken up by receptor-mediated endocytosis and are transported in coated vesicles to lysosomes where they are uncoated at low pH, thereby releasing viral genomes into the host cell cytoplasm. The 5′ two-thirds of the RNA genome is translated into a small polypeptide and a large polyprotein, and the latter is cleaved into four nonstructural proteins which together form the RNA-dependent RNA polymerase. This enzyme directs the transcription of full-length (−) sense RNA from which in turn two types of RNA are produced: full-length progeny genomic RNA, and a sub-

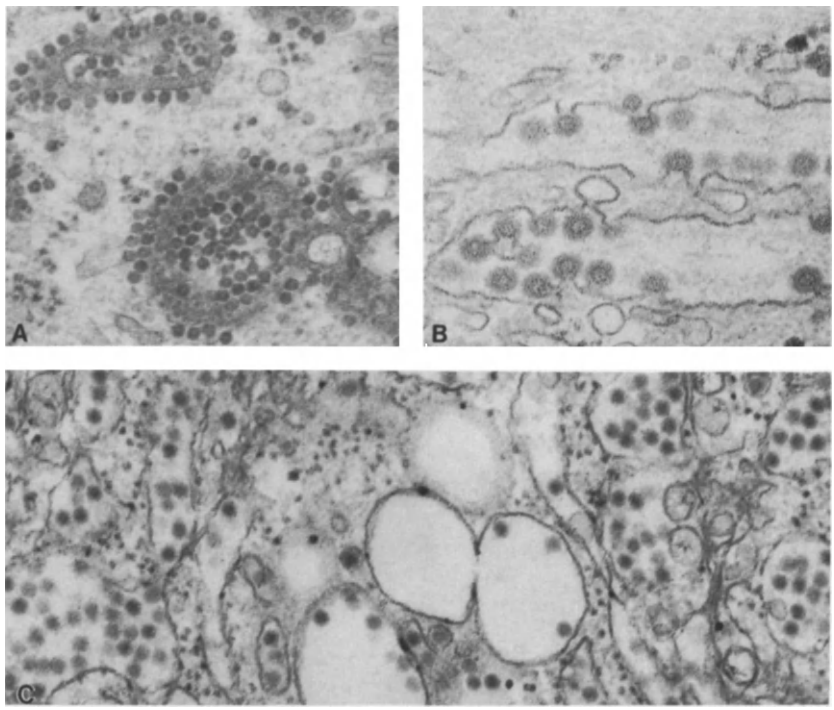

PLATE 25-2. *Electron micrographs of thin sections of cells showing contrasting modes of maturation of alphaviruses and flaviviruses in vertebrate cells. (A) Alphavirus capsids in cytoplasm. (B) Alphavirus nucleocapsids acquiring an envelope as they bud from the plasma membrane. (C) Mature virions of a flavivirus can be seen within the cisternae of the endoplasmic reticulum, but budding is not seen.*

genomic mRNA corresponding to the 3′ one-third of the genomic RNA. The latter is translated into another polyprotein which has protease activity and cleaves itself to yield the three viral structural proteins: C, E_1, and E_2.

Genomic RNA and nucleoprotein form icosahedral nucleocapsids in the cytoplasm (Plate 25-2A), and these in turn migrate to the plasma membrane. The peplomer structural proteins are glycosylated in stepwise fashion as they progress from the endoplasmic reticulum through the Golgi complex to the plasma membrane (see Fig. 4-10). Virion assembly takes place via budding of nucleocapsids through peplomer-modified plasma membrane (Plate 25-2B).

The replication strategies of member viruses of the genera *Pestivirus* and *Arterivirus* have not been studied in detail, but it seems clear that they are in general similar to that of the alphaviruses.

Flavivirus Replication

Flavivirus replication is much slower than that of alphaviruses, titers of virus produced in cultured cells and *in vivo* are usually lower, and host cell protein and RNA synthesis are only partially shut off (or in mosquito cells not at all). No subgenomic RNA has been found in flavivirus-infected cells; it is believed that genomic-length RNA is the only mRNA species. The genomic RNA contains a remarkably long single open reading frame of more than 10,000 nucleotides, which encodes a polyprotein of some 3300 amino acids. The three structural proteins are encoded by the 5′ end of the genome, seven nonstructural proteins by the rest of the genome. This genome organization implies that mature viral proteins are produced by posttranslational cleavage and modification of a polyprotein. Virion assembly occurs in vertebrate cells upon membranes of the endoplasmic reticulum and in mosquito cells upon the plasma membrane also, but unlike the alphaviruses, preformed capsids and budding are not seen. Instead, in vertebrate cells fully formed virions appear within cisternae of the endoplasmic reticulum (Plate 25-2C) and are released upon cell lysis. Virions are formed similarly in arthropod cells but also on plasma membranes, and the host cell is not damaged by their release.

PATHOGENESIS OF ALPHAVIRUS AND FLAVIVIRUS ENCEPHALITIS

Encephalitis is the most important clinical feature of disease due to the alphaviruses and flaviviruses. Following the introduction of an arbovirus into an animal by the bite of a vector arthropod, viral replication occurs in cells near the entry site and/or in regional lymph nodes infected via lymphatic drainage from the bite site. The resulting primary viremia allows virus to invade specific extraneural tissues and organs, leading to a high-titer secondary viremia, which permits the infection of arthropod vectors. The infection of extraneural organs and tissues may directly cause clinical disease; for example, the replication of Venezuelan equine encephalitis virus in reticuloendothelial tissues, and perhaps in striated muscle and connective tissue, is the probable cause of the systemic febrile disease in horses, and the replication of yellow fever virus in hepatocytes is the cause of the human disease. Other extraneural tissues and organs known to be important in the pathogenesis of arbovirus diseases include cardiac muscle, pancreatic epithelium, brown fat, and lymphoid tissues.

Routes of entry of viruses into the central nervous system are dis-

cussed in Chapter 7. Encephalitis due to alphaviruses and flaviviruses is probably due to hematogenous spread and subsequent entry of virus by one of several alternative routes: (1) passive diffusion of virus through the endothelium of capillaries in the central nervous system, (2) viral replication in vascular endothelial cells and release of progeny into the paraenchyma of the central nervous system, (3) viral invasion of the cerebrospinal fluid with infection of the choroid plexus and ependyma, or (4) carriage of virus in inflammatory or lymphoid cells which may migrate into the parenchyma of the central nervous system. An alternative possibility for the invasion of the central nervous system by arboviruses has recently received renewed support. Immunofluorescence studies show that in animals in which viremia is transient and reaches only a low titer, flaviviruses may replicate extensively in the olfactory epithelium, whence they invade the brain parenchyma via axonal spread to the olfactory bulbs.

Once virus enters the parenchyma of the central nervous system there are no anatomical or physiological impediments to spread in the narrow but open intercellular spaces throughout the central nervous system. Lesions are a result of direct infection, damage, and dysfunction of neurons. Virus produced in the central nervous system is trapped and does not reenter the circulation; hence, it is not involved in transmission.

DISEASES CAUSED BY TOGAVIRUSES

The diseases of veterinary importance caused by togaviruses are listed in Table 25-1. Alphaviruses cause encephalitis in horses and occasionally in humans, pestiviruses cause serious generalized diseases in cattle, sheep, and swine, and equine arteritis virus produces a mild infection in horses which may, however, cause abortion.

Equine Encephalitis

Clinical Features. Infection of horses with eastern, western, or Venezuelan equine encephalitis viruses produces a range of clinical manifestations; infection may be subclinical or may present with only fever, anorexia, and depression. Progressive systemic disease often leading to death with only minor neurological manifestations is most common in Venezuelan equine encephalitis. Neurological disease, marked by profound depression (typically with wide stance, hanging head, drooping ears, flaccid lips), is most severe in eastern equine encephalitis. In horses, the case fatality rate of eastern equine encephalitis virus infection is about 90%, western equine encephalitis virus infection about 20

to 40%, and Venezuelan equine encephalitis virus infection about 50 to 80%.

Pathogenesis. Further understanding of the clinical manifestations seen in equine encephalitis has been developed from studies of experimentally infected horses. For example, when horses are inoculated with eastern equine encephalitis virus, there is a biphasic febrile response starting 24–48 hours after infection and lasting 1 day (Fig. 25-1). Fever returns at the onset of neurological signs 4–6 days after infection and lasts 1–4 days. Neurological signs are the same as noted in field cases; there is severe weight loss, and death occurs in 5 to 10 days. In horses that recover, there are often neurological sequelae indicative of cerebral deficit and dysfunction. The pathogenetic basis for the initial systemic febrile disease lies in the tropism of the virus for muscle, connective tissue, myeloid tissue, and lymphoreticular tissue; the basis for the neurological disease is viral invasion of the central nervous system from the

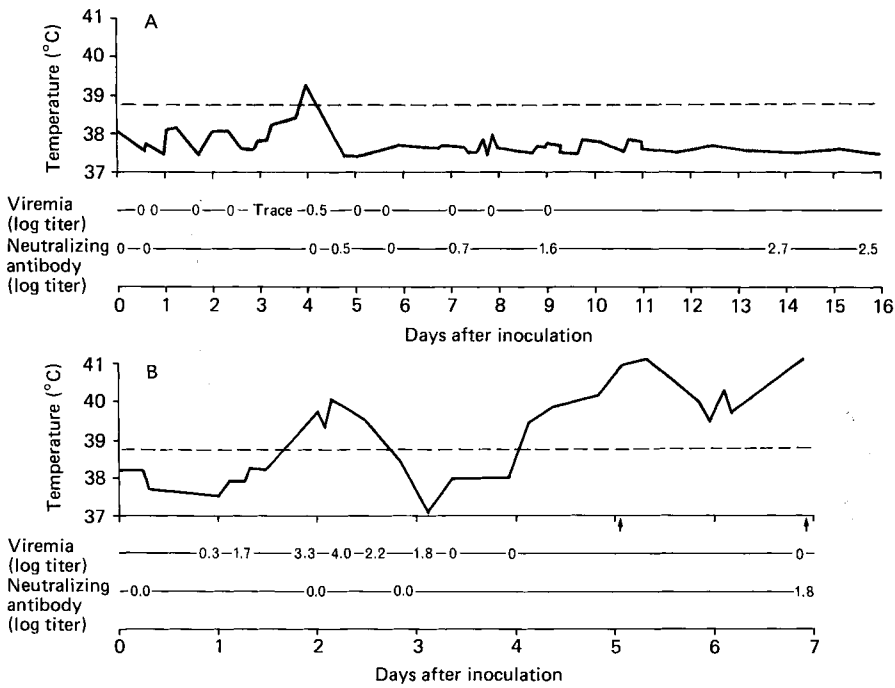

FIG. 25-1. *Clinical and laboratory features of eastern equine encephalitis infection in horses. (A) Acute infection followed by recovery. Low fever, low viremia, good antibody response. (B) Biphasic disease with high fever, high viremia, poor antibody response, central nervous system signs, and death. (Based on work of Dr. R. Kissling.)*

bloodstream with consequent viral replication in and destruction of neurons. Typical pathological features of these encephalitides in horses include neuronal necrosis with neuronophagia and intense perivascular and interstitial mononuclear inflammatory infiltration. Infection, whether clinically evident or silent, leads to long-lasting immunity.

Laboratory Diagnosis. Because of their sporadic occurrence and the importance of identifying the first seasonal cases of eastern and western equine encephalitis, laboratory diagnosis is necessary. Isolation of virus in cell culture or infant mice is attempted from equine blood and brain tissue, but because viral titers often decline before encephalitis develops, negative results are not helpful. In the face of an outbreak, isolation of virus from mosquitoes collected systematically in the area as part of a comprehensive diagnostic surveillance program is most useful.

In an epizootic of Venezuelan equine encephalitis, clinical diagnosis usually suffices, but confirmation in the laboratory may be attempted by trying to isolate virus from the blood of early febrile cases. Identification of viral isolates is done by serological methods. Serology can also be used for retrospective diagnosis of cases, but because of confusion caused by prior unrecognized infection and immunization, this requires demonstration of a titer change between paired serum samples.

Epidemiology. The life cycle of virtually every arbovirus is unique, involving variations in arthropod and vertebrate hosts and other ecological factors. As an example we have selected the life cycle of eastern equine encephalitis virus. In North America this virus is maintained in freshwater swamp habitats by an enzootic mosquito vector, *Culiseta melanura* (Fig. 25-2). This mosquito is responsible for amplification of the virus by transmission between wild birds. The infection has no apparent effect on most wild bird species, but there is a high viremia of several days duration in certain species, from which mosquitoes are infected. It is not clear, however, which of several mosquito species transmit virus to clinically important hosts (horses, humans), because *Culiseta melanura* feeds almost exclusively upon birds. In coastal New Jersey the salt-marsh mosquito *Aedes sollicitans* has been implicated because it feeds upon horses as well as birds, but this virus–vector relationship does not operate in other areas. Likewise, the overwintering mechanism of eastern equine encephalitis virus remains a mystery. Several lines of evidence exclude the possibility that virus is reintroduced from the tropics in Central and South America each year by migrating birds, and there have been repeated failures to demonstrate transovarial transmission of virus in *Culiseta melanura* mosquitoes. Other theories, such as persistent infection of reptiles and amphibians or birds, have not been confirmed.

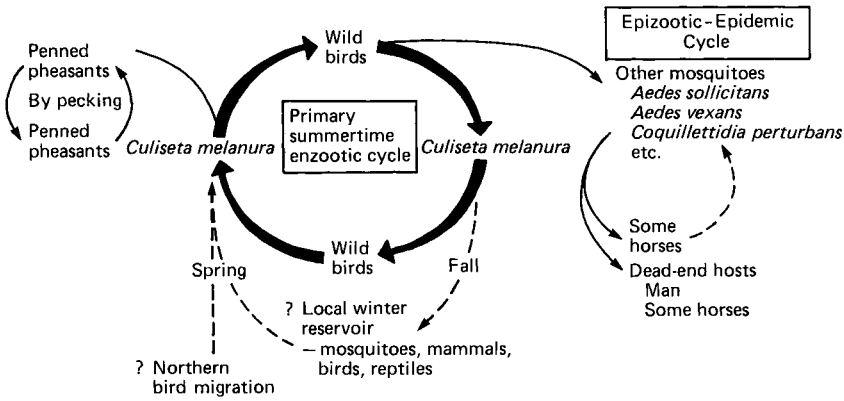

FIG. 25-2. *Features of the transmission cycle of eastern equine encephalitis virus in the United States. Known parts of the cycle in solid lines, speculative parts in broken lines. Mode of overwintering unknown. (Courtesy Dr. T. P. Monath.)*

Epizootic vector mosquitoes, capable of transmitting virus between horses, have also not been clearly identified, but during an outbreak of equine disease in Michigan in 1983, the mosquito *Coquillettidia perturbans* was implicated by virus isolation and by determination of equine and bird blood meal preference.

Western equine encephalitis virus, in the eastern United States, is believed to employ the same vertebrate and arthropod hosts as eastern equine encephalitis virus—sharing the same unanswered life cycle mysteries. In the western United States, Western equine encephalitis virus is transmitted between birds and to horses and humans by *Culex pipiens* mosquitoes, which may reach great population densities when climatic conditions or irrigation practices are suitable.

Control. Immunization of horses with inactivated cell culture vaccines for eastern, western, and Venezuelan equine encephalitides, and with an attenuated live-virus vaccine (TC-83) for Venezuelan equine encephalitis, form the basis of control measures. The inactivated bivalent or trivalent vaccines are given annually in the spring, in two doses 7–10 days apart. The attenuated live-virus Venezuelan equine encephalitis vaccine produces long-lasting immunity, but for practical purposes it also is given annually. Vector control is used mainly for short-term emergency control during outbreaks; aerial spraying with ultralow-volume insecticides, such as malathion or synthetic pyrethrins, has been effective in interrupting virus transmission. Prohibition of the movement of horses is also used in the face of outbreaks.

In an unprecedented event in the history of veterinary medicine, an

epizootic of Venezuelan equine encephalitis, which had started in northern South America in 1969 and by 1971 had resulted in the deaths of hundreds of thousands of horses throughout Central America, Mexico, and Texas, was brought to a complete halt by the large-scale combined use of: (1) a surveillance system to target control activities, (2) the then-experimental attenuated live-virus equine vaccine (TC-83), and (3) ultra-low-volume aerial spraying of insecticides. As a result of this control program and the ending of the 1971 vector season, the virus disappeared completely from Central and North America, and has not been detected in these areas since. It was estimated that the control program cost $20 million (U.S.). Much was learned which would allow a much earlier, quicker, and perhaps less expensive response to any future emergence of the epizootic serotype of Venezuelan equine encephalitis virus from its still-unknown interepizootic ecological niche in northern South America.

Infections of Pheasants. In eastern North America, many outbreaks of eastern equine encephalitis virus infection have occurred in pheasants, resulting in a mortality rate in flocks of 5 to 75%. The virus is introduced into flocks via mosquitoes and is spread when healthy birds peck upon sick, viremic birds. Mortality has also been observed in other domestic fowl, such as Pekin ducks. Prevention has been attempted by use of insecticides on premises and vaccine (equine vaccine) in valuable birds, but because of the sporadic occurrence of the disease, usually nothing is done systematically to prevent loss.

Human Infections. The equine encephalitis viruses are zoonotic and cause significant human disease. Eastern equine encephalitis virus causes sporadic severe, often fatal cases of neurological disease, often with sequelae in survivors, the overall case fatality rate among clinical cases being about 70%. Western equine encephalitis virus is usually less severe and has a case fatality rate among clinical cases of about 1%. Venezuelan equine encephalitis virus causes a systemic febrile illness, and about 1% of those affected develop clinical encephalitis. In the absence of adequate medical care, case fatality rates as high as 25 to 30% have been reported to young children with encephalitis. The risk posed for veterinarians by these viruses is small, but care should be taken to avoid penetration of the skin when working with sick horses or performing equine necropsies when there is suspicion of these infections.

Bovine Virus Diarrhea

Bovine virus diarrhea and mucosal disease are clinically dissimilar disease syndromes, and were originally described as separate diseases.

Two names were used, even after a common viral etiology was proven in 1959. There is now a consensus that the disease should be called bovine virus diarrhea and the causative agent bovine virus diarrhea virus (family *Togaviridae*, genus *Pestivirus*). The disease is an important cause of morbidity and mortality worldwide in dairy and beef cattle; recent advances in diagnosis and in the understanding of the epidemiology of the syndrome have brought about a greater appreciation of its economic importance.

Clinical Features. The acute infection of fully susceptible cattle, seen as bovine virus diarrhea, may occur at any age and is usually a trivial illness lasting a few days, whereas the persistent infection, mucosal disease, is acquired *in utero* and is characterized by high mortality and low morbidity rates, specific immune tolerance, and immunosuppression. Mucosal disease often is first recognized clinically in a herd by fever, anorexia, watery diarrhea, and erosive stomatitis, sometimes complicated by lameness and pneumonia, usually in only a few animals.

Detailed clinical and pathological manifestations of infection in individual cattle must be described in relation to age and pregnancy status. Three situations are considered: postnatal infection of nonpregnant cattle, infection of pregnant cows, and presistently infected cattle.

Postnatal Infection of Nonpregnant Cattle. After an incubation period of 5–7 days there is fever and leukopenia, but otherwise the infection is usually subclinical. Some animals in a susceptible herd may exhibit diarrhea, which may be explosive in character, some animals may have a nasal and ocular discharge and an erosive stomatitis, and in dairy cows there may be a considerable drop in milk yield. This disease is referred to as bovine virus diarrhea. Because of the immunosuppression associated with infection, this disease in calves may be manifested by increased problems with opportunistic respiratory and intestinal infections. When infection of susceptible cows occurs via infected semen from a persistently infected bull, there is usually embryo death and transient repeated breeding problems, often not recognized in usual husbandry systems.

Infection of Pregnant Cows. As described above, infection of adult cattle is usually of little consequence, except that there is a high frequency of transplacental spread of virus to the fetus. This may result in any one of several outcomes: depending on the age (immunological maturity) of the fetus and the strain of virus, infection may result in fetal death and mummification or abortion, congenital anomalies, the birth of a weak undersized calf ("weak calf syndrome"), or the birth of a clinically normal calf. Infection before 100 days of gestation usually results in destructive lesions, and retardation in growth of organs and tissues, resulting in

death or low birthweight. Between 100 and 150 days of gestation infection often affects organogenesis of the eye and central nervous system, seen as cerebellar hypoplasia, cavitation of the cerebrum, and retinal dysplasia. Surviving calves that have been infected *in utero* before the development of immunological competence remain infected for life. They never mount an effective immune response to the virus; this is persistent tolerant infection. Such calves, which remain seronegative to all tests, shed large amounts of virus in all body secretions and excretions and are very efficient in transmitting virus to susceptible cattle in the herd. These animals also have a high probability of developing clinical mucosal disease. Surviving animals that have been infected *in utero* after the development of immune competence (at about 125 days of gestation), whether manifesting pathological damage or not, usually develop neutralizing antibody and eliminate the virus.

Persistently Infected Cattle. In susceptible herds to which the virus has been recently introduced, a very high proportion of calves born in the next calving season may be persistently infected. Mortality in these calves often reaches 50% in the first year of life, due to the various manifestations of mucosal disease. In these calves there is chronic fever, anorexia, profuse watery diarrhea, nasal discharge, and erosive or ulcerative stomatitis. There is dehydration and emaciation, and death usually follows at a few weeks to a few months. Pathologically, there are multiple erosions with little surrounding inflammation, occurring from the mouth to the abomasum. In the intestine, discoloration of mucosal folds due to mucosal hyperemia and hemorrhage may occur, giving a striped appearance to the luminal surface. Histological examination confirms the epithelial necrosis seen visibly, and also indicates a massive destruction of lymphoid tissue.

Epidemiology. The virus is transmitted easily from animal to animal and from herd to herd by indirect means through feed contaminated with urine or oronasal secretions, feces, or aborted fetuses and placentae. The virus is transmitted directly to susceptible cattle rather inefficiently from acutely affected animals and very efficiently from persistently infected animals. Some persistently infected females survive to breeding age and may give birth to persistently infected offspring, thereby perpetuating the transmission pattern. Where infection has been present in a herd for some time and the majority of animals are immune, the introduction of susceptible animals, typically heifers, results in sporadic losses, often continuing over a period of years if husbandry practices remain unchanged. Where infection is absent in a herd, the introduction of a persistently infected animal is often followed by dramatic losses. Since the infection also occurs in sheep and goats, as well as

swine, deer, bison, and other wild ruminants, these species may also be sources of virus for the initiation of infection in cattle herds.

Laboratory Diagnosis. A presumptive diagnosis can be made on the basis of clinical history, examination of herd reproduction records, clinical signs, and gross and microscopic lesions. When present, oral lesions are especially suggestive of this disease. Confirmatory diagnosis is based upon virus isolation in cell cultures, viral antigen detection in tissues, and serology. Specimens submitted for virus isolation include feces, nasal exudates, blood and tissues collected at necropsy, and aborted fetuses. Because the virus is noncytopathogenic in cell cultures, its presence is determined by immunofluorescence. The same technique may be used to detect viral antigen in tissues. Also, paired acute and convalescent sera may be examined serologically, usually by a neutralization test, but caution must be used in the interpretation of negative results, because of the seronegativity of persistently infected tolerant animals.

Prevention and Control. The economic importance of bovine virus diarrhea is clear, especially in feedlots and in dairy herds, but there is no treatment and control is far from satisfactory. The major objective of control measures is to prevent the further occurrence of persistently infected animals in the herd. This requires the identification and elimination of such animals and the avoidance of further introductions by quarantine—a process that may be economically unrealistic. In such areas, an alternative is to allow the introduction into the herd of immune animals only.

In most areas, immunization is the only control strategy used, but vaccines have several drawbacks. Inactivated-virus vaccines, containing detergent-"split" virus produced in cell cultures, have met with only limited success. Attenuated live-virus vaccines, also produced in cell cultures, are more widely used, but there are unresolved problems:

1. There is morbidity and mortality in calves receiving some vaccines, caused by insufficient attenuation of vaccine virus or reversion of vaccine virus to wild type during vaccine production passage.
2. There are questions of vaccine viruses causing immunosuppression, with decreased responsiveness to other vaccines (often formulations include bovine virus diarrhea vaccine virus in combination with other attenuated live-virus vaccines), and increased incidence of opportunistic infections.
3. There have been problems with wild-type, virulent viruses being present in the calf serum, even irradiated serum, used in vaccine production cell cultures, causing disease in vaccine recipients.

Given these problems, many authorities do not advise use of present vaccines. Clearly, there is great need for a safe, efficacious vaccine.

Border Disease

The same togavirus that causes bovine virus diarrhea in cattle (or a variant virus indistinguishable from it) also infects sheep worldwide. The disease in sheep was first described on farms in border areas between England and Scotland, hence the ovine disease is known as border disease, and also, because of its clinical signs, as "hairy shaker disease." Where the disease has been recognized, its incidence is low and it is not a significant economic problem. In adult sheep the infection is always subclinical, but infection of pregnant ewes results in the delivery of dead or deformed lambs. Newborn lambs are characterized by excessive hairiness of birth coat, poor growth rate, and neurological disease evidenced by erratic gait and continuous trembling of limbs. These signs are due to defective myelination of nerve fibers in the central nervous system. In some lambs an immune response to the virus starts *in utero,* while in others there is infection of the lymphoreticuloendothelial system resulting in immunosuppression and permanent seronegativity. The latter animals, whether exhibiting clinical signs of infection or not, may be carriers and shed virus continuously in all body excretions. Control has been attempted in an investigational setting using either inactivated or attenuated live-virus bovine virus diarrhea vaccines, but in the practical setting no control measures are economically worthwhile.

Hog Cholera

Hog cholera, once also called European swine fever, is economically the most important contagious disease of swine worldwide; where the disease is present losses are severe, and where immunization and eradication programs are in place there are large costs for maintenance of public disease control agencies. The name "swine fever," used as a vernacular term in Europe ever since the disease was first recognized in the nineteenth century, was changed to "European swine fever" in the 1970s so as to distinguish it from African swine fever. However, in the rest of the world and in the scientific literature the disease is called hog cholera and its etiological agent is called hog cholera virus (family *Togaviridae,* genus *Pestivirus*). The virus is serologically related to bovine virus diarrhea virus.

Clinical Features. In its classical form, hog cholera is an acute infection accompanied by high fever, depression, anorexia, and con-

junctivitis. These signs appear after an incubation period of 2 to 4 days and are followed by vomiting, diarrhea and/or constipation, opportunistic bacterial pneumonia, and nervous system dysfunctions such as paresis, paralysis, lethargy, circling tremors, and, occasionally, convulsions. Light-skinned swine exhibit a diffuse hyperemia and purplish discoloration of the skin on the abdomen and ears. There is severe leukopenia, occurring early and reaching levels unmatched in any other disease of swine. In a susceptible herd, clinical signs are usually seen first in a few animals, often as huddling and piling up of all the animals in a pen, as if they were cold. Then over the course of about 10 days nearly all animals in the herd become sick. Young swine may die peracutely without clinical signs; older animals may die within a week of onset from the effects of the viral infection or later from the added effects of opportunistic bacterial superinfection. The herd mortality rate may reach 100%.

Less dramatic, subacute, or chronic forms of disease have been recognized in which there is a prolonged incubation period, an extended period of clinical disease with death occurring weeks or months afterward. These forms of disease have been associated with viruses of low or moderate virulence. When such viruses are inoculated into susceptible swine they may cause subclinical infection with the development of immunity or nonlethal disease with sequelae. Often there is a transient recovery phase of 2 to 6 weeks which is then followed by a relapse with emaciation, dermatitis, purpura, and death.

Pathology. In peracute cases there may be no gross changes noted at necropsy; in acute cases there are submucosal and subserosal petechial hemorrhages which are most evident under the capsule of the kidney, in intestinal serosa, and in the cortex of lymph nodes. Congestion and infarction are seen in spleen, liver, bone marrow, and lungs. These lesions are caused by viral infection and degeneration of the endothelium of small vessels.

In subacute or chronic cases, there is necrotic ulceration of the mucosa of the large intestine and evidence of opportunistic bacterial pneumonia and enteritis. This syndrome is associated with a high incidence of abortion, fetal death and mummification, and congenital anomalies. Liveborn piglets, whether healthy or abnormal, are persistently infected, immunologically tolerant, and lifelong virus shedders.

Laboratory Diagnosis. A positive diagnosis of hog cholera is difficult to make without laboratory confirmation. This is particularly true of the subacute and chronic forms of the disease. An acute, highly infectious, hemorrhagic disease with a high mortality rate should always arouse suspicion of hog cholera, no matter what the herd vaccination status or

the area or national eradication program status. When hog cholera is suspected, disease control authorities must be notified and tissue specimens (pancreas, lymph nodes, tonsil, spleen, blood) must be submitted to an authorized laboratory. Immunofluorescence, agar gel precipitin, and ELISA techniques allow rapid detection of viral antigens in tissues. Virus isolation and neutralizing antibody assays are done in porcine cell cultures, but because the virus is not cytopathogenic such assays are complex, employing immunofluorescence to detect the presence of virus. Where these specific, sensitive, and rapid diagnostic techniques are not available, tissues are fixed in 10% formalin for histological diagnosis.

Epidemiology. Hog cholera is transmitted by close contact with infected swine, or indirectly via excretions and secretions of infected swine. Shoes, clothing, and vehicles have been incriminated in the transportation of virus between herds. Carriage of virus between herds by inapparently infected carrier animals is also important. Garbage and kitchen scrap feeding was at one time an important mode of virus transmission between herds; this was especially important because many pigs were shipped for slaughter when they showed the first signs of disease and pork scraps containing high titers of virus were then fed to swine. Garbage-cooking regulations now in place in many countries have stopped this and several other similar disease problems.

Prevention, Control, and Eradication. For many years, control of hog cholera involved quarantine and immunization. The first immunization schemes employed virulent virus inoculated together with enough immune serum to prevent clinical disease. Later, in the 1960s, attenuated live-virus vaccines prepared in cell cultures were used widely in many countries. In many developed countries this control strategy has given way in recent years to the strategy of eradication; by use of "test and slaughter," hog cholera has been eradicated from the United States, Canada, Australia, and many European countries.

Eradication programs have been extremely expensive, but they have been very successful. Several factors have contributed to their success:

1. Hog cholera virus infection is restricted to domestic swine and the virus has not been transmitted to any important extent by feral swine or wild pig species; therefore, reintroduction of virus from uncontrollable sources has not been a problem.
2. Effective herd immunity induced by vaccines reduced the incidence of infection to a level where the amount of slaughtering needed to complete the eradication of the virus was economically feasible.
3. The system for surveillance of the swine population was supported by good diagnostic techniques and reliable clinical diagnosis.

4. It was clear that persistent infections with chronic virus shedding, masked by the presence of vaccine viruses, would always lead to disease and economic losses whenever herd immunity waned.
5. There was good support from the swine industries in all countries where eradication programs were introduced.

Equine Arteritis

Equine arteritis is a systemic febrile disease, usually observed only on breeding farms. Serological studies indicate that the virus, equine arteritis virus (family *Togaviridae*, genus *Arterivirus*), occurs worldwide and is widespread in equine populations, but disease has been documented only in the United States, Austria, Switzerland, and Poland. From serological studies, it is presumed that most infections are subclinical. The clinical features of overt infections are acute depression with fever, leukopenia, anorexia, palpebral edema, conjunctivitis, catarrh, and edema of legs, genitalia, and abdomen. The basic lesion caused by the virus is a segmental necrosis of small arteries throughout the body. The disease is rarely fatal, but 40–80% of pregnant mares at risk abort.

Transmission in via aerosol and contact with infected horses or aborted fetuses and placentae. Persistent infections are suspected as being important in the transmission of the virus, but have not been proven by repeated virus isolation. Immunization of horses with an attenuated live-virus vaccine prepared in cell cultures produces long-lasting immunity and no untoward effects. In view of the very rare occurrence of clinical outbreaks of disease, the need to immunize equine populations is disputed, but because of the effect of infections on reproductive outcome, immunization of valuable breeding mares may be warranted.

DISEASES CAUSED BY FLAVIVIRUSES

There are some 60 recognized flaviviruses and although some of them cause diseases of major importance in humans only three produce diseases of veterinary importance, namely, louping ill, Wesselsbron virus disease, and Japanese encephalitis (see Table 25-2).

Louping Ill

Louping ill is an infectious encephalomyelitis of sheep that occurs in the British Isles. Louping ill virus is a member of a serocomplex of 14 related tick-borne viruses that includes Russian spring–summer encephalitis virus, Central European encephalitis virus, Omsk hemorrhagic

fever virus, and Powassan virus. The viral life cycle involves transmission between sheep by the tick *Ixodes ricinus*, with occasional involvement of horses, cattle, grouse, and deer. The disease occurs in spring and summer. Infected sheep develop a prolonged viremia and a biphasic febrile response, the second peak of which coincides with the development of nervous system dysfunction—cerebellar ataxia, tremors, hyperexcitability, and paralysis. The disease gains its name from the peculiar leaping gait of ataxic sheep. Few animals that develop neurological signs survive, and most survivors exhibit neurological deficits. Control of the disease involves immunization of lambs, using an inactivated, concentrated virus vaccine produced in cell cultures, dipping all sheep with residually active acaricides, and environmental control of ticks. Louping ill virus is zoonotic, transmitted to humans by ticks or occupationally by contact with infected sheep tissues. The human disease is biphasic: the first phase is influenzalike, and the second phase is a meningoencephalitic syndrome that resolves without sequelae in 4 to 10 days.

Wesselsbron Disease

Wesselsbron virus is the cause of an important disease of sheep in many parts of sub-Saharan Africa. The clinical disease and its epidemiology resemble Rift Valley fever. The most susceptible species is the sheep, in which infection is marked by fever, depression, hepatitis with jaundice, and subcutaneous edema. Abortions are frequent, and mortality is high in pregnant ewes and newborn lambs. Cattle, horses, and swine are infected subclinically. The virus is transmitted in summer and fall by various *Aedes* mosquitoes; the disease is a particular problem in low-lying humid areas where mosquito density is greatest. Control involves immunization of lambs with an attenuated live-virus vaccine which is often combined with Rift Valley fever vaccine. Wesselsbron virus is zoonotic, causing in humans a febrile disease with headache, myalgia, and arthralgia.

Japanese Encephalitis

Japanese encephalitis virus is a member of a serocomplex containing three other related viruses: St. Louis encephalitis virus, Murray Valley encephalitis virus, and West Nile virus. Each represents an important human disease problem in a different part of the world, but only Japanese encephalitis causes significant disease in domestic animals. Japanese encephalitis virus is the most important mosquito-borne human pathogen in Japan, China, Korea, Thailand, and other countries of

southeastern Asia, and it has recently extended its range westward into India.

The mosquito *Culex tritaeniorhynchus*, which breeds in fresh water and irrigated rice fields, and feeds upon birds, swine, and humans, is the most common vector. Swine are the most abundant species of domestic animals in many parts of Asia; they have a short life span and continuously provide generations of susceptible animals. The mosquito–swine–mosquito transmission cycle serves as an efficient mode of virus amplification.

In several Asian countries Japanese encephalitis infection in swine causes considerable losses because of a high abortion rate in sows and a high neonatal mortality rate. Adult swine, horses, cattle, and sheep act as amplifiers. In Japan, control of the human and swine diseases has been very successful; a national program is based upon immunization of children and swine with different inactivated virus vaccines produced in cell cultures. Attenuated live-virus vaccines for human and animal use are under development; these vaccines offer the possibility of lower costs and may be suitable for use in large areas of southeastern Asia.

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CHAPTER 26

Orthomyxoviridae

The family *Orthomyxoviridae* comprises the genus *Influenzavirus*, which contains two species, A and B; and an unnamed genus which contains influenza C virus. Only influenzavirus A is of interest to veterinarians, as the cause of swine influenza, equine influenza, and avian influenza (also called fowl plague). These are acute respiratory diseases which usually occur as epizootics. Although influenza viruses are among the best studied of all animal viruses, efficient control of influenza has not been achieved.

Swine influenza virus was isolated in the midwestern United States in 1931. This was followed by the isolation of human influenza A virus in ferrets in London in 1933. Fowl plague had been known in Europe since the nineteenth century and was largely eradicated from that continent by 1930. The causative virus was not recognized as an influenza A virus until 1955. Equine influenza virus was first isolated in Prague in 1956.

PROPERTIES OF ORTHOMYXOVIRUSES

The typical enveloped virion is spherical and about 100 nm in diameter (Plate 26-1), but larger, more pleomorphic forms are commonly seen.

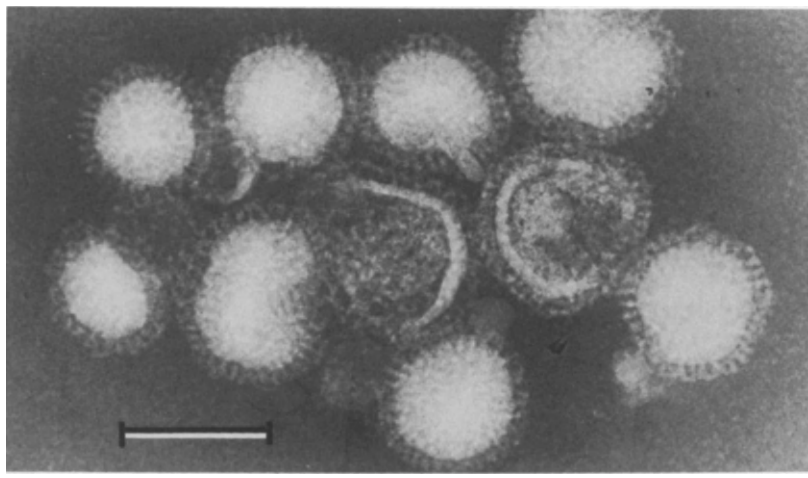

PLATE 26-1. Orthomyxoviridae. *Negatively stained preparation of virions of influenza A virus (bar = 100 nm).*

Filamentous forms up to several micrometers in length are characteristic of many strains upon first isolation. The envelope consists of host lipid from which project rod-shaped hemagglutinin (H) and mushroom-shaped neuraminidase (N) peplomers, both of which are glycoproteins. Beneath the envelope there is a matrix (M) protein, and within the envelope–matrix protein coat there is a ribonucleoprotein (NP), with helical symmetry. NP and M proteins determine species specificity, i.e., they distinguish between influenzaviruses A and B, but being internal they have no role in attachment and antibodies to them are not protective. Subtype-specific antigenic determinants are carried by H and N peplomers (Table 26-1). Antibodies to the hemagglutinin confer immunity to reinfection with any viral strain containing the same hemagglutinin.

The (−) sense ssRNA genome occurs as eight separate molecules,

TABLE 26-1
Properties of Orthomyxoviruses

Pleomorphic spherical or filamentous virion, diameter 80–120 nm
Envelope containing hemagglutinin and neuraminidase peplomers
Nucleoprotein of helical symmetry
Linear (−) sense ssRNA segmented genome, eight segments, total size 13.6 kb
Capped 5′ termini of cellular RNA used as primers for mRNA transcription
Nuclear transcription; cytoplasmic maturation by budding from plasma membrane
Defective interfering particles and genetic reassortment frequently occur

most of which code for a single protein. Because of the segmented genome, genetic reassortment can occur in cells infected with two different strains of influenza A virus (see Chapter 5). Reassortment of the genes for hemagglutinin or neuraminidase produces antigenic shift. Mutations in these genes cause antigenic drift. An understanding of these genetic phenomena is essential, since they constitute the two fundamental mechanisms for the generation of antigenic diversity among influenza viruses in nature and thus of epidemics and epizootics.

The three influenzavirus species A, B, and C have no shared antigens; indeed influenza C virus is sufficiently distinct to be classified as a separate genus. Influenzavirus A is divided into subtypes, all of which share a common nucleoprotein and matrix protein but differ in their hemagglutinin (H) and/or neuraminidase (N). So far, 13 subtypes of H (H1–H13) and 9 of N (N1–N9) have been described in birds, animals, or humans. Because novel subtypes of influenza A can arise by genetic reassortment, any combination of H and N subtypes is theoretically possible; however, only limited ranges of subtypes have been found so far in each species of animal, except for birds. Viral isolates are codified as type (A or B), animal species, place, number of the isolate, year of isolation, followed in parentheses by the H and N subtypes. Thus the virus first isolated from horses in Prague in 1956 is designated A/equi/Prague/1/56(H7N7).

Influenza viruses are sensitive to heat (56°C, 30 minutes), acid treatment (pH 3), and treatment with lipid solvents, and are thus very labile under ordinary environmental conditions.

VIRAL REPLICATION

Influenza virus adsorbs to glycoprotein receptors whose oligosaccharide side chains terminate in sialic acid. Following endocytosis of the virion, a conformational change in the hemagglutinin, occurring only at the pH pertaining in the endosome (pH 5), facilitates membrane fusion, thus triggering uncoating. The nucleocapsid migrates to the nucleus where viral mRNA is transcribed by a unique mechanism. A viral endonuclease cleaves the capped 5′ terminus from heterogeneous nuclear RNA, and this serves as a primer for transcription by the viral transcriptase. Of the eight primary RNA transcripts so produced, six are monocistronic mRNAs and are translated directly into the proteins representing H, N, NP, and the three components of the viral polymerase. The other two primary RNA transcripts undergo splicing, each yielding two mRNAs which are translated in different reading frames. Thus the

eight virion RNA molecules code for 10 proteins: 7 structural and 3 nonstructural.

The H and N polypeptides become glycosylated (see Fig. 4-10). In permissive cells, the hemagglutinin is eventually cleaved, but the two resulting chains remain linked by disulfide bonds (see Fig. 9-5). The virus matures by budding from the apical surface of the cell (see Fig. 4-11). It is not known by what mechanism one copy of each of the eight genes is selected for incorporation into a virion. Defective interfering particles, originally known as "incomplete virus," are often produced following infection at high multiplicity (see Chapter 5).

PATHOGENESIS AND IMMUNITY

The pathogenesis of swine and equine influenza virus infections follows a similar pattern and resembles the disease in humans (see Chapter 10). Infection occurs via the respiratory tract, probably by aerosols. Virions attach to the cilia of mucosal cells of the nose, trachea, and bronchi. Within 2 hours, viral antigen can be demonstrated by immunofluorescence in respiratory tract epithelial cells, and 2 hours later in alveolar septae. The virus spreads throughout the respiratory tract within 1 to 3 days. Transient viremia has been detected in equine influenza, but appears to be rare. Cellular necrosis coincides with the most severe clinical signs, hyperemia and pulmonary consolidation. Bronchi contain exudate with degenerated and detached infected mucosal cells and neutrophil leukocytes. At this time lobular atelectasis, emphysema, and focal coagulative necrosis of bronchial epithelium may occur. Thickening of the alveolar septae, interstitial pneumonia, and hyperplasia of bronchial epithelium are prominent 5 days after infection, the cellular reaction in the bronchial epithelium, alveolar septae, and peribronchial and perivascular areas being maximal on the ninth day. As in human influenza, the viral infection lowers resistance to secondary infection, which may cause bronchopneumonia.

Since many species of birds and several different subtypes of influenza A virus are involved in avian influenza, it is not surprising that a wide variety of responses is observed. The most notable difference between avian influenza and the disease in mammals is that the virus replicates in the intestinal tract as well as the respiratory tract. In infections with virulent strains ("fowl plague") viremia occurs, with lesions in the liver, spleen, heart, and kidney. Hemorrhagic lesions may occur on the combs and wattles of turkeys. Avian influenza is often complicated by secondary bacterial infection or mixed viral infections.

In all species, antibodies detectable by hemagglutination inhibition and neutralization tests appear about 7 days after infection, reaching a peak during the third week of the disease. They may persist for up to 18 months. Complement-fixing antibodies develop much later and the titers remain low. The antibody response in young animals is slower and less pronounced than in adults. Antibodies transferred from the dam to newborn piglets or foals via the colostrum protect the newborn animals for 30 to 35 days after birth.

Secretory antibodies (IgA) are also produced in the respiratory tract, appearing 8 days after infection and reaching maximal titers a week later. IgA titers decrease more rapidly than do the titers of antibodies in the serum. During the second week after infection, local and systemic cell-mediated immune responses can also be detected.

Antibody assays in birds are complicated by the fact that adult birds may have experienced infections with a variety of subtypes. Antibody titers are often low, especially in ducks.

LABORATORY DIAGNOSIS

All influenza viruses replicate well in 10-day-old embryonated eggs, using either the amniotic or the allantoic route of inoculation and incubating at 35° to 37°C for 3 to 4 days. Viral replication is detected by the demonstration of hemagglutinating activity in the harvested amniotic or allantoic fluid. Cell culture systems used for research include chick embryo fibroblasts and the canine kidney cell line, MDCK.

The best material for viral isolation in swine and equine influenza is nasal mucus taken early in the development of the infection, or lung material obtained at necropsy. Avian influenza virus is often best isolated from cloacal swabs, but viruses must be tested for virulence if their significance is to be properly evaluated (see below). The isolate is identified by hemagglutination inhibition tests, using a panel of subtype-specific sera. However, since birds may be infected with a variety of subtypes, with different neuraminidase as well as different hemagglutinin antigens, the viral isolate should also be characterized by neuraminidase inhibition tests.

Retrospective serological diagnosis can be made, using the hemagglutination inhibition test with paired serum samples, which must be suitably treated to eliminate nonspecific inhibitors. Serological methods are not of much value in birds, because of their frequent infections with a multiplicity of serotypes.

SWINE INFLUENZA

Experimentally, swine are susceptible to several subtypes of influenza A virus, including some derived from humans, and influenza virus C. However, the principal naturally occurring disease is caused by swine influenza virus, subtype H1N1. Swine influenza was first observed in 1918 in the north central United States and was for a long time limited to this area, where annual outbreaks occurred during the winter. Outside the United States, outbreaks were reported in Canada, South America, Asia, and Africa, beginning in 1968. In Europe, swine influenza was observed in the 1950s in Czechoslovakia, the United Kingdom, and West Germany; then the virus disappeared until an outbreak in 1976 in northern Italy, whence it spread to Belgium and southern France in 1979. Since then it has spread rapidly to other European countries.

Swine influenza virus isolates made in Europe during and after 1979 are related to, but clearly distinct from, the classical strains from the United States. Antigenically and genetically the recent European isolates, except those from Italy, are closely related to H1N1 virus isolates from birds. Thus two distinct antigenic variants of swine influenza viruses, both of subtype H1N1, are currently circulating in swine in different parts of the world.

Clinical Features

After an incubation period of 1 to 3 days, clinical signs, mainly restricted to the respiratory tract, appear suddenly in the majority of swine within a herd. The animals do not move freely and tend to huddle together. Rhinitis, nasal discharge, sneezing, and conjunctivitis develop, and weight loss is apparent in all sick swine. Infected swine have a paroxysmal cough, often associated with an arched back; breathing is rapid, labored, and often of abdominal type. Marked apathy, anorexia, and prostration are common, and the temperature rises to 41° to 41.5°C. After 3 to 6 days the swine usually recover quickly, eating normally by 7 days after appearance of the first clinical signs. If sick swine are kept warm and free of stress, the course of disease is benign with very few complications and a case fatality rate of less than 1%, but some cases develop severe bronchopneumonia, which may result in death. Even when the animals recover, the economic consequences of swine influenza are considerable, in that sick swine lose weight or their weight gains are reduced.

Epidemiology and Control

Swine influenza generally appears with the introduction of swine from an infected into a a susceptible herd. Frequently the disease appears simultaneously on several farms within an area, outbreaks being explosive, with all swine in a herd becoming sick at the same time. Outbreaks commence when the temperature begins to fall during late fall and are worst during the winter. Virus is shed in the nasal secretions, and animal-to-animal transmission probably occurs through droplets or small-particle aerosols. Close contact between swine, meteorological factors, and other causes of stress contribute to spread.

The problem of interepidemic survival has been a matter of intensive investigation for many years but is still unsolved. Recent investigations suggest that swine influenza virus circulates in swine throughout the year and that some swine become carriers, manifest disease occurring when the weather becomes colder.

Swine influenza virus also infects turkeys and humans. The infection in turkeys may induce clinical signs with respiratory disease or a decline in egg production and an increase in the number of abnormal eggs. Infections of humans with swine influenza virus may cause respiratory disease, but transmission to humans seems to be sporadic and person-to-person spread is limited.

Symptomatic treatment may help prevent complications due to secondary infections. Recovery is more rapid if stress is avoided. Attenuated live temperature-sensitive swine influenza vaccines and inactivated virus vaccines have been developed, but, as in human influenza, the results have not been very satisfactory.

EQUINE INFLUENZA

Epidemics of influenzalike diseases affecting horses have been reported for centuries. The differentiation of equine influenza from other respiratory diseases was established in 1956 when influenza virus [A/equi/1/Prague/56(H7N7)] was isolated from one of the epidemics. A second equine virus [A/equi/2/Miami/63(H3N8)] was first recovered from horses in the United States. The hemagglutinin of subtype 1 (H7) is related to that of some strains of fowl plague virus, whereas the hemagglutinin of subtype 2 (H3) is antigenically related to human and some avian H3 strains. Each subtype has a different neuraminidase antigen (N7 and N8). Neither subtype has undergone significant antigenic

change since it was first isolated, except that antigenic drift has been detected in subtype 2, first in 1972 in South America and, more recently, in 1980 in the United States and Europe.

Equine influenza has a wide distribution but has not been reported in Australia or New Zealand.

Clinical Features

Signs of illness appear after an incubation period of 1 to 3 days, with reddening of the nasal mucosa, conjunctivitis, and increased nasal and conjunctival exudate. Usually there is transient swelling of the pharyngeal lymph nodes before a sudden elevation of temperature to between 39.5° and 41°C. The fever persists for up to 36 hours, and there is a dry, harsh, paroxysmal cough. Recovery begins after 1 or 2 weeks, provided sick horses are kept at rest and free of stress. Secondary bacterial or mixed infections may occur, with intervals of normal temperature alternating with periods of fever. Such chronic cases are characterized by purulent nasal exudate and catarrhal bronchopneumonia. In some epizootics of equine influenza subtype 2, uncomplicated cases may also show a more severe disease course.

Clinical diagnosis of acute cases is straightforward, but diagnosis in partially immune animals is more difficult. Influenza can be confused with equine herpesvirus 4, adenovirus, and rhinovirus infections.

Epidemiology

Equine influenza viruses are highly contagious and are spread rapidly in a stable or stud by infectious exudate that is aerosolized by frequent coughing. Virus is excreted during the incubation period, and horses remain infectious for at least 5 days after clinical disease begins. Close contact between horses seems to be necessary for rapid transmission. Equine populations that are frequently moved, such as racehorses, breeding stock, show jumpers, and horses sent to sales, are at special risk. The rapid international spread of equine influenza is caused by year-round transport of horses for racing and breeding purposes, both in western Europe and between Europe and North America. Although clinical manifestations of equine influenza normally begin in the cold season, epizootics occur mostly during the main racing season, i.e., between April and October in the northern hemisphere. Outbreaks are usually caused by one subtype. Horses are the only known reservoir of equine influenza viruses.

Control

Stables and courses where equine influenza outbreaks occur should be put under quarantine for at least 4 weeks. Movement of persons associated with the diseased horses should also be limited. After all horses have recovered, cleaning and disinfection of boxes and stables, equipment,and transport vehicles is necessary.

Prevention of equine influenza can be achieved by vaccination with a bivalent, inactivated vaccine, which is administered three times, the first two vaccinations 8–12 weeks apart, the third 6 months later. Revaccination is carried out at 9-month intervals. However, immunity to subtype 2 is weak and lasts only a short time. The experience following revaccination of racehorses every 3–6 months is excellent, and outbreaks of influenza no longer occur in such populations.

AVIAN INFLUENZA

Avian influenza viruses occur worldwide. Various species of wild birds, mainly waterfowl, constitute an important reservoir. Outbreaks of disease after infection with avian influenza viruses have been reported in chickens, turkeys, ducks, pheasants, quail, pigeons, geese, and various wild species. The signs, course, and pathological findings in domestic birds are extremely variable. Infections can remain subclinical, develop into a mild, self-limiting respiratory disease, or induce severe acute generalized disease with high morbidity and mortality. The classical clinical signs of infection with highly virulent viruses are the sudden onset and a generalized disease.

Avian influenza may cause tremendous economic losses in chickens. The outbreak in Pennsylvania and Virginia in 1983–1984, caused by an H5N2 virus (see Chapter 5), cost in indemnity alone approximately $40 million (U.S.). Over the last decade, influenza virus infection of turkeys has become economically the most important disease in this species in many parts of the United States. Losses arise from the high mortality rate, the drastic drop in egg production, and the costs of control measures that include depopulation.

Properties of the Virus

A large number of different strains of influenza A have been isolated from birds, comprising all 13 known hemagglutinin subtypes and all 9 neuroaminidase subtypes, in all possible combinations, from poultry as

well as wild birds. Except for some H5 and H7 strains, isolates from wild birds do not induce disease, but strains isolated from domestic fowl are usually pathogenic. Highly pathogenic strains (called fowl plague) contain a cleaved hemagglutinin (see Chapter 4) and have a wide host cell spectrum *in vitro*. In countries where turkeys and ducks are reared for meat production, numerous isolations have been made from them, comprising almost all hemagglutinin subtypes including those of H5, H7, and swine influenza viruses (H1). Occasionally avian strains infect mammals, as in outbreaks in seals in waters northeast of the United States in the winter of 1979–1980 and in later years, which caused a high mortality.

Clinical Features

The incubation period varies from a few hours to a few days, depending on the dose and virulence of the strain. Infection of chickens and turkeys with highly virulent viruses is characterized by the sudden onset of high mortality, and also by cessation of egg laying, respiratory signs, rales, excessive lacrimation, sinusitis, edema of the head and face, cyanosis, especially visible on the combs and wattles, and diarrhea. Less virulent viruses may also cause considerable losses, particularly in turkeys, because of decreased egg production, respiratory disease, anorexia, depression, sinusitis, and a low mortality. Sometimes only some of these signs are seen. However, the effects may be greatly exacerbated by concurrent infections (e.g., Newcastle disease, various bacterial and mycoplasma infections), the use of live-virus vaccines, or environmental stress (e.g., poor ventilation and overcrowding). In ducks the most frequent signs are sinusitis, diarrhea, and increased mortality.

Laboratory Diagnosis

Clinical diagnosis is usually not possible except in an epizootic. Because of variability of signs and the resemblance to other avian diseases, virus isolation is essential to establish the cause of an outbreak, since serological diagnosis is unreliable. The widespread occurrence of avian influenza viruses of varying virulence renders determination of the virulence of any isolates made from domestic birds essential, in order to assess their significance. Of the various tests suggested, the intracerebral pathogenicity index and intravenous pathogenicity index tests, in day-old chicks and 6-week-old chickens, respectively, are probably the most useful. Highly pathogenic isolates are defined as those that produce not less than 75% mortality within 8 days when at least eight

healthy susceptible chickens, 4–8 weeks old, are inoculated with bacteria-free infectious allantoic or cell culture fluids by either the intramuscular, intravenous, or caudal air sac routes.

Epidemiology

The epidemiology of avian influenza is poorly understood because of the role of wild birds, the great variety of different strains, and the variable effects in different host species. Wild ducks and geese are regarded as refractory to disease, but wild ducks probably represent the most important reservoir of avian influenza viruses. Among domestic birds, chickens and turkeys are the species most likely to develop disease, but pheasants, quail, guinea fowl, and partridges also develop clinical illness.

The virus is shed in secretions from the respiratory tract and conjunctiva, and in feces. Transmission requires very close contact between birds, and airborne spread does not seem to play an important role. Since virus is excreted in large amounts in feces, in which it can survive for rather long periods, mechanical spread may occur, via birds, humans, fomites, water, and food. Avian influenza viruses appear to be introduced into susceptible flocks periodically by interspecies transmission between chickens and turkeys, and from wild birds, especially wild ducks. Influenza in turkeys is seen principally in countries where the birds are kept in an environment to which wild birds have access.

Control

Initial control is aimed at preventing the introduction of avian influenza viruses. Legislative measures, including quarantine and trade limitations for birds and products, are provided by many countries to prevent their introduction. However, such measures do not affect wild birds, particularly migratory species, and in high-risk areas measures are therefore taken to prevent access of wild birds to poultry farms. These are not always successful, as evidenced by the Pennsylvania outbreak of 1983–1984, described in Chapter 5.

In the United States, Australia, and most European countries, virulent avian influenza virus is handled as an exotic virus; once diagnosed, quarantine and eradication programs are implemented. To minimize secondary spread, strict hygienic measures must be introduced which include cleaning and disinfection, an interval between slaughter and repopulation, and controlled movement of people and animals. Vaccination has also been employed, but as with influenza in other animals

there are problems in selecting the appropriate vaccine strain. Because of the risk of reassortment with "wild" viruses, only inactivated vaccines should be used and because of the diversity of serotypes these must be polyvalent. However, experimental polyvalent vaccines are expensive and not very efficient. Local quarantine and depopulation of affected farms remain the only effective way to eradicate virulent influenza from chicken and turkey farms.

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CHAPTER 27

Paramyxoviridae

Paramyxoviruses and orthomyxoviruses were originally grouped together as the "myxoviruses," because of the morphological similarity of the virions and the fact that the prototype viruses, Newcastle disease virus and influenza virus, each carry a hemagglutinin and a neuraminidase. However, it was later realized that the viruses of each group differ in such basic properties as genome structure and mode of replication, hence they were separated into two families.

The family *Paramyxoviridae* contains three genera, *Paramyxovirus, Morbillivirus,* and *Pneumovirus,* and includes some of the most important pathogens of domestic animals and humans (Table 27-1). Of diseases caused by the member viruses, the most significant economic losses are caused by Newcastle disease virus, rinderpest virus, and bovine respiratory syncytial virus.

Newcastle disease virus has caused devastating epizootics in poultry in many countries. First recognized in Java in 1926, and later observed in many parts of the world, the disease represents a serious threat to chicken and turkey industries in countries free of virulent strains of the virus. Where virulent strains are enzootic, Newcastle disease is a major cause of economic loss.

TABLE 27-1
Diseases of Domestic Animals Caused by Paramyxoviruses

Genus	Virus	Animal species affected	Disease
Paramyxovirus	Parainfluenzavirus 1 (Sendai)	Mouse, rat, rabbit	Severe respiratory disease in laboratory rats and mice
	Parainfluenzavirus 2 (SV5)	Dog	Respiratory disease in dogs
	Parainfluenzavirus 3	Cattle, sheep, other mammals	Respiratory disease in cattle and sheep
	Newcastle disease (avian parainfluenzavirus 1)	Domestic and wild fowl	Severe generalized disease with central nervous system signs
Morbillivirus	Rinderpest virus	Cattle, wild ruminants	Severe generalized disease
	Peste des petits ruminants virus	Sheep, goat	Like rinderpest
	Canine distemper virus	Canidae, Procyonidae, Mustelidae	Severe generalized disease; central nervous system signs
Pneumovirus	Bovine respiratory syncytial virus	Cattle, sheep	Respiratory disease

Rinderpest virus has caused catastrophic losses in cattle in many parts of the world for centuries. The disease was first described in the fourth century and was not eliminated from Europe until the nineteenth century; today it is still the cause of great economic loss in Africa, the Middle East, and parts of Asia. It has been suggested on the basis of monoclonal antibody studies that rinderpest virus is the archetype morbillivirus, having given rise to canine distemper and human measles viruses some 5000–10,000 years ago.

Bovine respiratory syncytial virus disease seems to be an "emerging disease," the cause of more and more pneumonia, interstitial pulmonary edema, and emphysema, especially in recently weaned calves and young cattle. Unlike parainfluenzavirus 3, its role in shipping fever is not well understood.

Canine distemper is the most important viral disease of dogs, producing high morbidity and mortality in unvaccinated populations, worldwide.

PROPERTIES OF PARAMYXOVIRUSES

The virions of member viruses of the family *Paramyxoviridae* are pleomorphic, usually roughly spherical or filamentous, with a diameter of 150 to 300 nm or more (Plate 27-1). They consist of a lipid-containing bilayer envelope with glycoprotein peplomers surrounding a helically symmetrical "herringbone" nucleocapsid with a diameter of 12 or 18 nm in different genera (Table 27-2).

The genome is a single linear molecule of (−) sense ssRNA, of 18 to 20 kb, and there are five to seven proteins. There are two types of envelope

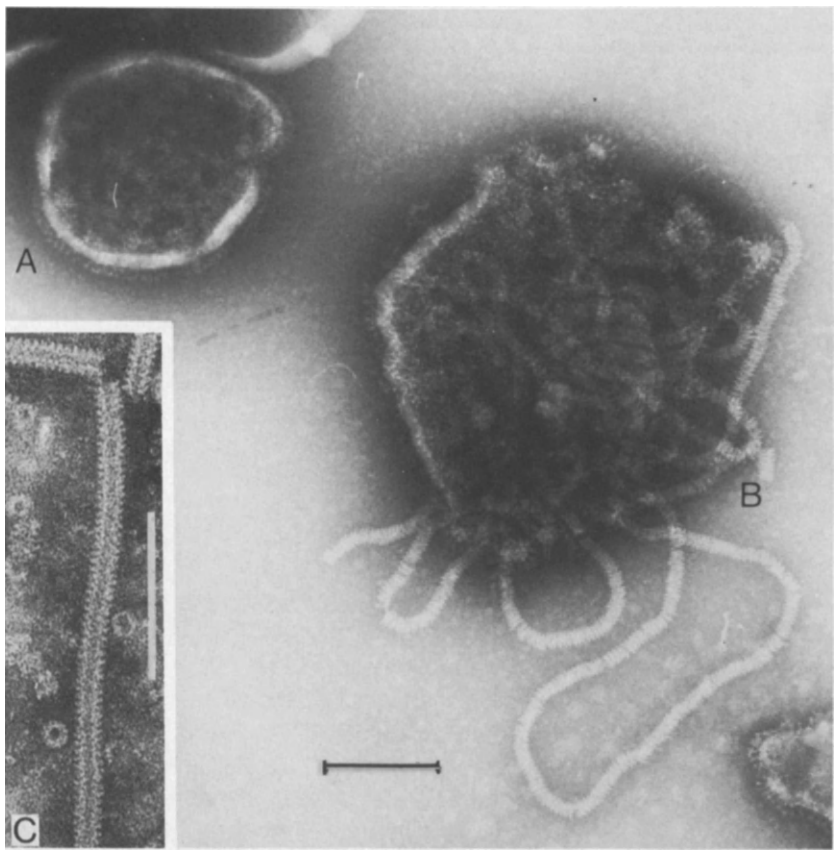

PLATE 27-1. *Negatively stained virions of a paramyxovirus. (A) Intact virion; peplomers visible at lower edge. (B) Partially disrupted virion, showing nucleocapsid. (C) Enlargement of portion of nucleocapsid, in longitudinal and cross section (bars = 100 nm). (Courtesy Dr. A. J. Gibbs.)*

TABLE 27-2
Properties of Members of the Family Paramyxoviridae

Pleomorphic virion with lipoprotein envelope, diameter 150–300 nm, sometimes filamentous
Nucleocapsid with helical symmetry, 18 nm in diameter in *Paramyxovirus* and *Morbillivirus;* 12 nm in diameter in *Pneumovirus*
Linear (−) sense ssRNA genome, 18–20 kb
Five to seven structural proteins, including an F (fusion) protein and either a hemagglutinin-neuraminidase (HN) or hemagglutinin (H) or glycoprotein (G) (see Table 27-3)
Transcriptase in virion
Sensitive to heat (56°C), pH 3, and lipid solvents
Cytoplasmic replication, budding from plasma membrane
Syncytium formation, intracytoplasmic inclusion bodies

glycoproteins with three functions: a fusion function is mediated by an F (fusion) protein, while hemagglutinin and neuraminidase functions are mediated in different viruses by either HN (hemagglutinin, neuraminidase), H, or G (glycoprotein) proteins (Table 27-3). HN protein mediates viral attachment and release from cells; F protein also assists in viral attachment, but in addition it mediates cell–cell fusion and allows infection to spread without virus release and even in the presence of antibody. There is no detectable neuraminidase activity in morbilliviruses and no detectable hemagglutinin or neuraminidase activity in pneumoviruses, but in each case virion attachment and other functions are carried out by analogous proteins. In cell culture and *in vivo* the activity of F protein causes cells to fuse together into syncytia which are characteristic of this family (see Plate 27-2).

To acquire biological activity the F protein must be cleaved by a cellular protease into two disulfide-linked polypeptides, F_1 and F_2; when this fails to occur, as in some cell cultures, the virus formed is not infectious. Since the F protein is essential for viral penetration of the host cell by fusion of the viral envelope with the plasma membrane, and for direct intercellular spread by cell-to-cell fusion, it plays a key role in the pathogenesis of paramyxovirus infections, including persistent infections. Paramyxovirus vaccines, to be maximally effective, must elicit antibodies against the F protein as well as against HN.

In contrast to the antigenic variability of the envelope glycoproteins of the orthomyxoviruses (see Chapters 4 and 26), the peplomers of paramyxoviruses are antigenically remarkably stable, geographically and over time.

TABLE 27-3
Functions and Terminology of Virion Proteins in the Three Genera of the Family Paramyxoviridae

Function	Genus		
	Paramyxovirus	*Morbillivirus*	*Pneumovirus*
Hemagglutinin—cell attachment, induction of immunity (antibody)	HN	H (only measles virus)	G (no hemagglutinin activity)
Neuraminidase—virion release	HN	None	None
Fusion protein—cell fusion, cell penetration, cell–cell spread, contribution to induction of protective immunity (antibody)	F	F	F
Nucleoprotein—protection of genome RNA	NP	NP	N
Transcriptase—RNA genome transcription	L and P	L and P	L and P
Matrix protein—virion envelope stability	M	M	M
Other—functions unknown	SH	—	M_2

VIRAL REPLICATION

Several different substrates are used to grow different paramyxoviruses. Cell cultures derived from homologous species are usually used for morbilliviruses and pneumoviruses, but these viruses are not readily cultivated and adaptation by passage is usually necessary. Viral replication in cell cultures is usually lytic, but carrier cultures readily arise in many virus–host cell systems. Syncytium formation is a characteristic feature of the cytopathology, as is the formation of acidophilic inclusions in the cytoplasm. Even though their replication is entirely cytoplasmic, morbilliviruses also produce acidophilic intranuclear inclusions (Plate 27-2). Hemadsorption is readily demonstrable with parainfluenzaviruses and some morbilliviruses (see Plate 3-2), but not with pneumoviruses.

Adsorption to cells and penetration of viruses of the family *Paramyxoviridae* occurs by mechanisms that are different from those of the

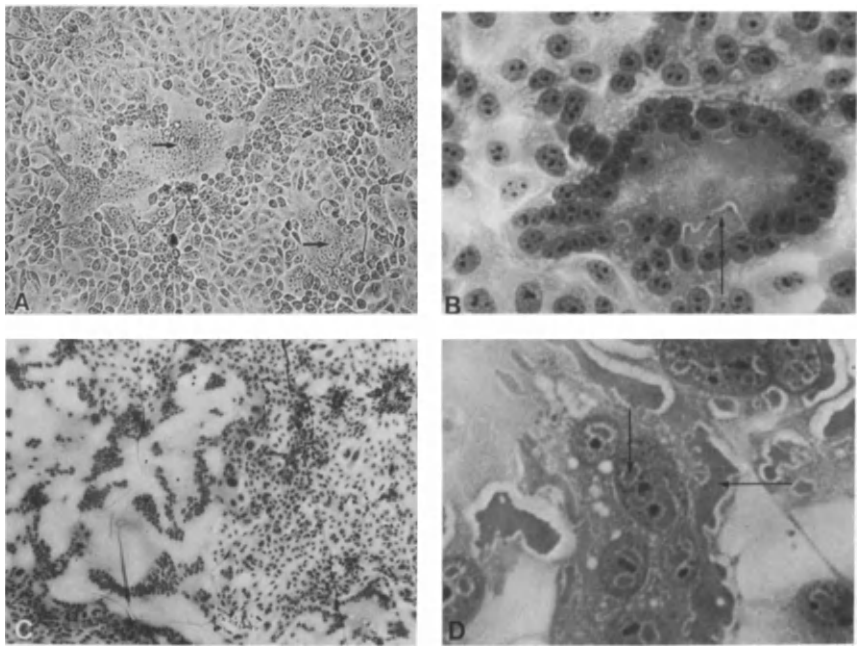

PLATE 27-2. *Cytopathic effects induced by paramyxoviruses. (A) Respiratory syncytial virus in HEp-2 cells (unstained, ×200). Note two syncytia, resulting from cell fusion. (B) Respiratory syncytial virus in HEp-2 cells (H and E stain, ×400). Syncytium containing many nuclei and acidophilic cytoplasmic inclusions (arrow). (C) Morbillivirus in kidney cells (H and E stain, ×30). Huge syncytium containing hundreds of nuclei. This monolayer was embedded in nitrocellulose before being stripped from the culture tube and stained. (D) Morbillivirus in kidney cells (H and E stain, ×400). Note multinucleated giant cell containing acidophilic nuclear (vertical arrow) and cytoplasmic (horizontal arrow) inclusions. (Courtesy I. Jack.)*

orthomyxoviruses. The orthomyxoviruses do not have a fusion function that is active at the cell surface; they attach via their HA protein and are internalized through endosomes in which the low pH activates a fusion function of their HA protein. Virion envelope fusion with the endosome membrane leads to entry of the ribonucleoprotein complex into cytoplasm. The paramyxovirus ribonucleoprotein complex, on the other hand, enters the cytoplasm directly, as the HN (or H or G) protein-mediated attachment is coordinated with F protein-mediated fusion of virion envelope and host cell membrane.

The replication strategy of the (−) sense RNA viruses (see Chapter 4) was discovered with Newcastle disease virus. The 18-kb (−) sense ssRNA genome of paramyxoviruses is transcribed by a virion-associated

RNA-dependent RNA polymerase (transcriptase) into some six or seven (+) sense mRNAs that code for the viral polypeptides. Full-length (+) sense RNA is also synthesized and serves as template for the replication of (−) sense viral genomic RNA. Control of these processes is mainly at the level of transcription. The gene coding for the nucleoprotein (NP) is located at the proximal 3′ terminus of the genome, the gene for the transcriptase at the 5′ terminus. More NP mRNA and NP protein are made than is the case for the transcriptase. Virion maturation involves: (1) the incorporation of viral glycoproteins (HN or H or G, and F) into host cell plasma membrane, (2) the association of the matrix protein (M) with the inner surface of the altered host cell membrane, (3) the alignment of nucleocapsid (RNA plus NP plus L plus P) beneath the M protein, and (4) the formation and release via budding of mature virions from the sites of modified plasma membrane (see Fig. 4-11).

DISEASES CAUSED BY MEMBERS OF THE GENUS *PARAMYXOVIRUS*

Parainfluenza viruses and Newcastle disease virus cause important infections of domestic animals (see Table 27-1).

Parainfluenzaviruses

These viruses occur worldwide in animals and humans and are associated with local infections of the respiratory tract, which are usually subclinical, unless associated with secondary bacterial infections. The three serotypes that affect domestic animals have a wide host range.

Parainfluenzavirus 1 Infections. Parainfluenzavirus 1, also known as Sendai virus, produces natural infections in humans, monkeys, guinea pigs, rabbits, rats, and mice. Whereas in most species infection remains subclinical, severe respiratory disease with high mortality can occur in breeding colonies of laboratory mice and rats. It is a significant disease in laboratory rodents used for biomedical research, and is difficult to control or eliminate because cross-infection occurs between different rodent species.

Parainfluenzavirus 2 Infections. Parainfluenzavirus 2, also known as Simian virus 5 (SV5), infects humans, monkeys, and dogs, and probably cattle, sheep, swine, and cats. Parainfluenzavirus 2 infections play a role in the kennel cough syndrome of dogs. Infections of dogs with parainfluenzavirus 2 alone are common and either cause mild clinical signs or remain subclinical. More serious disease develops when additional

microbial or viral agents, poor hygiene, or stress complicate parainfluenzavirus 2 infections, and is characterized by the sudden onset of serous nasal secretion, cough, and fever, lasting for 3 to 14 days. In severe cases (mostly in malnourished or young dogs) there are also conjunctivitis, tonsillitis, anorexia, and lethargy. Since a number of other infections can induce similar clinical signs, definitive diagnosis depends on virus isolation from nasal or throat swabs.

Parainfluenzavirus 3 Infections. Antibodies to parainfluenzavirus 3 have been demonstrated in humans, cattle, sheep, water buffaloes, deer, pigs, dogs, cats, monkeys, guinea pigs, and rats. The prevalence in cattle varies between 60 and 90%. Parainfluenzavirus 3 causes clinical disease in cattle and sheep independently of its role of predisposing to secondary bacterial infections of the respiratory tract. In calves and lambs infection is marked by fever, lacrimation, serous nasal discharge, depression, dyspnea, and coughing. Many animals may exhibit minimal clinical signs, but some may develop interstitial pneumonia. In the latter, lesions consisting of inflammatory consolidation are usually present only in the anterior lobes of the lungs. Because of the variety of agents that can cause these kinds of clinical and pathological manifestations, etiological diagnosis can only be achieved by virus isolation from nasal swabs or postmortem material. Virus isolation is done in cell cultures of bovine origin and virus is identified serologically (by immunofluorescence, hemagglutination inhibition, or virus neutralization).

The uncomplicated respiratory infection caused by parainfluenzavirus 3 runs a clinical course of 3 to 4 days, with complete recovery the rule. However, the true importance of this infection in cattle and sheep derives from its role in enzootic pneumonia, called in cattle in many countries "shipping fever." In this case the viral respiratory infection alone, or in concert with other viral infections (adenovirus, infectious bovine rhinotracheitis, bovine respiratory syncytial virus infections), predisposes to secondary bacterial invasion, especially by *Pasteurella haemolytica*. Poor hygiene, crowding, transport, harsh climatic conditions, and other causes of stress exacerbate parainfluenzavirus 3 infections and associated *Pasteurella haemolytica* infections (see Chapter 10).

Antibiotics, which help control secondary bacterial infections, and immunization are used to control shipping fever. Attenuated live-virus and inactivated parainfluenzavirus 3 vaccines are available, usually given combined with other antigens, e.g., infectious bovine rhinotrachetitis virus, bovine adenovirus, and bovine virus diarrhea virus vaccines.

Newcastle Disease

Newcastle disease was first observed in Java in 1926, and in autumn of that year the virus spread to England, where it was first recognized in Newcastle, hence the name. It was then thought to be classical fowl plague (avian influenzavirus A; see Chapter 26); the two viruses were not differentiated until 1949.

Nine serotypes of avian parainfluenzaviruses are recognized, but only avian parainfluenzavirus 1, Newcastle disease virus, is associated with a clearly defined disease. Newcastle disease is a highly contagious infection of many avian species which can lead to substantial losses in chickens and turkeys. Recently, acute disease due to Newcastle disease virus has also been observed in pigeons, mainly in Europe.

Newcastle disease outbreaks vary in clinical severity and transmissibility. In some outbreaks, especially in adult chickens, clinical signs may be minimal; this form of disease is termed *lentogenic.* In other outbreaks, the disease may have a mortality rate of up to 25%, often higher in young birds; this form of disease is termed *mesogenic.* In yet other outbreaks there is a very high mortality rate, sometimes approaching 100%; this form of the disease is termed *velogenic.*

The acute clinical disease is associated with respiratory distress, circulatory disturbances, and severe diarrhea. Central nervous system signs dominate in chronic cases. Economic consequences arise from the high mortality associated with the mesogenic and velogenic forms of disease, and from the reduced weight gains and production losses in survivors of any form of the disease. In most countries with developed poultry industries the lentogenic form is most common and the velogenic form is considered exotic. Although Newcastle disease has lost some of its importance during the last decade due to the success of strict control measures, it remains a threatening disease in industrialized countries and the cause of substantial losses in developing countries.

Properties of Newcastle Disease Virus. There is only one serotype, but individual virus strains vary considerably in virulence. Virulence is measured as a "neuropathic index" (NI) determined by intracerebral inoculation of day-old chicks. Lentogenic ($NI \leq 0.25$; avirulent to mildly virulent), mesogenic ($NI = 0.6–1.8$; intermediate virulence), and velogenic ($NI \geq 2.0$; highly virulent) strains are differentiated.

Compared with most paramyxoviruses, Newcastle disease virus is relatively heat stable, a feature of great importance in relation to its epidemiology and control. It remains infectious in bone marrow and muscles of slaughtered chickens for at least 6 months at −20°C and for

up to 4 months at refrigerator temperature. Infectious virus may survive for months at room temperature in eggs laid by infected hens, and for over a year at 4°C. Similar survival times have been observed for virus on feathers, and virus may remain infectious for long periods in contaminated premises. Quaternary ammonium compounds, 1–2% Lysol, 0.1% Cresol, and 2% formalin are used for disinfection.

Clinical Features. The incubation period in natural infections is 4–6 days. Variability in virulence determines the course of the disease. Peracute disease associated with velogenic virus strains is usually lethal. Acute and subacute disease associated with mesogenic and lentogenic virus strains are most common in developed countries with modern poultry industries. Disease commences with anorexia, elevated temperature (up to 43°C; normal 41.6°C), dullness, and thirst, and ruffled feathers, a hemorrhagic comb, closed eyes, and dry larynx and pharynx. Sick birds sneeze and show respiratory distress, and have watery diarrhea. A drop in egg production can last up to 8 weeks. Eggs laid during this phase are small and soft-shelled, and the albumen is watery. Surviving birds may exhibit central nervous system signs, characterized by paresis of limbs, ataxia, torticollis, and circling movements, or by myoclony and tremors.

In pigeons a severe, rapidly spreading disease occurs, with anorexia, diarrhea, polyuria, conjunctivitis, edema, and central nervous system signs including paresis of legs and wings. In turkeys clinical signs are similar to those in chickens, whereas in pheasants, ducks, and geese mainly central nervous system involvement is observed.

Pathogenesis and Immunity. Initially the virus replicates in the mucosal epithelium of the upper respiratory and intestinal tracts; shortly after infection virus spreads via the blood to the spleen and bone marrow, producing a secondary viremia. This leads to infection of other target organs: lung, intestine, and central nervous system. Respiratory distress and dyspnea result from congestion of the lungs and damage to the respiratory center in the brain. Gross pathological findings include ecchymotic hemorrhages in the larynx, trachea, esophagus, and throughout the intestine. The most prominent histological lesions are necrotic foci in the intestinal mucosa and the lymphatic tissue and hyperemic changes in most organs, including the brain.

Antibody production is rapid. Hemagglutination-inhibiting antibody can be detected within 4 to 6 days of infection and persists for at least 2 years. The level of hemagglutination-inhibiting antibody is a measure of immunity. Serum antibodies of the hen are transferred to chicks via the yolk, and protect chicks for 3 to 4 weeks after hatching. Serum IgG does

not prevent respiratory infection; locally produced IgA antibodies play an important role in protection in both the respiratory tract and the intestine.

Laboratory Diagnosis. Since the signs are relatively nonspecific, diagnosis must be confirmed by virus isolation and serology. The virus may be isolated from spleen, brain, or lungs by allantoic inoculation of 10-day-old embryonated eggs, the virus being differentiated from other viruses by hemadsorption and hemagglutination inhibition tests. Determination of virulence is essential for field isolates. In addition to the neuropathic index, the mean death time of chicken embryos and the intravenous pathogenicity index in 6 week-old chicks are also used. The hemagglutination inhibition test is used for the diagnosis of chronic Newcastle disease, in countries where this form of the disease is enzootic.

Epidemiology. The host spectrum of Newcastle disease virus includes gallinaceous birds (domestic chicken, turkey, guinea fowl, peacock), and pheasants, quail, partridges, and pigeons. Geese and ducks rarely develop disease. Wild birds represent a potentially important but unknown reservoir, virus having been isolated from a wide range of species. Occasional human infections occur, as an occupational disease, with conjunctivitis and sometimes laryngitis, pharyngitis, and tracheitis.

In birds that survive, virus is shed in all secretions and excretions for at least 4 weeks. Trade in infected avian species and products plays a key role in the spread of Newcastle disease from infected to noninfected areas, and importation of the virus to various countries in frozen chickens has occurred. Virus may also be disseminated with uncooked kitchen refuse, foodstuffs, bedding, manure, and transport containers. By comparison, the epidemiological role of live vectors such as wild birds or possibly mites is less important, although the former may carry virus into previously uninfected countries. Transmission occurs by direct contact between birds, by the airborne route via aerosols and dust particles, and via contaminated feed and water. Mechanical spread between flocks is favored by the relative stability of the virus and its wide host range. With lentogenic strains transovarial transmission is important, and virus-infected chicks may hatch from virus-containing eggs.

Prevention and Control. Newcastle disease is a notifiable disease in most countries, so that legislative measures constitute the basis for control. Where the disease is enzootic, control can be achieved by good hygiene combined with immunization, live-virus vaccines of naturally

occurring lentogenic strains being most commonly used. These are effective and safe, even in chicks. They may be administered via drinking water, which must not contain chlorine or other disinfectants, and are first given at 4 to 6 months of age. Laying hens are revaccinated every 4 months. Protection against disease can be expected about a week after vaccination. Vaccinated birds excrete the vaccine virus for up to 15 days after vaccination, hence in some countries birds cannot be moved from vaccinated flocks until 21 days after vaccination. Furthermore, vaccinated birds can shed wild-type virus after natural infection for up to 40 days, and may thus represent an important virus reservoir. Inactivated vaccines, administered subcutaneously, are usually used for pigeons.

DISEASES CAUSED BY MORBILLIVIRUSES

The close relationships between the three major morbilliviruses, measles, canine distemper, and rinderpest—including their antigenic cross-reactions—are reflected in the very similar pathogenesis of the diseases that they cause in humans, dogs, and cattle, respectively.

Rinderpest

Rinderpest is a highly infectious, acute or subacute, systemic disease of ruminants, which is characterized by necrosis and erosion of the mucosa in the respiratory and digestive tracts. Early constipation is followed by diarrhea. Death is usually preceded by dehydration and prostration. The disease can cause catastrophic economic loss due to the high mortality. Today, rinderpest occurs only in Africa and Asia; a dramatic increase of rinderpest has recently been observed in West Africa.

Properties of Rinderpest Virus. There is only one serotype, which is antigenically stable and exhibits extensive cross-reactivity with the other morbilliviruses. The virus is labile and is rapidly inactivated in decaying carcasses—within a few hours under tropical conditions. In manure the virus remains infectious for about 48 hours, whereas meat, spleen, and lymph nodes at 5°C remain infectious for 2 to 3 days. For disinfection, sodium hydroxide, detergents, and all commercial disinfectants are effective.

Clinical Features. Clinical signs are variable depending on the susceptibility of the breed or species of ruminant and the immune status of the animal. After an incubation period of 4 to 15 days, the temperature rises to 41°C, and anorexia, weakness, and depression develop. There is increased lacrimal and nasal secretion, accompanied by salivation. Focal

necrosis, superficial erosions, and petechiae appear in the mucosa of the mouth. Dyspnea, coughing, and diarrhea occur between days 4 and 7 of fever. Feces are watery and contain blood and sloughed mucosa; dehydration develops in severe cases. Death usually occurs between 6 and 12 days after the onset of the clinical signs. In highly susceptible cattle populations, all infected animals become sick, and a mortality rate of up to 90% has been observed. Indigenous breeds in Africa have a lower mortality, up to 50%. Surviving cattle recover some 4–5 weeks after the onset of disease and are immune for life; there is no carrier state.

Pathogenesis and Immunity. After intranasal infection, virus replicates and viral antigen can be demonstrated in tonsils and mandibular and pharyngeal lymph nodes 24 hours after infection. Viremia develops 2–3 days after infection and 1–2 days before the animal becomes febrile. After systemic spread, virus can be demonstrated in lymph nodes, spleen, bone marrow, and mucosa of the upper respiratory tract, lung, and the digestive tract. Thereafter, it replicates in the nasal mucosa, causing necrosis, erosions, and fibrinous exudation.

Cattle that survive rinderpest have a lifelong immunity. Neutralizing antibodies appear 6–7 days after the onset of clinical signs, and maximum titers are reached during the third and fourth weeks.

Laboratory Diagnosis. In countries where rinderpest is enzootic, clinical diagnosis is usually sufficient. In countries free of the disease but subject to importations, it can be confused with other diseases affecting the mucosa, such as bovine virus diarrhea and malignant catarrhal fever, and in the early stages, differentiation from infectious bovine rhinotracheitis and foot-and-mouth disease is difficult. The virus infects a wide range of cells, but isolation for laboratory diagnosis is routinely carried out in bovine kidney cell cultures. The neutralization test is used for serological diagnosis.

Epidemiology. The host range includes domestic cattle, water buffalo, sheep, and goats. Camels are susceptible but do not play an important role in the epidemiology of the disease. Domestic pigs can develop clinical signs and are regarded as an important virus reservoir in Asia. Among wild animals, all species of the genus *Artiodactyla* are susceptible.

In enzootic areas the disease spreads from animal to animal by contact, infection occurring through aerosol droplets. Virus is shed in secretions from the nose, throat, and conjunctiva as well as in feces, urine, and milk. Infected cattle excrete virus during the incubation period, before clinical signs occur, and in Africa and Asia such animals are the

most important source for the introduction of rinderpest into disease-free areas. Because the virus is thermolabile, indirect spread via fresh meat and meat products, food, and transport vehicles is unusual.

Prevention and Control. In rinderpest-free countries, veterinary public health measures are designed to prevent introduction of the virus. Importation of uncooked meat and meat products from infected countries is forbidden, and zoo animals must be quarantined before being transported to such countries. In countries with enzootic rinderpest, or where the disease has a high probability of being introduced, attenuated live-virus vaccines are used.

Because of the great economic impact of rinderpest, some of the earliest efforts in the science of immunology were directed toward protecting cattle from this disease. Early in this century these efforts proved fruitless, but over the years a progression of better and better approaches were developed until today we have a practical, safe, and efficacious vaccine. This is a strain of virus adapted to rabbits and then serially passaged in calf kidney cells, resulting in a vaccine that is safe because it is not shed from recipients, efficacious because it induces lifelong immunity, and inexpensive to produce. It is one of the best vaccines available for any animal disease, but it is thermolabile and requires a "cold chain," a difficult practical problem in many areas where rinderpest occurs.

Using the attenuated live-virus vaccine grown in cell culture, antibody can be first detected 7–17 days after vaccination, and neutralizing antibodies persist for life.

Peste des Petits Ruminants

Peste des petits ruminants is a highly contagious, systemic disease of goats and sheep very similar to rinderpest and caused by a closely related morbillivirus. Unlike rinderpest, however, many infections are subclinical. It occurs mainly in West Africa, although outbreaks have also been described elsewhere. After an incubation period of 5 or 6 days, clinical signs develop, including fever, anorexia, a necrotic stomatitis with gingivitis, and diarrhea. The course of the disease may be peracute, acute, or chronic; however, the virus does not persist. Peste des petits ruminants has economic consequences, in that mortality in goats can reach 95% and in sheep only slightly less.

Transmission of the virus is similar to that of rinderpest. Wild animals are not believed to play a role in the spread of virus. Control depends on vaccination. Because of the close antigenic relationship to rinderpest

virus, rinderpest virus vaccines are employed, and protect sheep and goats for at least a year.

Canine Distemper

Canine distemper is a highly infectious, acute or subacute, febrile disease of dogs and other carnivores, which occurs worldwide and has been known since 1760. Edward Jenner first described the course and clinical features of the disease in 1809; its viral etiology was demonstrated in 1909.

Properties of Canine Distemper Virus. There is only one serotype of the virus, but strains vary in virulence. Growth of the virus in cell cultures from dog, ferret, monkey, and human, or in embryonated chicken eggs, is possible only after adaptation. Inactivation is rapid at 37°C and occurs after a few hours at room temperature. Disinfectants readily destroy viral infectivity.

Clinical Features. Peracute cases with sudden onset of fever and sudden death are rare, but the acute disease is common. After an incubation period of 3 to 7 days, infected dogs develop a biphasic rise of temperature up to 41°C. Anorexia, catarrh, conjunctivitis, and depression are common during this stage. Some dogs show primarily respiratory signs, others intestinal signs. The first signs of the pulmonary form are a catarrhal inflammation of the larynx and bronchi, tonsillitis, and a cough. Later bronchitis or catarrhal bronchopneumonia develop, and sometimes pleuritis. Gastrointestinal signs include severe vomiting and watery diarrhea. After the onset of the disease, central nervous system signs are observed in some dogs, characterized by behavioral changes, forced movements, local myoclony, tonic–clonic spasms, epileptoid attacks, ataxia, and paresis. The duration of disease varies, depending on complications caused by secondary bacterial infections. The mortality rate ranges between 30 and 80%, but surviving dogs often have permanent central nervous system sequelae. In old dogs, an unusual encephalitis may occur as a late complication of distemper (see Chapter 10). Another late complication is "hard-pad" disease, in which hyperkeratosis of foot pads and the nose occurs; this syndrome usually leads to death.

Pathogenesis and Immunity. The pathogenesis of canine distemper was described in Chapter 10 (see Fig. 10-3). Briefly, it is a generalized infection in which, after initial replication in the oropharyngeal lymphoid tissue, cell-associated viremia occurs and virus is distributed

throughout the body. Some dogs develop an early immune response and recover quickly; in others viral infection of the respiratory, intestinal, and urogenital tracts leads to death. Some dogs develop a demyelinating encephalomyelitis about a month after infection; very rarely virus persists in the brain to cause old dog encephalitis years later.

Dogs surviving distemper have life-long immunity to reinfection. Antibodies can first be demonstrated after 6 to 9 days and reach their peak 2–4 weeks after infection, persisting with unchanged titers for about 2 years. Neutralizing antibodies are transferred to offspring in colostrum.

Laboratory Diagnosis. Clinical signs of canine distemper are no longer considered pathognomonic where vaccination is widely practiced, since the disease is so rare, hence laboratory diagnosis is necessary to exclude infectious canine hepatitis, canine parvovirus disease, leptospirosis, toxoplasmosis, and rabies. The most useful diagnostic method is the demonstration by immunofluorescence of antigen in impression smears of the conjunctiva or in peripheral blood lymphocytes (antemortem), or lung, stomach, intestinal, and bladder tissue (postmortem).

Epidemiology. The host range of canine distemper virus embraces all species of the families Canidae (dog, dingo, fox, coyote, jackal, wolf), Procyonidae (raccoon, panda), and Mustelidae (weasel, ferret, mink, skunk, badger, marten, otter).

Canine distemper virus is shed with all secretions and excretions from the fifth day after infection, which is before the onset of clinical signs, sometimes for weeks. Transmission is mainly via direct contact and droplet infection. Young dogs are more susceptible than old ones, the highest susceptibility being between the ages of 4 and 6 months, after they have lost maternal antibody.

There are differences between urban and isolated dogs with respect to epidemiology. Infections are frequent in urban dogs, in kennels and in other situations where close contact between dogs occurs. Serological investigations show that 80% of all puppies born to vaccinated urban bitches have antibodies to distemper virus up to the age of 8 weeks. This rate decreases to 10% by the age of 4 or 5 months, after which the percentage with antibodies slowly increases again, reaching 85% at the age of 2 years. In rural areas the number of dogs is too small to support a continuing chain of infection, so that highly susceptible dog populations develop, a situation which leads to catastrophic epizootics affecting dogs of all ages.

Prevention and Control. Immediately after exposure to distemper, hyperimmune serum or immune IgG can be used prophylactically. Antibiotic therapy generally has a beneficial effect by lessening the effect of secondary opportunistic bacterial infections. Immunization is recommended for the effective control of distemper, using attenuated live-virus vaccines at the age of 8 weeks and again at 12 to 16 weeks. Annual revaccination is usually recommended by vaccine manufacturers. Neutralizing antibodies are detectable 6 days after immunization, reaching a peak 3–5 weeks later.

DISEASES CAUSED BY PNEUMOVIRUSES

Respiratory Syncytial Virus Infections

Respiratory syncytial viruses cause mild to severe respiratory tract disease in cattle and sheep. Bovine respiratory syncytial virus was first detected in Japan, Belgium, and Switzerland in 1970, and was isolated a little later in England and the United States. The virus probably occurs worldwide. Sheep are also susceptible to bovine respiratory syncytial virus.

Clinical Features. Respiratory syncytial virus disease is particularly important in recently weaned calves and young cattle, in which infection is characterized by sudden onset of fever, hyperpnea, lethargy, rhinitis, and cough. Bronchiolitis and multifocal and interstitial pneumonia may be associated with interstitial edema and emphysema, and cases progressing to severe bronchopneumonia may end in death. The highest mortality often occurs in calves on a high plane of nutrition, leading to the speculation that certain feedstuffs such as corn silage may predispose cattle to the effects of infection. In general, in outbreak situations morbidity is high but mortality is low.

Pathogenesis and Pathology. In calves infected experimentally, the virus causes complete loss of the ciliated epithelium 8–10 days after infection, so that pulmonary clearance is compromised, with consequent secondary infections (see Chapter 10). At autopsy, subpleural and interstitial emphysema may be seen in all lobes of the lungs; if secondary bacterial infection is present there may be areas of consolidation. A characteristic finding is the presence of syncytial cells in the lungs, which are usually larger than those associated with parainfluenzavirus 3 infection.

Laboratory Diagnosis. Bovine respiratory syncytial virus grows in a variety of bovine cell cultures, best in those derived from respiratory tract cells. The cytopathic effect is similar to that of parainfluenzavirus 3; syncytia and intracytoplasmic inclusions are prominent. However, since viral infectivity is thermolabile and sensitive to freeze–thaw cycles, virus isolation is difficult. Immunofluorescent detection of viral antigen in lung tissue from early cases of the disease is sensitive and reliable.

Epidemiology and Control. Most commonly, respiratory syncytial virus infections occur during the winter months when cattle and sheep are housed in confined conditions. However, there have been important outbreaks in cow–calf operations in summer as well. The virus spreads rapidly—probably through aerosols or droplets of respiratory tract excretions. Reinfection of the respiratory tract is not uncommon in calves with antibody. Preexisting antibody, whether derived passively from maternal transfer or actively by prior infection, does not prevent viral replication and excretion, although clinical signs may be mild or inapparent where antibody titer is high. Even then, the stress of transport, etc., may result in acute disease if virus is reintroduced.

The role of respiratory syncytial virus is enzootic pneumonia ("shipping fever") in cattle and sheep is not clearly understood. The virus has been isolated from the respiratory tract of sick calves and lambs after arrival in the feedlot, and antibody prevalance studies have indicated that infection at this time is widespread, probably because the virus is enzootic in this environment, along with other viral respiratory tract pathogens. The unresolved question is whether or how often infection leads to the fibrinous pneumonia caused by *Pasteurella haemolytica*, the true end event in shipping fever (see Chapter 10).

For control, it should be kept in mind that clinical disease of the respiratory tract is often caused by several factors acting together. Therefore, careful diagnosis is needed before control measures are applied. A commercial attenuated live-virus vaccine is available for use in cattle.

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CHAPTER 28

Coronaviridae

The coronaviruses are ssRNA viruses that infect a wide range of mammalian and avian species; they are important causes of respiratory and enteric disease, encephalomyelitis, hepatitis, serositis, and vasculitis in domestic animals (Table 28-1). In humans coronaviruses are one of several groups of viruses that cause the common cold.

The prototype of the family, avian infectious brochitis virus, is one of the most infectious of all viruses, and causes an acute respiratory disease which in young chicks can cause very high mortality. Outbreaks can be explosive, involving nearly every bird in the flock at about the same time, because of respiratory transmission and a very short incubation period. In many ways, transmissible gastroenteritis virus of swine and mouse hepatitis virus behave similarly, affecting young animals most severely, spreading very quickly to all animals at risk, and causing major economic losses before control strategies can be put into place.

TABLE 28-1
Antigenic Relationships and Diseases Caused by Coronaviruses[a]

Antigenic group	Virus	Disease
Mammalian group 1	Human coronavirus 229E	Common cold
	Transmissible gastroenteritis virus of swine	Gastroenteritis
	Feline infectious peritonitis virus	Peritonitis, pneumonia, meningoencephalitis, panophthalmitis, wasting
	Canine coronavirus	Enteritis
Mammalian group 2	Human coronavirus OC43	Common cold
	Mouse hepatitis virus (many serotypes)	Hepatitis, encephalomyelitis, enteritis
	Bovine coronavirus	Gastroenteritis
	Porcine hemagglutinating encephalomyelitis virus	Vomiting, wasting, and encephalomyelitis
Avian group 1	Infectious bronchitis virus of chickens (at least eight serotypes)	Tracheobronchitis, nephritis
Avian group 2	Bluecomb disease virus of turkeys	Enteritis

[a]Coronaviruses have been associated with infections of the respiratory, enteric, and central nervous systems in monkeys, rats, rabbits, and other species.

PROPERTIES OF CORONAVIRUSES

The coronaviruses were so named because the unusually large club-shaped peplomers projecting from the envelope give the particle the appearance of a solar corona (Plate 28-1). Though typically about 100 nm in diameter, the virion is pleomorphic and can range in size from 75 to 160 nm. The helical ribonucleoprotein, difficult to discern in electron micrographs, seems to be connected directly to an unusual transmembrane glycoprotein (E1) that performs the role normally filled by matrix protein in other enveloped viruses. A second glycoprotein (E2) forms the prominent club-shaped peplomers and is important in cell attachment. The third structural protein is a phosphoprotein (N) which encapsidates the viral RNA to form a long, flexible ribonucleoprotein with helical symmetry (Table 28-2).

The genome consists of a single linear molecule of (+) sense ssRNA 17–24 kb in size, which is capped and polyadenylated, and is infectious.

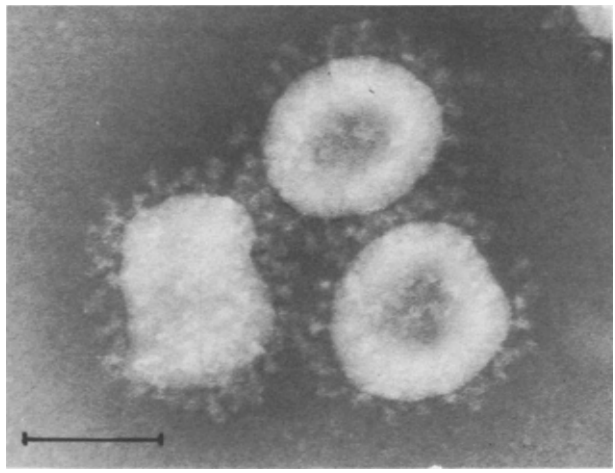

PLATE 28-1. Coronaviridae. *Negatively stained preparation of virions (bar = 100 nm).*

The family contains one genus, *Coronavirus,* which has been divided by serological methods into mammalian and avian groups, each of which can be further subdivided (Table 28-1).

VIRAL REPLICATION

The strategy of expression of the coronavirus genome is unique. The input virion RNA molecule is translated directly, one of the products being an RNA polymerase which then transcribes a full-length (−) sense copy RNA, from which in turn is transcribed a 3′-coterminal "nested set" of subgenomic mRNAs (Fig. 28-1). The nested set comprises six overlapping species of mRNAs which extend for different lengths from a

TABLE 28-2
Properties of Coronaviruses

Pleomorphic spherical virion, 100 (75–160) nm in diameter
Envelope with large, widely spaced, club-shaped peplomers
Helical nucleocapsid 10–20 nm in diameter
Genome: linear (+) sense ssRNA, 17–24 kb, capped and polyadenylated, infectious
Three structural proteins: peplomeric glycoprotein E2 (180–200K), transmembrane glycoprotein E1 (50–60K), nucleocapsid phosphoprotein N (50–60K)
Replicates in cytoplasm, budding into endoplasmic reticulum and Golgi cisternae

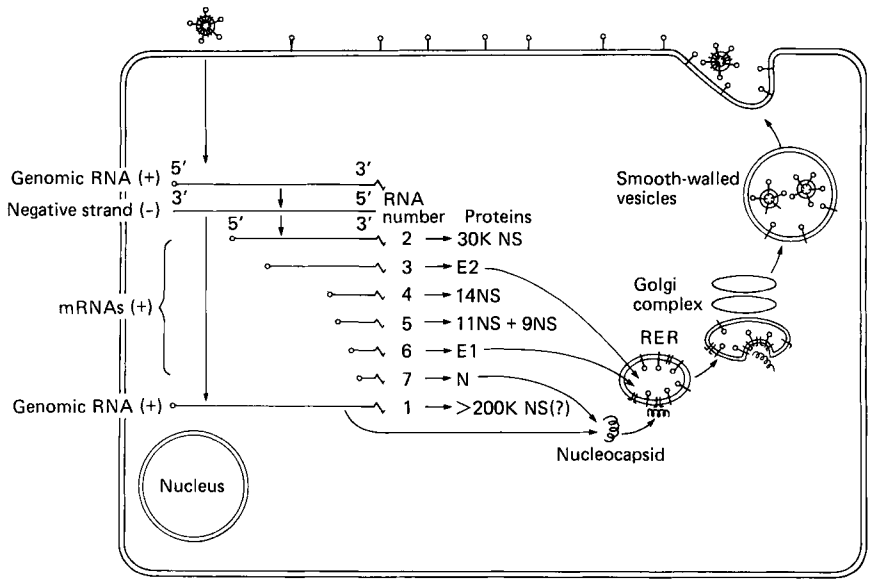

FIG. 28-1. *Major features of coronavirus replication. E1, Transmembrane glycoprotein. E2, Peplomer glycoprotein. N, Nucleoprotein. NS, Nonstructural protein. [From L. S. Sturman and K. V. Holmes,* Adv. Virus Res. **28,** *35 (1983) and K. V. Holmes, personal communication (1986); Courtesy Dr. K. V. Holmes.]*

common 3′ terminus. Only the unique sequence toward the 5′ end, which is not shared with the next smallest mRNA in the nested set, is translated, each product therefore being a unique protein.

The whole of the replication cycle occurs in the cytoplasm. The envelope is acquired by budding through those membranes that contain the viral transmembrane glycoprotein, namely cisternae of the endoplasmic reticulum and Golgi complex; the virions are then transported in vesicles to the plasma membrane for release from the cell.

BOVINE CORONAVIRUS DIARRHEA

Rotaviruses are regarded as the major cause of viral diarrhea in the young calf, but coronaviruses are also important. Coronaviruses were first reported as a cause of diarrhea in calves in the United States in 1973; since that time the virus has been recognized worldwide. Initially, diagnosis was based on detection of virus by electron microscopy, but subsequently the addition of trypsin to the culture medium was shown to facilitate the isolation of virus in cell cultures. A variety of bovine cell

cultures and Vero cells are susceptible, and viral growth can be recognized by hemadsorption.

The pathogenesis is similar to that of rotavirus diarrhea (see Chapter 10). Disease is most commonly seen in calves at about 1 week of age, the time when antibody in the dam's milk has fallen to a low level. The diarrhea usually lasts for 4 or 5 days. The destruction of the absorptive cells of the intestinal epithelium of the small intestine, and to a lesser extent the large intestine, leads to the rapid loss of water and electrolytes. Glucose and lactate metabolism is affected; hypoglycemia, lactic acidosis, and hypervolemia can lead to acute shock, heart failure, and death, although coronavirus diarrhea is generally less severe than that caused by rotaviruses.

Available vaccines are not effective; they do not appear to contain sufficient antigenic mass, and they cannot be given early enough. Alternatives to vaccinating calves are to immunize the dam to promote elevated antibody levels in the colostrum or to feed colostral antibody directly to the calf in colostrum. Monoclonal antibody to control *E. coli* infections in valves is already available commercially; similar preparations could be used to control coronavirus and rotavirus diarrhea.

TRANSMISSIBLE GASTROENTERITIS OF SWINE

This disease was first recognized in the United States in 1964, but is now seen in Europe and several other countries. It usually occurs in the winter months and is characterized by an explosive outbreak of vomiting and profuse diarrhea. Transmissible gastroenteritis is one of the major causes of death in young piglets in the midwestern United States. Mortality is high, vaccines are of limited efficacy, and it appears to be difficult to prevent the introduction of the virus into herds.

Clinical Features

The disease is usually recognized at farrowing time. The incubation period is short, usually 1–3 days, and all litters within the farrowing house are commonly affected at the same time. The clinical signs in piglets are vomiting, followed by a watery diarrhea and rapid loss of weight. The diarrhea is profuse, with an offensive odor, and often contains curds of undigested milk. Piglets under 7 days of age generally die within 2 to 7 days of the onset of signs; piglets over 3 weeks of age usually live, but may be unthrifty for several weeks. In growing, finishing, and adult swine the disease is commonly associated with inappetance and diarrhea of a few days duration, and may even go unnoticed.

Sows infected late in pregnancy may develop pyrexia, but are otherwise normal and rarely abort.

Laboratory Diagnosis

A presumptive diagnosis of transmissible gastroenteritis can be made from the sudden appearance of a rapidly spreading and often fatal disease of young piglets accompanied by vomiting and diarrhea. Confirmatory diagnosis can be by a range of techniques: identification of virus by electron microscopy, demonstration of specific antigen by immunofluorescence, isolation of virus in a range of cell types, and demonstration of rising antibody titers in paired sera from sows with affected litters or from pigs that have recovered from the disease.

Epidemiology and Control

Transmissible gastroenteritis occurs most commonly in the winter months (in North America between November and April), but its source is unknown. Unproven hypotheses concerning its origin include carriage by starlings, dogs, and foxes, or persistent infection of some swine, which may excrete virus only when stressed by cold weather. Its presence becomes apparent only when large numbers of piglets are born at a time when weather conditions favor transmission.

Control is different, although good management of the farrowing house can reduce the risk. The most widely used vaccination regimen involves vaccinating the sow with attenuated vaccines 3 weeks before farrowing. This approach provides high levels of protective antibody in the colostrum during the critical first few days of the piglet's life.

The coronavirus CV-777 also causes diarrhea in pigs. In general, the disease is less dramatic than transmissible gastroenteritis.

PORCINE HEMAGGLUTINATING VIRUS ENCEPHALOMYELITIS

This disease was first reported in Canadian swine in 1958, but it has now been recognized in the United States and Europe. Many of its clinical and pathological features are indistinguishable from those of porcine polioencephalomyelitis, which is caused by a picornavirus (see Chapter 23). The disease, which is seen principally in piglets under 2 weeks of age, is characterized by anorexia, hyperesthesia, muscle tremors, paddling of the legs, vomiting, and depression often leading to emaciation and death. In contrast to transmissible gastroenteritis, diarrhea is not commonly seen. Mortality in young piglets is high; older

litters often survive but remain permanently stunted. Serological surveys indicate that the virus is present in many swine herds, in many of which clinical disease has not been recorded.

The virus infects pigs via the upper respiratory tract and pharynx, from where it spreads to the brain via peripheral nerves. In the central nervous system, virus is first detected in the sensory nuclei of the trigeminal and vagal nerves, with subsequent spread to the brain stem, cerebral hemispheres, and cerebellum. The infection of other organs does not contribute significantly to the pathogenesis of the disease.

A clinical diagnosis of porcine hemagglutinating virus encephalomyelitis can be confirmed by the isolation of virus in primary cultures of porcine cells. Growth of the virus in cell culture can be detected by hemagglutination.

Since no vaccines are available, good husbandry is essential for the prevention and control of the disease.

EQUINE CORONAVIRUS DIARRHEA

Apart from the observation that coronavirus particles have been observed by electron microscopy in the feces of foals with diarrhea, little is known about the importance or geographical distribution of the virus. Until the virus has been isolated in cell culture and compared with other coronaviruses, it is premature to conclude that horses are infected with a separate species of coronavirus.

CANINE CORONAVIRUS DIARRHEA

Canine coronavirus usually produces a mild gastroenteritis from which the dog recovers. Although originally described before the first occurrence of canine parvovirus enteritis in 1978 (see Chapter 22), it now commonly occurs in association with canine parvovirus infection, which causes a more severe and sometimes fatal diarrhea. The virus commonly infects pups and is probably worldwide in distribution. Epizootics of coronavirus enteritis have been recorded in wild species of dogs. The disease is similar in progression to that caused by other enteric coronavirus infections such as calf coronavirus disease.

Serologically, canine coronavirus is closely related to transmissible gastroenteritis virus of swine. Although canine coronavirus does not infect pigs, transmissible gastroenteritis virus produces a subclinical infection in dogs.

Since there are many causes of diarrhea in dogs, clinical suspicion of

canine coronavirus infections should be confirmed by virus identification using electron microscopy or virus isolation in primary canine cell culture. Detection of antibody to canine coronavirus in the sera of pups is of limited value, since it may be of maternal origin and unrelated to the cause of the diarrhea.

An inactivated vaccine is available for the control of canine coronavirus infection.

FELINE INFECTIOUS PERITONITIS

Feline infectious peritonitis is an important disease that occurs in cats of all ages and in all parts of the world. A coronavirus was identified by electron microscopy as the cause of the disease in the early 1970s, but it was almost a decade before the virus was successfully grown in cell cultures. Serological surveys have now established that the virus is widely distributed in wild and domestic cats. For example, in catteries it is not unusual to find over 90% of cats with antibody to the virus. However, the incidence of clinical disease is much lower (<10%), indicating that subclinical infections are common.

Feline infectious peritonitis often occurs in association with other diseases, particularly those likely to cause immunosuppression, such as feline leukemia, feline panleukopenia, and feline syncytial virus infections.

Clinical Features

The clinical onset of feline infectious peritonitis is insidious; the cat loses its appetite, is depressed, and may have a fever. Progressive debility follows, and in the classical ("wet") form of the disease (Plate 28-2), abdominal distention is seen as a result of the peritonitis, although only a proportion of clinically diseased cats develop peritonitis. Pleuritis causing dyspnea is observed in some cats, and there are reports of neurological and ocular disease occurring in others. Affected cats die within 1 to 8 weeks. Peritoneal fluid from cats with peritonitis clots, contains high concentrations of protein, and is often flecked with fibrin.

Pathogenesis and Pathology

Most cats with feline infectious peritonitis have antibody to the virus—often to very high titer—and immune complexes have been demonstrated in the renal glomeruli. These and other observations support the concept that at least some of the pathology of feline infectious

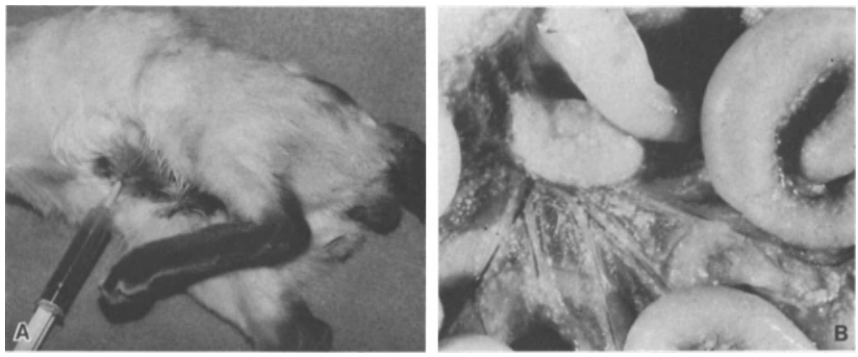

PLATE 28-2. *Feline infectious peritonitis, "wet" form. (A) Peritoneal fluid has been withdrawn. Note enlarged scrotum due to inflammation of tunica vaginalis and other serosal surfaces. (B) White fibrinous deposits on mesentery. (A, courtesy Dr. V. P. Studdert.)*

peritonitis is immunologically mediated. Until recently, it had been assumed that antibody to coronaviruses in cats was due exclusively to prior exposure to feline infectious peritonitis virus. It is now realized that transmissible gatroenteritis virus of swine produces subclinical infection in cats and, further, that cats can be infected with a feline enteric coronavirus (as yet not fully characterized and recognized only in California). Both these viruses can sensitize cats to feline infectious peritonitis virus and induce the rapid onset of clinical disease if infection occurs.

Laboratory Diagnosis

Clinical diagnosis of the classical form of feline infectious peritonitis is not difficult. When doubt exists, virus isolation can be attempted in feline embryonic lung cultures from peritoneal exudate, blood, and homogenates of abdominal and thoracic organs. Antibody can be detected in sera by several techniques, but in view of the frequency of inapparent infections with infectious peritonitis virus, interpretation of such data is difficult.

Epidemiology and Control

Under natural conditions, feline infectious peritonitis virus probably spreads by aerosol from clinically diseased cats. The importance of fecal excretion of the virus and subclinically infected cats in the epidemiology of the disease have not been critically examined.

The fact that some cats with actively or passively acquired antibody develop a more rapidly progressive form of the disease than seronegative cats inoculated with the same dose of virus, represents a major hurdle to the development of effective vaccines, and no vaccines are presently available. Control of feline infectious peritonitis depends on segregation of infected cats from susceptible cats. Any cat with antibody to the virus must be regarded as persistently infected.

AVIAN INFECTIOUS BRONCHITIS

Avian infectious bronchitis was first recognized and shown to be of viral etiology in the United States in the 1930s. It has now been recorded in almost every country of the world and is regarded as one of the most important viral diseases of chickens. It is an acute, highly infectious disease of the respiratory system of chickens, characterized by sneezing, coughing, tracheal rales, the accumulation of excess mucus in the bronchi, and depression.

Clinical Features

Outbreaks of infectious bronchitis are explosive; the virus spreads rapidly to involve the entire flock within a few days. Chickens between 1 and 4 weeks of age show the most severe disease, which is recognized initially by coughing, sneezing, nasal discharge, and respiratory distress—gasping (Plate 28-3A). Mortality in young chicks is usually 25–30%, but in some outbreaks can be as high as 75%. In older birds the disease often goes unnoticed, but in laying hens there is a marked drop in egg production, with many soft-shelled and malformed eggs being laid.

Pathology and Pathogenesis

The course of the disease in young chicks is from 7 to 21 days depending on the severity of the disease. Autopsy of young chicks dying from infectious bronchitis shows sinsusitis, catarrhal tracheitis (Plate 28-3B), bronchitis, and congestion and edema of the lungs. Caseous plugs may be present in the bronchi.

The primary target for viral replication is the trachea, but the virus also replicates in the lungs, kidneys, ovaries, and lymphoid tissue. Infectious bronchitis virus can establish persistent infection in some chickens which results in shedding of virus in the feces for several months after initial exposure to the virus. When virus persists in the presence of

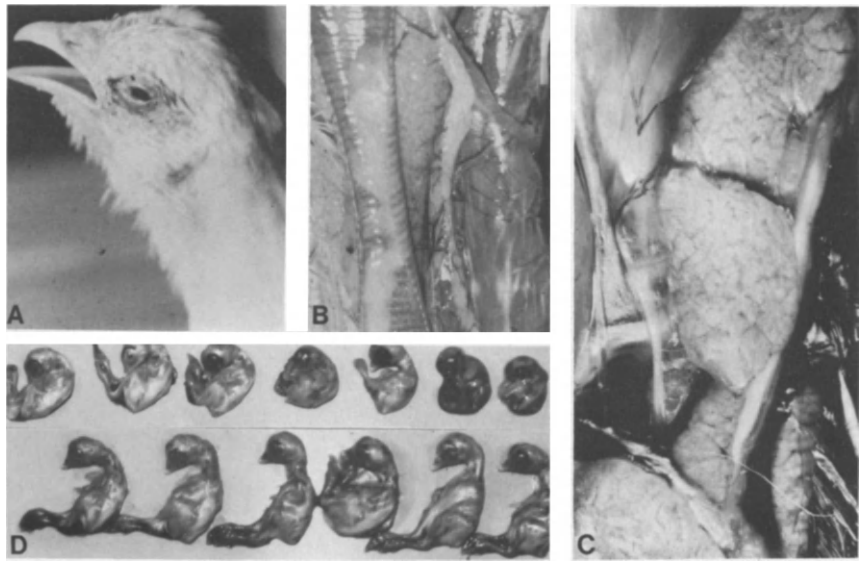

PLATE 28-3. *Avian infectious bronchitis. (A) One synonym for the disease is "gasping disease." (B) Thick mucopurulent exudate in the trachea. (C) Nephrosis. The kidney is pale and enlarged to about five times normal size. (D) Embryos from embryonated hen's eggs inoculated via the allantoic cavity with serial dilutions of virus when 9 days old, and examined 11 days later. Amounts of virus diminish in pairs from right to left in the top row, and from left to right in the bottom row. (Courtesy R. J. H. Wells.)*

high levels of antibody, severe nephritis can occur—possibly an immune complex-mediated disease (Plate 28-3C).

Laboratory Diagnosis

In contrast to several of the coronaviruses, infectious bronchitis virus can be easily isolated, principally by the allantoic inoculation of 9- to 12-day-old embryonated eggs obtained from seronegative hens. The virus rarely causes embryonic death in the first passage, but egg-adapted strains kill the embryo within 48 hours. Infected embryos are to a variable degree stunted or curled tightly (Plate 28-3D). A range of cell and organ cultures can be used also for virus isolation.

At least eight serotypes of infectious bronchitis virus exist and fall into two major groupings by cluster analyses based on neutralization data. This grouping is supported by the observation that the electrophoretic migration patterns of the virion glycoproteins of representative viruses

from each of the groups are distinct. Within these antigenic groups are virus isolates of widely differing pathogenicity.

Epidemiology and Control

Infectious bronchitis virus spreads between birds by aerosol and by ingestion of food contaminated with feces. Outbreaks of infectious bronchitis have declined in recent years due to the wide use of vaccines; however, it may occur even in vaccinated flocks following the introduction of infected replacement chicks from another farm. To minimize this risk, most poultry farms purchase only day-old chicks and rear them in isolation.

Attenuated vaccines, administered in the drinking water or as aerosols, are in wide use to protect chicks and are usually given between 7 and 10 days, and again at 4 weeks. Vaccination earlier than 7 days may be unsuccessful because most chicks have passive immunity up to this age. The presence of several serotypes would at first appear to make vaccine formulation difficult; however, no correlation between serotypic variation and resistance to infection has been shown. Local immunity in the respiratory system is critical for protection and can be generated by heterotypic vaccine strains.

Control of infectious bronchitis is difficult because of the presence of persistently infected chickens in many flocks; vaccination programs should be tailored to the type of poultry operation and the strains of virus prevalent in the area.

BLUECOMB DISEASE

Bluecomb was first recognized in turkeys in the United States in 1951 and has now been recorded in other countries. The disease affects turkeys of all ages but is most severe in 1- to 6-week-old poults. The onset is characterized by loss of appetite, constant chirping, diarrhea, weight loss, and depression. The skin of the head and neck may become cyanosed. The lesions in the digestive tract are very similar to those seen in coronavirus infections in mammals, and younger poults may die.

Only one serotype of bluecomb virus is recognized; the virus can only be isolated and grown in embryonated eggs of turkeys and chickens or in turkey embryo intestinal organ culture.

An inactivated vaccine is available, but it is generally considered to be ineffective. Some turkeys may shed virus in their feces for several months.

MOUSE HEPATITIS

Mouse hepatitis virus, first isolated in 1949 and later classified as a coronavirus, is a highly contagious and ubiquitous virus of laboratory mice, which is enzootic in many mouse colonies throughout the world. It is of major concern to mouse breeders and biomedical research workers. Though often subclinical, it can cause severe disease, and just as important, mouse coronavirus infection may greatly distort experimental results.

Many strains of mouse hepatitis virus have been isolated. All share cross-reacting antigens, but each possesses strain-specific antigenicity, with considerable overlap. However, serological or genetic relatedness is not a reliable predictor of biological behavior. Infection with different serotypes of the virus can cause hepatitis, encephalomyelitis, enteritis, and nephritis, the type and severity of the disease being dependent on the strain of virus and the age and strain of the mouse.

Some viral strains, transmitted by the respiratory route, usually cause illness associated with hepatitis and encephalitis. Other strains, transmitted by the fecal–oral route, cause lesions and disease referable to the intestine. The behavior of the virus in mouse colonies in which it is enzootic has created the impression that infections are chronic and latent, since many experimental manipulations, such as immunosuppression or infection with other agents, will precipitate acute mouse coronavirus disease. However, this is probably because most infections are subclinical, and experimental manipulations that exacerbate disease are applied coincidentally during active infection. Except in the athymic nude mouse, infections appear to be acute and self-limited, without viral persistence or a carrier state. Maintenance of infection in a mouse population therefore requires continual exposure of new, susceptible mice, either by introduction from outside or by breeding. If neonatal mice are brought into enzootically infected mouse rooms, they suffer high mortality, but if introduced as weanlings they usually sustain subclinical infections, which maintain the enzootic situation.

In contrast with most of the coronaviruses, the various serotypes of mouse coronavirus can be isolated and grown in any of several lines of mouse cells, the characteristic cytopathic effect being the formation of syncytia.

Since moust hepatitis virus causes acute, nonpersistent infections, control is achieved by breaking the infectious cycle by cessation of breeding or quarantine without the introduction of new mice. However, it is very difficult to prevent the reentry of virus into facilities receiving

mice from outside sources. Because of the multiplicity of serotypes, vaccination is impractical.

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CHAPTER 29

Bunyaviridae

Paradoxically, the largest family of mammalian viruses was one of the last to be recognized. Once a collection of poorly characterized "left-over" arboviruses, tied together by weak serological cross-reactions, the family *Bunyaviridae* now contains over 200 viruses, in five genera. Nearly all of these viruses are arthropod-borne, replicating in and being transmitted by either mosquito, tick, sandfly, or *Culicoides* vectors in specific life cycles involving mammalian or avian vertebrate host species. Only a few bunyaviruses are important pathogens of animals or humans (Table 29-1), but some, notably Rift Valley fever virus, can cause epizootics and human epidemics of such scale and severity as to command particular attention from national and international disease control and regulatory agencies.

Many bunyaviruses persist in their arthropod vectors via transovarial transmission—that is, virus is transmitted to eggs so that larvae, nymphs, and adults of succeeding generations are infected and capable of transmission to the vertebrate host. As this occurs in a particular ecological niche, the virus evolves by genetic drift and shift.

TABLE 29-1

Bunyaviruses Affecting Domestic Animals and Humans

Virus	Genus	Distribution	Arthropod vector	Animals affected	Syndrome in domestic animals
Rift Valley fever	*Phlebovirus*	Africa	Mosquito	Sheep, cattle, buffalo, humans	Hepatitis, abortion—zoonosis
Akabane	*Bunyavirus*	Australia, Israel, Japan	Mosquito	Cattle	Congenital infection
Cache Valley	*Bunyavirus*	United States	Mosquito	Cattle, sheep	Congenital infection
Nairobi sheep disease	*Nairovirus*	Eastern Africa	Mosquito	Sheep and goats	Hemorrhagic enteritis
Crimean hemorrhagic fever	*Nairovirus*	Central and southwestern Asia, India	Tick	Sheep, cattle, goats, humans	Nil—zoonosis
Hantaan	*Hantavirus*	Asia, Europe	Nil	Rodents, humans	Nil—zoonosis

TABLE 29-2
Properties of Bunyaviruses

Spherical, enveloped virion, 90–100 nm in diameter
Glycoprotein peplomers but no matrix protein in envelope
Three circular nucleocapsids with helical symmetry
Segmented (−) sense ssRNA genome, three segments with complementary 3′ and 5′ termini, total size 13.5–21 kb; part of one segment of *Phlebovirus* is ambisense
Cytoplasmic replication; budding into Golgi vesicles
Genetic reassortment occurs

PROPERTIES OF BUNYAVIRUSES

The member viruses of the family *Bunyaviridae* are spherical, approximately 90–100 nm in diameter, and are composed of a lipid bilayer envelope covered with glycoprotein peplomers surrounding three helical circular nucleocapsids (Table 29-2). The genome consists of three linear segments of ssRNA, large (L), medium (M), and small (S), of 9 to 15, 3 to 6, and 1.2 to 2.4 kb, respectively, each formed into a circle by hydrogen bonding of the ends. Most of the genome is of (−) sense, but it has been demonstrated with some member viruses (genus *Phlebovirus*) that the 5′ end of the S segment is of (+) sense; the term ambisense has been coined to describe this genome. Virions contain four major proteins: a transcriptase (designated L, 150–200K), a nucleoprotein (N, 25–50K), and two glycoproteins (G1 and G2, 40–120K) which form the surface peplomers. Epitopes for the complex antigenic interrelationships of the viruses are expressed on the glycoprotein peplomers as well as on the nucleoprotein.

Classification

Many of the viruses now included in the family *Bunyaviridae* were originally placed together into serogroups on the basis of antigenic relationships shown by hemagglutination inhibition or complement fixation. Serological relationships have now been complemented by measurements of genome segment size, structural protein size, and genetic relatedness; closely related viruses can exchange their genome segments and yield infectious progeny—genetic reassortment (see Chapter 5). By comparing all of these characteristics, five genera have been defined. Viruses within a given genus are with few exceptions antigenically related, and viruses of one genus are not antigenically related to viruses in another genus. As with other families of viruses, the definition of a viral

species or serotype is based on a uniquely specific reaction in the neutralization test. The five genera are listed below:

1. *Bunyavirus* contains 16 serogroups and more than 100 viruses, most of which are mosquito-borne, but some are transmitted by sandflies or *Culicoides*. The genus includes pathogens of domestic animals and humans such as Akabane, California encephalitis (La Crosse), Bunyamwera, and Oropouche viruses.
2. *Phlebovirus* contains more than 30 viruses, all of which are transmitted by sandflies or mosquitoes. It includes the pathogens that cause Rift Valley fever in several species of animals and sandfly fever in humans.
3. *Nairovirus* contains more than 30 viruses, mostly tick-borne, including the pathogens Crimean–Congo hemorrhagic fever, Nairobi sheep disease, and Dugbe viruses.
4. *Uukuvirus* contains about 10 viruses, all of which are tick-borne, and none of which are known to be pathogenic in animals or humans.
5. The proposed genus *Hantavirus* contains about 10 viruses, none of which are arthropod-borne. All are transmitted via urine from their persistently infected reservoir rodent hosts in nature or from rats in laboratory colonies. Several of these viruses cause human hemorrhagic fever with renal syndrome and similar diseases.

VIRAL REPLICATION

Bunyaviruses replicate well in many kinds of cell cultures, e.g., Vero (African green monkey) cells, BHK-21 (baby hamster kidney) cells, and mosquito (*Aedes albopictus*) cells. Many also replicate to high titer in suckling mouse brain.

Viral entry into its host cell is via fusion of the viral envelope with the cell membrane; all steps in replication take place in the cytoplasm. After penetration of the host cell, the virion transcriptase associated with each of the three circular nucleocapsids is activated and transcribes subgenomic mRNAs from each virion RNA species. After translation of these mRNAs, replication of the virion RNA and a second round of transcription occur. The L RNA segment codes for the virion transcriptase and the M segment codes for the G1 and G2 glycoproteins. In the genus *Bunyavirus*, the S RNA segment codes for two proteins from overlapping reading frames, the nucleoprotein and a nonstructural protein. In the genus *Phlebovirus*, the S RNA segment also codes for two proteins, but it employs an ambisense transcription strategy. In the transcription of this segment, the (−) sense 3′ half of the virion RNA species is transcribed into mRNA, which is then translated into the nucleocapsid protein. The 5′ half of this RNA species is not translated directly, but

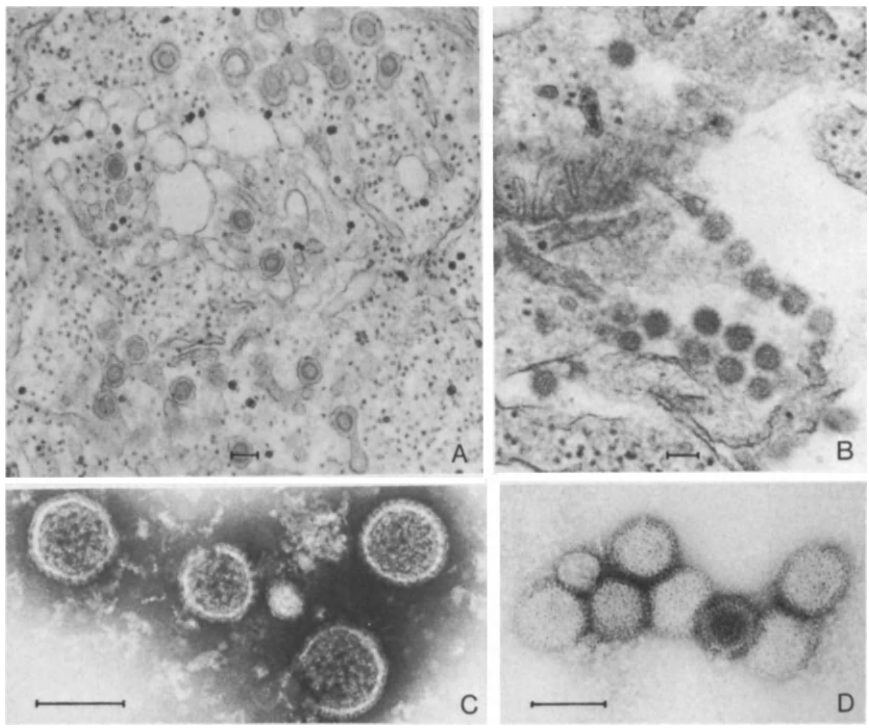

PLATE 29-1. Bunyaviridae. *(A and B) Sections of cultured cells. (A) Virions in Golgi vesicles. (B) Extracellular virions. (C and D) Negatively stained preparations. (C) Hantaan virus. (D) Rift Valley fever virus (bars = 100 nm). (C, courtesy Drs. J. McCormick and E. L. Palmer; D, courtesy Dr. E. L. Palmer.)*

following replication of the whole RNA molecule the 3′ half of the complementary strand is transcribed into an mRNA, which codes for another protein, as yet uncharacterized. Translation of this RNA segment also involves overlapping reading frames for the synthesis of two proteins, the nucleoprotein and a nonstructural protein.

The viruses mature by budding into Golgi vesciles and are released by fusion of the vesicle membrane with the plasma membrane and exocytosis, or by cytolysis (Plate 29-1).

RIFT VALLEY FEVER

Epizootics in sheep, goats, and cattle have been recognized in southern and eastern African countries from the time when intensive livestock husbandry was introduced at the beginning of this century.

Between 1950 and 1976 there were at least 16 major epizootics at various places in sub-Saharan Africa. An exceptionally devastating epizootic–epidemic occurred in Egypt in 1977 and 1978, resembling in detail the biblical description of one of the plagues of ancient Egypt. There were many hundreds of thousands of cases in sheep and cattle and more than 200,000 human cases with 600 deaths. The extent and severity of this epizootic–epidemic may have been due to the high population densities of fully susceptible animals and humans.

Clinical Features

Infected sheep develop fever, inappetance, vomiting, mucopurulent nasal discharge, and bloody diarrhea. Under field conditions, 90–100% of pregnant ewes abort, and there is a mortality rate of 90% in lambs and 20–60% in adult sheep. The clinical disease and outcome are similar in goats. In cattle the disease is somewhat less severe, but pregnant cows always abort, and the mortality rate in calves and cows is less than 10–30%.

Pathogenesis and Immunity

Vertebrate Hosts. After entry by mosquito bite or through the oropharynx, there is an incubation period of 29 to 72 hours, during which virus invades the parenchyma of the liver and reticuloendothelial organs, leading to widespread severe cytopathic effects. At necropsy of terminally affected sheep, it is not uncommon to find nearly total hepatocellular destruction. The spleen is enlarged and there are gastrointestinal and subserosal hemorrhages. Encephalitis, evidenced by neuronal necrosis and perivascular inflammatory infiltration, is a late event, seen in a small proportion of animals surviving the hepatic infection. Recovery from encephalitis is quick, there are no sequelae, and immunity is long-lasting.

Mosquito Vectors. By extrapolation to Rift Valley fever virus of results obtained from laboratory studies with other bunyaviruses, some indication of the pathogenesis of the infection in mosquito vectors is possible. The situation is dramatically different from that in the vertebrate host. Virus ingested by a mosquito in a viremic blood meal first infects gut epithelial cells. Progeny virus from this infection invades the hemolymph and nerves, from where it can infect other organs including the salivary glands and sex organs. It usually takes about a week for this infection cycle to be completed; the infection is not cytopathic in any mosquito tissue, and it persists for the life of the arthropod host. Virus

produced in the salivary glands is injected into the vertebrate host during subsequent blood meals. Virus produced in sex organs invades eggs as they are formed, thereby initiating the transovarial transmission cycle.

Laboratory Diagnosis

Laboratory work is done to confirm clinical and pathological diagnosis, especially when the disease is not currently causing an epizootic. The virus can be isolated in mice or in cell cultures and identified serologically. Retrospective serological diagnosis is possible by demonstrating a rise in antibody titer in surviving animals.

Epidemiology

In eastern and southern Africa, Rift Valley fever virus survives as a silent or minimally evident enzootic cycle for many years and then, when there is exceptionally heavy rainfall, explodes in epizootics–epidemics of great magnitude. Although such outbreaks had been studied for many years, it was not until recently that there has been any satisfactory explanation of this phenomenon. In an epizootic–epidemic, Rift Valley fever virus is transmitted by many species of *Culex* and *Aedes* mosquitoes; important vector species have been proven by epidemiological and laboratory evidence. These mosquitoes are very numerous after heavy rains or when improper irrigation techniques are used; they are infected when feeding on viremic sheep and cattle (and humans). A very high level of viremia is maintained for 3 to 5 days in infected sheep and cattle, allowing many mosquitoes to become infected. This amplification of the transmission cycle, together with mechanical transmission by biting flies, results in infection and disease in a very high proportion of animals (and humans) at risk.

These epizootic–epidemic cycles are started by infected mosquitoes occupying an unusual ecological niche. Throughout the grassy plateau regions of eastern and southern Africa, there are dry depressions in which floodwater *Aedes* species live, surviving long periods of drying as eggs and emerging only when the depressions are filled by exceptional rainfall. These mosquitoes are transovarially infected with Rift Valley fever virus and capable of transmitting the virus to a few sheep, cattle, and wild ruminants, and thus starting epizootics–epidemics which are maintained and amplified by other mosquito species.

In its epizootic cycles the virus may also be spread directly by fomites, by direct contact, and mechanically by arthropods such as tabanid flies. Infected sheep have a very high level of viremia, and transmission at the

time of abortion via contaminated placenta and fetal and maternal blood is a particular problem. Abattoir workers and veterinarians (especially those performing necropsies) are often infected directly.

The capacity of Rift Valley fever to be transmitted without the involvement of an arthropod vector raises clear concerns over the possibility for its importation into nonendemic areas via infected, viremic animals or humans, or even via animal products. Although the virus has never appeared outside Africa, it is not clear why this is so. The source of the virus which initiated the 1977 epizootic–epidemic in Egypt was never found. As was the case in Egypt, mosquito species capable of efficient transmission are present in most of the livestock-producing areas of the world.

Prevention and Control

Vaccination is used for the protection of sheep and cattle; vector control may be attempted to try to control severe epizootics in farm animals or epidemics in humans.

Vaccination. Attenuated live-virus Rift Valley fever vaccines for use in sheep, produced in mouse brain and in embryonated eggs, are effective and inexpensive, but cannot be used in pregnant animals because they cause abortions. Inactivated-virus vaccines produced in cell cultures avoid the problem of abortion. Both types of vaccines have been produced in Africa in large quantities, but vaccines must be delivered in a systematic way to entire animal populations—preferably on a regular schedule before the start of the mosquito season, or at least at the first indications of viral activity. These requirements make control very expensive and demand a skilled disease control infrastructure, an almost impossible proposition in the areas of the world where the disease exists. Even if used only in the face of an epizootic, outbreaks occur with such explosive speed that it is difficult to deliver enough vaccine fast enough. Moreover, even when vaccine is delivered quickly, there is often not enough time for protective immunity to develop.

Vector Control. Insecticide use must be comprehensive because of the involvement in epizootics of a wide range of vectors, and must be distributed over large areas and throughout vector breeding seasons. This is virtually impossible in Africa, but would be called into action if Rift Valley fever occurred outside that continent.

Disease in Humans

Rift Valley fever virus is zoonotic and causes an important human disease that occurs coincidentally with outbreaks in ruminants. The

human disease is marked by fever, chills, severe headache, "back-breaking" myalgia, diarrhea, vomiting, and hemorrhages, usually with a course of 4 to 6 days and complete recovery. In a small proportion of cases there is meningoencephalitis, hemorrhagic lesions, and/or retinitis. The overall case fatality rate in humans is about 0.1%, but in the rare hemorrhagic fever syndrome it may be 10%.

AKABANE DISEASE (CONGENITAL ARTHROGRYPOSIS—HYDRANENCEPHALY SYNDROME)

Seasonally, in Australia, Japan, and Israel there are epizootics in cattle of fetal or newborn arthrogryposis and hydranencephaly, abortions, and fetal death, caused by Akabane virus, a mosquito-borne member of the genus *Bunyavirus*. The virus can cause the same disease in sheep and goats. Although the clinical disease has been described in only three countries, there is evidence that the virus is present in Kenya, South Africa, and countries of the southwest Pacific region. In Japan, between 1972 and 1975, more than 42,000 bovine cases were recorded.

Following the bite of an infected mosquito, the virus infects the pregnant cow without producing clinical signs and reaches the fetus from the maternal circulation. The primary fetal infection is an encephalomyelitis and polymyositis. Severely affected fetuses die and are aborted, but survivors develop hydrocephalus and neurogenic arthrogryposis (Plate 29-2). Calves infected between the third and fourth months of gestation

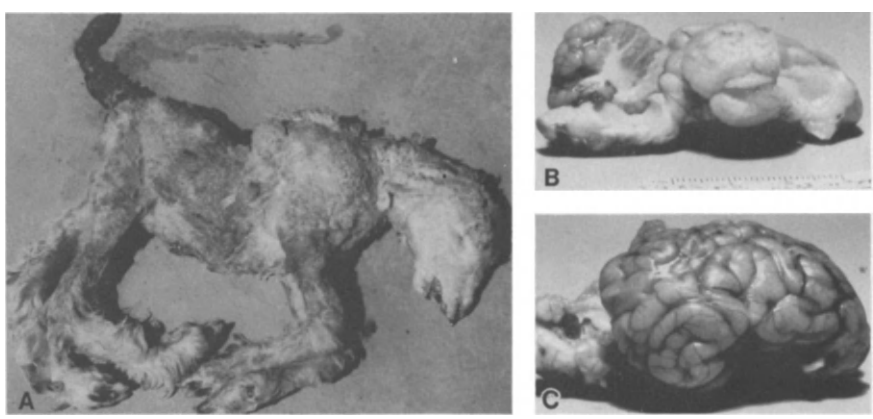

PLATE 29-2. *Akabane virus infection. (A) Arthrogryposis in a lamb born during an outbreak of Akabane infection in Australia. (B) Micrencephaly, which involves mainly the cerebral hemispheres. (C) Normal brain (part of the cerebellum has been removed). (Courtesy Dr. Ian M. Parsonson.)*

show hydranencephaly at birth; those infected between the fourth and sixth months develop arthrogryposis.

Diagnosis is suggested by clinical, pathological, and epidemiological observations, and may be confirmed by detection of specific antibody in serum taken from aborted fetuses or presuckle serum from deformed calves, or by detection of a titer rise between paired maternal sera. Virus can be isolated from placenta or fetal brain or muscle by inoculation of cell cultures or by intracerebral inoculation of suckling mice, viral isolates being identified serologically.

In Japan, Akabane virus is transmitted by *Aedes* and *Culex* mosquitoes, and in Australia by the midge, *Culicoides brevitarsis*. It is not known whether the virus is transmitted transovarially in any of these vectors. An inactivated-virus vaccine produced in cell culture has proved safe and efficacious in Japan and Australia.

NAIROBI SHEEP DISEASE

Nairobi sheep disease virus, the prototype of the genus *Nairovirus*, is the cause of disease in sheep and goats in eastern Africa. Related viruses occur in Nigeria (Dugbe virus of cattle) and India (Ganjam virus in sheep and goats). The virus is transmitted by all stages of the tick *Rhipicephalus appendiculatis*, in which transovarial infection occurs. The vertebrate reservoir host of the virus remains unknown; the virus has not been found in wild ruminants or other animals in the area.

In Kenya, sheep and goats acquire the infection when they are transported from northern districts to the Nairobi area. After a short incubation period there is high fever, hemorrhagic enteritis, and prostration. Affected animals may die within a few days, and pregnant ewes abort. Subclinical infections also occur, and recovered animals are immune. Control depends primarily on dipping to control the tick vector *Rhipicephalus*, which is also the vector of the economically important protozoal disease, East Coast fever. Vaccines are effective in preventing the disease in sheep.

CRIMEAN–CONGO HEMORRHAGIC FEVER

Crimean hemorrhagic fever has been recognized for many years in central Asia and eastern Europe as a severe zoonotic disease affecting farmers and other people coming in contact with livestock, and woodcutters and other people coming in contact with ticks. The hemorrhagic fever is marked by fever, prostration, and subcutaneous, gastroenteric,

and genitourinary hemorrhage. There is a necrotizing hepatitis, and damage to the heart and central neverous system; the case fatality rate is about 10%. The causative virus is identical to a virus, originally named Congo virus, which causes a nonfatal febrile disease in central Africa. The distribution of Crimean–Congo hemorrhagic fever virus is now known to extend from China through central Asia to India, Pakistan, eastern Europe, and the Middle East, and throughout most of sub-Saharan Africa. The virus is maintained by a cycle involving transovarial transmission in *Hyalomma* ticks and tick transmission to domestic and wild ruminants and humans. There is no evidence that there is a clinical disease in animals. The virus is also transmitted to humans by direct contact with subclinically infected viremic animals, for example, during sheep shearing or veterinary procedures, and it is also transmitted between humans, especially nosocomially. This is an emerging problem, with more and more cases being reported each year from many parts of the world and more and more antibody being found in animal populations. For example, about 8% of cattle in parts of Africa have evidence of having been infected. Prevention, which involves vector control, is difficult because of the large areas of wooded and brushy tick habitat involved.

HEMORRHAGIC FEVER WITH RENAL SYNDROME

During the Korean war of 1950–1952, thousands of United Nations troops developed a disease marked by fever, headache, hemorrhagic manifestations, and acute renal failure with shock; the mortality rate following infection was 5–10%. The etiological agent of this disease remained a mystery until 1978 when a virus, named Hantaan virus, was isolated in Korea from the field rodent *Apodemus agrarius* and identified as a unique bunyavirus. Recently, related viruses have been found in other parts of the world in association with other rodents; these viruses comprise the genus *Hantavirus*. Some of these viruses have been associated with human diseases, with varying clinical manifestations and a variety of local names. Epidemiologically, there are three disease patterns: rural, urban, and laboratory-acquired. From a clinical standpoint, there are two disease patterns: severe (with significant mortality) and mild (without mortality). Each pattern is determined by the rodent–virus combination involved. The rural disease caused by viruses similar to the prototype virus is widespread in the Far East, where it causes severe disease (Korean hemorrhagic fever; in China, epidemic hemorrhagic fever), and in Europe, where it causes mild disease (in Scan-

dinavia, nephropathia epidemica). The urban disease, associated with house rats (*Rattus rattus* and *Rattus norvegicus*), is mild and occurs in Japan, Korea, and China.

From the veterinary point of view, the most important pattern of disease is that involving laboratory rats and transmission to animal caretakers and research personnel. There have been problems in Belgium, Korea, the United Kingdom, and Japan, where there have been more than 100 cases acquired in the laboratory and one death. Control of the rural and urban patterns of disease depends mainly on avoidance of contact between humans and rodents. Prevention of introduction of virus into laboratory rat colonies requires quarantined entry of new stock (or entry only of known virus-free stock), prevention of access by wild rodents, and serological testing.

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CHAPTER 30

Rhabdoviridae

The family *Rhabdoviridae* encompasses more than 100 viruses of vertebrates, invertebrates (mostly arthropods), and plants, the virions of all having a distinctive bullet-shaped morphology. Important animal pathogens occur in four subgroups of the family: the genus *Lyssavirus* (rabies and rabies-related viruses), the genus *Vesiculovirus* (vesicular stomatitis viruses), and two unnamed subgroups which include bovine ephemeral fever virus and the fish rhabdoviruses, respectively.

Rabies virus is the cause of one of the oldest and most feared diseases of humans and animals—recognized in Egypt before 2300 BC and in ancient Greece, where it was well described by Aristotle. Rabies has the dubious distinction of being the most lethal of all infectious diseases; it also has the distinction of having been the disease which stimulated one of the great early discoveries in biomedical research. In 1885, before the nature of viruses had begun to be comprehended, Louis Pasteur developed, tested, and applied a rabies vaccine and thereby opened the modern era of the prevention of viral diseases by vaccination.

Vesicular stomatitis of horses, cattle, and swine is also among the oldest known infectious diseases of animals, first recognized as distinct from foot-and-mouth disease early in the nineteenth century, and there-

after found to cause periodic epizootics throughout the Western Hemisphere. The disease was a significant problem in artillery and cavalry horses during the American Civil War. Bovine ephemeral fever was first recognized in Africa in 1867 and is now known to be enzootic or the cause of periodic epizootics in Africa, most of Southeast Asia, Japan, and Australia. Several fish rhabdoviruses are the cause of serious losses in the expanding aquaculture industries of North America, Europe, and Japan.

PROPERTIES OF RHABDOVIRUSES

Rhabdoviruses are approximately 75 nm wide, 180 nm long (although some viruses are longer, some shorter), and consist of a lipid-containing bilayer envelope with glycoprotein peplomers surrounding a helically wound nucleocapsid (Table 30-1). This helically wound nucleocapsid gives the viruses their distinctive bullet-shaped morphology (Plate 30-1); it contains a single linear molecule of (−) sense ssRNA, 13–16 kb. During rhabdovirus replication, defective interfering (DI) virus particles are commonly formed which are shorter and have a smaller RNA molecule than normal infectious particles, with complex deletion mutations in their genome (see Chapter 5). The virion contains five proteins (molecular mass values for vesicular stomatitis virus), including a transcriptase (L, 190K), a nucleoprotein (N, 50K), a matrix protein (M, 29K), and a glycoprotein (G, 69K) which forms the surface peplomers and contains the epitopes against which neutralizing antibodies are directed.

Classification

Based on the properties of the virions and on serological interrelationships, two genera have been defined within the family:

1. The genus *Lyssavirus* contains rabies, Mokola, Lagos bat, Duvenhage, Kotonkan, and Obodhiang viruses. Except for rabies virus,

TABLE 30-1
Properties of Rhabdoviruses

Bullet-shaped enveloped virion, 180 × 75 nm, with matrix protein and glycoprotein peplomers
Nucleocapsid with helical symmetry
Linear (−) sense ssRNA genome, 13–16 kb
Cytoplasmic replication

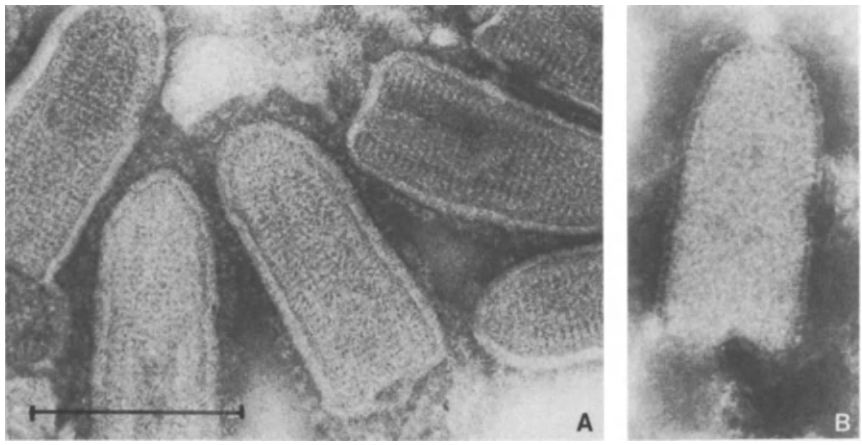

PLATE 30-1. Rhabdoviridae. *Negatively stained virions of (A) vesicular stomatitis virus and (B) rabies virus (bar = 100 nm).*

members of the genus have been found only in Africa. Each of them, with the possible exceptions of Kotonkan and Obodhiang viruses, must be considered capable of causing rabieslike disease in humans and animals.

2. The genus *Vesiculovirus* contains a growing number of serologically distinct viruses, all of which must be considered capable of causing vesicular disease in horses, cattle, and swine (see tabulation below).

Virus	Geographical Distribution
Vesicular stomatitis—Indiana	North, Central, and South America
Vesicular stomatitis—New Jersey	North, Central, and South America
Cocal	Trinidad, Brazil
Alagoas	Argentina
Brazil	Brazil
Piry	Brazil
Chandipura	India, Nigeria
Isfahan	Iran

VIRAL REPLICATION

"Fixed" (laboratory-adapted) rabies virus and vesicular stomatitis viruses replicate well in many kinds of cell cultures, e.g., Vero (African green monkey kidney) cells, BHK-21 (baby hamster kidney) cells, which are the most common substrate for animal rabies vaccines, chick embryo

fibroblasts, which are also a common substrate for vaccines, and human diploid fibroblasts (WI-38, MRC 5), which are the substrate for modern human vaccines. Laboratory-adapted ("fixed") rabies virus and vesicular stomatitis virus, as well as wild-type ("street") rabies virus and bovine ephemeral fever virus (after adaptation), replicate to high titer in suckling mouse and suckling hamster brain.

Viral entry into its host cell occurs via fusion of cell membrane with the viral envelope; all replication steps take place in the cytoplasm. Viral transcriptase, which is associated with the nucleocapsid, initiates the transcription of five subgenomic mRNA species, which are then translated into the five structural viral proteins. Replication of genomic RNA and attachment of nucleoprotein molecules to it leads to the formation of helically wound nucleocapsids. These in turn are formed into mature virions by budding through cell membranes modified by the insertion of viral glycoprotein. Vesicular stomatitis virus replication usually causes cytopathic effects rapidly, but the replication of rabies and bovine ephemeral fever viruses is usually noncytopathic because these viruses do not shut down host cell protein and nucleic acid synthesis.

RABIES

Rabies virus can infect all warm-blooded animals, and in nearly all instances the infection ends in death. Cattle rabies is important in Central and South America, where it is estimated that more than 1 million animals die each year. Dog rabies is still important in many parts of the world; infected dogs cause most of the estimated 75,000 human rabies cases that occur each year worldwide. In many countries of Europe, and in the United States and Canada, wildlife rabies has been increasing in importance. Rabies is present throughout the world, with the exception of Australia, Japan, Great Britain, and many smaller islands such as Hawaii and most of the islands of the Caribbean basin.

Clinical Features

The clinical features of rabies are similar in most species, but there is great variation between individuals. Following the bite of a rabid animal the incubation period is usually between 14 and 90 days, but may be considerably longer, and incubation periods of over a year have been documented. There is a prodromal phase prior to overt clinical disease, which is often overlooked in animals or is recalled only in retrospect as a change in temperament. Two clinical forms of the disease are recognized: furious and dumb or paralytic. In the furious form, the animal

becomes restless, nervous, aggressive, and often dangerous as it loses all fear of humans and bites at anything that gains its attention. The animal often cannot swallow, giving rise to the synonym for the disease, "hydrophobia." There is often excessive salivation, exaggerated responses to light and sound, and hyperesthesia. As the encephalitis progresses, fury gives way to paralysis, and the animal manifests the same clinical features as seen throughout in the dumb form of the disease. Terminally, there are often convulsive seizures, coma, and respiratory arrest, with death occurring 2–7 days after the onset of clinical signs. A higher proportion of dogs, cats, and horses exhibit fury than is the case for cattle or other ruminants or laboratory animal species.

Pathogenesis and Immunity

Rabies virus enters the body in the bite or occasionally the scratch of a rabid animal, or when virus-laden saliva from a rabid animal enters an open wound. Viral replication in the bite site, in muscle, is followed by invasion of peripheral nerve endings and central movement of viral genome in the cytoplasm of axons to the central nervous system (see Chapter 10). Viral entry into the spinal cord and then the brain (particularly the limbic system) is associated with clinical signs of neuronal dysfunction. Usually, at about the same time that central nervous system infection causes fury, virions are also shed from the apical end of mucus-secreting cells in the salivary glands and are delivered in high concentrations into saliva. In some cases, virus is excreted into the saliva several days (rarely up to 14 days) before clinical signs develop; in other cases virus may never be present in the saliva, even during the terminal stages of the disease.

Throughout the course of rabies, host inflammatory and specific immune responses are only minimally stimulated; the most likely reasons for this are because the infection is noncytopathic in muscle and in nerve cells and because the infection is largely concentrated in the immunologically sequestered environment of the nervous system. At death, except for a moderate mononuclear inflammatory infiltration in the nervous system, there is little histological evidence of a host response to infection. Further, in experimentally infected animals neutralizing antibody reaches significant levels only as death approaches, when it is too late to be of help and may be the cause of immunopathological disease.

Laboratory Diagnosis

It is important that the laboratory diagnosis of rabies in animals be undertaken in approved laboratories by qualified, experienced person-

nel, since in many cases decisions on human treatment and/or animal indemnification are involved. Postmortem diagnosis involves direct immunofluorescence of touch impressions of brain tissue (medulla, cerebellum, and hippocampus) from the suspect animal (Plate 30-2). There are no reliable antemortem diagnostic techniques suitable for use on suspect animals; if there is human exposure or suspected animal-to-animal transmission, and/or if clinical observation suggests rabies, the animal must be killed and brain tissue collected for testing. A comprehensive algorithm is available to guide the public health worker and physician in deciding on the course of human postexposure treatment following exposure to a potentially infectious animal (Table 30-2).

Epidemiology, Prevention, and Control

Rabies virus is not stable in the environment and in usual circumstances is only a risk when transmitted by the bite or scratch of a rabid animal, although in bat caves the virus can be transmitted via aerosol.

The control of rabies in different countries of the world poses greatly different problems, depending on whether they are free of the disease, whether they are industrialized or developing countries, and whether vampire bat rabies is a problem.

Rabies-Free Countries. Quarantine, rigidly enforced and involving segregation of dogs and cats in licensed premises for 6 months, is effectively used to exclude rabies from Australia, Japan, New Zealand, Hawaii, and several other islands. Rabies did not become enzootic in wildlife in the United Kingdom and was eradicated from dogs in that country in 1902 and again in 1922, after its reestablishment in the dog population in 1918. Since then it has been excluded by a well-administered quarantine system.

Developing Countries. In most countries of Asia, Latin America, and Africa, enzootic dog rabies is a serious problem, marked by significant domestic animal and human mortality. In these countries very large numbers of doses of human vaccines are used, and there is a need for comprehensive, professionally organized, and publicly supported agencies active in the following areas: (1) stray dog and cat elimination and control of the movement of pets (quarantine may be called for in emergencies), (2) immunization of dogs and cats, so as to break the chains of virus transmission, (3) laboratory diagnosis, so as to confirm clinical observations and obtain accurate incidence data, (4) surveillance, to measure the effectiveness of all control measures, and (5) public education programs to assure cooperation.

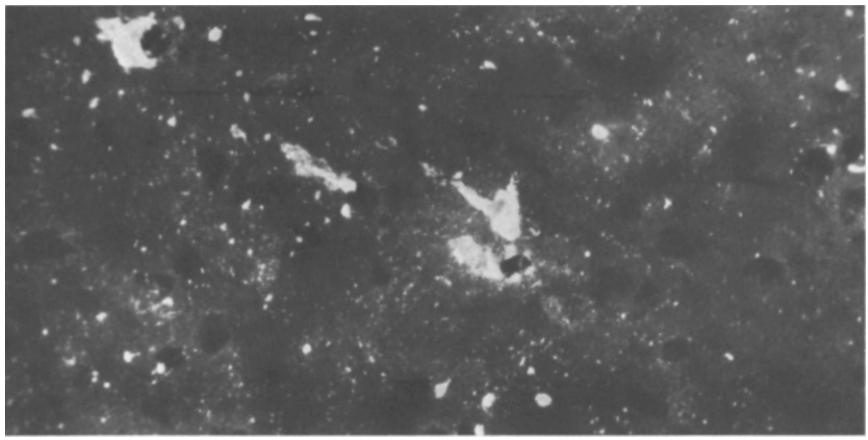

PLATE 30-2. *Rabies; strongly positive diagnostic immunofluorescence (IF). Tissue impressions are made from medulla, cerebellum, and hippocampus of the suspect animal by lightly touching tissues to clean microscope slides. Duplicate impressions are made on each slide, one for test, one for control. Slides are air-dried and fixed in acetone at −20°C for 4 hours (in an emergency, one set of slides may be fixed for 10 minutes at room temperature; negatives are then confirmed by examining fully fixed slides). Control slides are prepared similarly from known rabies-positive and rabies-negative brain tissues (sensitivity and specificity controls). Slides are air-dried, and each impression is ringed with thick ink or nail polish to contain the fluorescein isothiocyanate (FITC)–conjugated antirabies globulin. One aliquot of pretitrated conjugated globulin is preabsorbed with normal brain tissue (test); a second aliquot is preabsorbed with rabies-infected brain tissue (another specificity control). One impression on each slide is covered with the absorbed conjugated globulin, the other with the mock-absorbed conjugated globulin. Slides are incubated for 30 minutes at 37°C in a humidified chamber, rinsed and soaked in two 10-minute changes of phosphate-buffered saline, pH 7.6, and then rinsed in distilled water to remove salts. A drop of phosphate-buffered glycerol (pH 8.5) is added to each impression as a mounting medium for a coverslip. A standard IF microscope, set for observing the fluorescent emission of FITC, is used to examine all impressions. Rabies antigen is identified by its specific apple-green fluroescence in test and positive control impressions, and by its absence in negative and absorbed controls. Viral antigen may appear as dustlike particles or as large masses (2- to 10-μm masses, equivalent to Negri bodies in histological sections). Viral antigen may also appear as threadlike forms when masses are disrupted and smeared when impressions are made.*

Comparative studies have shown that the IF technique has a 97–98% correlation with virus isolation techniques, which are very slow and cumbersome. Despite this proven reliability of the IF technique, some laboratories still confirm negative clinical specimens by virus isolation (inoculation of suspect brain tissue intracerebrally into newborn to 3-week-old mice and observation for 30 days).

TABLE 30-2
Rabies: Guide for Human Postexposure Prophylaxis[a]

Animal species	Condition of animal at time of attack	Treatment of exposed person[b]
Domestic: dog and cat	Healthy and available for 10 days of observation	None, unless animal develops rabies[c]
	Rabid or suspected rabid	Rabies immune globulin[d] and vaccine[e]
	Unknown (escaped)	Consult public health officials; if treatment is indicated, give rabies immune globulin and vaccine
Wild: skunk, bat, fox, coyote, raccoon, bobcat, and other carnivores	Regard as rabid unless proved negative by laboratory tests[f]	Rabies immune globulin[d] and vaccine[e]
Other: livestock, rodents, and lagomorphs (rabbits and hares)	Consider individually	Public health officials should be consulted on questions about the need for rabies prophylaxis; bites of squirrels, hamsters, guinea pigs, gerbils, chipmunks, rats, mice, other rodents, rabbits, and hares almost never call for antirabies prophylaxis

[a]In applying these recommendations, take into account the animal species involved, the circumstances of the bite or other exposure, the vaccination status of the animal, and presence of rabies in the region. Public health officials should be consulted if questions arise about the need for rabies prophylaxis.

[b]All bites and wounds should immediately be thoroughly cleansed with soap and water. If antirabies treatment is indicated, both rabies immune globulin and vaccine should be given as soon as possible, regardless of the interval from exposure.

[c]If during the 10-day observation period a dog or cat should exhibit clinical signs of rabies, it should be immediately killed and tested, and treatment of the exposed individual with serum and vaccine should be started.

[d]If rabies immune globulin is not available, use antirabies serum, equine. Do not use more than the recommended dosage. Anticipate possible need to treat for serum sickness.

[e]Five 1-ml intramuscular doses to be given on days 0, 3, 7, 14, and 28. WHO recommends an optimal sixth dose at 90 days. Local reactions to vaccines are common and do not contraindicate continuing treatment. Discontinue vaccine if fluorescent-antibody tests of the animal are negative.

[f]The animal should be killed and tested as soon as possible. Holding for observation is not recommended.

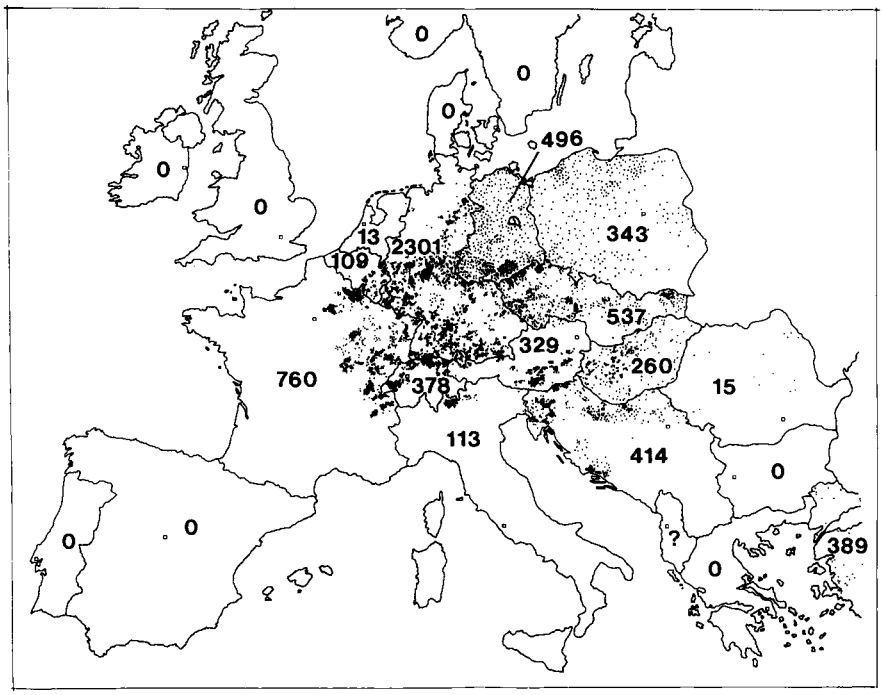

FIG. 30-1. *Occurrence of fox rabies in Europe during the last quarter of 1983. (Courtesy of the World Health Organization Collaborating Centre for Surveillance of Rabies, Tübingen, Federal Republic of Germany.)*

Industrialized Countries. In countries such as the United States, Canada, and the countries of Western Europe, endemic wildlife rabies now predominates. Fox rabies is a problem in the Appalachian mountain regions of the United States, in Ontario, Canada, in many European countries, and in polar areas inhabited by the Arctic fox (Figs. 30-1 and 30-2). Skunk rabies is common in the central areas of North America, from Texas to Saskatchewan, where it is the principal cause of rabies in cattle. Raccoon rabies in the United States began a gradual northern movement from Florida in the 1950s, reaching Georgia in the 1960s, and then causing an explosive epizootic in Virginia, Maryland, and the District of Columbia in the 1980s. The latter movement was caused by the importation of raccoons, for sporting purposes, from rabies-infected areas. Studies with monoclonal antibodies have shown that raccoon isolates from newly affected areas are the same as isolates from Florida and Georgia. In the past rabies control in wildlife has been based on animal population reduction by trapping and poisoning, but in the past

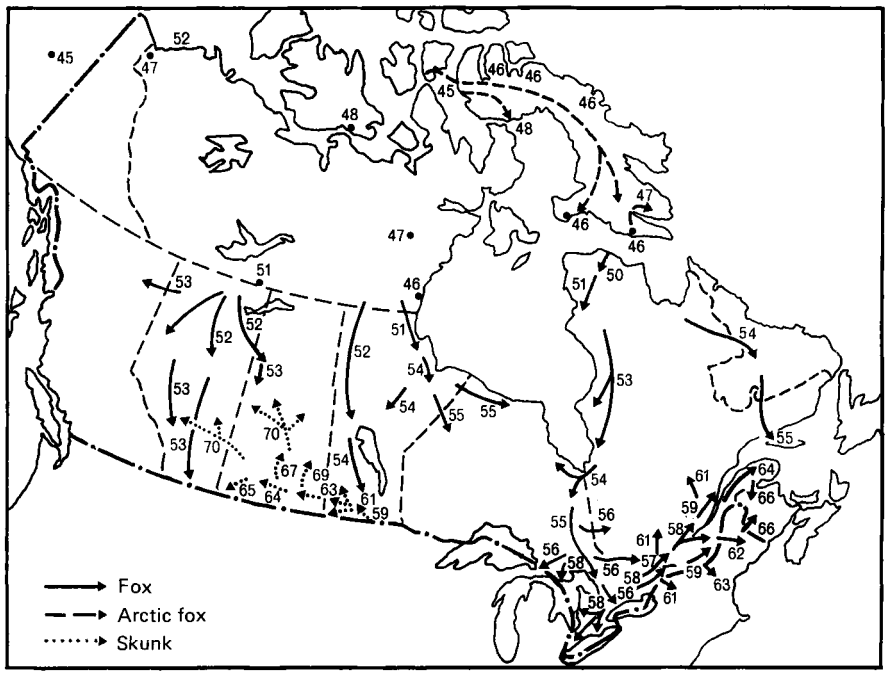

FIG. 30-2. *Rabies epizootics in Canada, 1945–1972. These epizootics involved Arctic foxes in the North, foxes in most of Canada, and skunk in south-central Canada. Numbers indicate year of epizootic. (Courtesy of* Canadian Journal of Comparative Medicine.*)*

few years, fox immunization, by the distribution of baits containing an attenuated live-virus rabies vaccine, has been used in Switzerland and Germany to interrupt virus transmission. The question of whether immunization of other wildlife species will be useful, especially in more complex ecosystems, will depend on further research on the safety and efficacy of orally ingested wildlife vaccines, on delivery systems appropriate for each reservoir host species, and on solution of legal and jurisdictional problems.

Latin America. In several countries of Latin America, vampire bat rabies is a problem to livestock industries (and to humans). Here, control efforts have depended on the use of bovine vaccines and more recently the use of anticoagulants (such as diphenadione and warfarin, injected in subtoxic doses into the reticulum of cattle). When vampire bats feed on the blood of treated cattle, they suffer fatal hemorrhages in their wing capillaries.

Vaccination. Animal rabies vaccines, produced by modern cell culture technology as either inactivated or attenuated live-virus products, are efficacious and safe. In the United States, an annual update of indications and contraindications for the use of each licensed animal rabies vaccine is published by an expert group (see "Further Reading").

Veterinarians and other individuals occupationally or otherwise at risk of rabies should be prophylactically immunized. Because of several variables in the level and nature of risk, the immediate availability of postexposure booster vaccination, etc., a comprehensive set of recommendations for prophylactic vaccines use has been produced by a group of experts and is updated each year (Table 30-3; see "Further Reading").

VESICULAR STOMATITIS

Originally, vesicular stomatitis was considered of interest only because of its role in the differential diagnosis of foot-and-mouth disease in cattle and the debilitating lameness it can cause in horses. More recently, however, the disease has been recognized as the cause of economically important losses in conditioning and milk production in cattle, especially as more dairying is undertaken in warmer climates.

Clinical Features

The clinical features of vesicular stomatitis infection vary greatly among animals in a herd. Lesions develop quickly after an incubation period of 1 to 5 days. Excess salivation and fever are often the first signs of infection in cattle and horses, and lameness is often the first sign in swine. Vesicular lesions on the tongue, the oral mucosa, teats. and coronary bands of cattle may progress to total epithelial denudation with secondary bacterial infection. Lesions may cause profuse salivation and anorexia, lameness, and rejection of the nursing calf. In horses, tongue lesions are most pronounced, often progressing to complete sloughing of the epithelium. In swine, vesicular lesions are most common on the snout and coronary bands. Lesions usually heal within 7 to 10 days, and there are no sequelae.

Pathogenesis and Immunity

The virus probably enters the body through breaks in the mucosa and skin, due to the minor abrasions caused, for example, by rough forage, or by the bites of flying arthropods. There does not seem to be a sys-

TABLE 30-3
Rabies: Criteria for Human Preexposure Immunization[a]

Risk category	Nature of risk	Typical populations	Preexposure regimen
Continuous	Virus present continuously, often in high concentrations; aerosol, mucous membrane, bite, or nonbite exposure possible; specific exposures may go unrecognized	Rabies research lab workers; rabies biologicals production workers	Primary preexposure immunization course; serology every 6 months; booster immunization when antibody titer falls below acceptable levels
Frequent	Exposure usually episodic, with source recognized, but exposure may also be unrecognized; aerosol, mucous membrane, bite, or nonbite exposure	Rabies diagnostic lab workers, spelunkers, veterinarians, and animal control and wildlife workers in rabies-epizootic areas	Primary preexposure immunization course; booster immunization or serology every 2 years
Infrequent (greater than population at large)	Exposure nearly always episodic with source recognized; mucous membrane, bite, or nonbite exposure	Veterinarians and animal control and wildlife workers in areas of low rabies endemicity; certain travelers to rabies-epizootic areas; veterinary students	Primary preexposure immunization course; no routine booster immunization or serology
Rare (population at large)	Exposure always episodic, mucous membrane, or bite with source recognized	Population at large in countries with animal rabies, including individuals in rabies-epizootic areas	No preexposure immunization

[a]Preexposure immunization consists of three doses of modern cell culture vaccine, 1.0 ml intramuscularly, one each on days 0, 7, and 28. Administration of routine booster doses of vaccine depends on exposure risk category as noted above. Preexposure immunization of immunosuppressed persons is not recommended.

temic, viremic phase of infection except in swine and small laboratory animals. Local vesiculation and epithelial denudation follow epithelial cell destruction and interstitial edema, which separates epithelium from underlying tissues. Spread of such lesions occurs by extension, such that it is common for the entire epithelium of the tongue or teat to be sloughed. High titers of infectious virus are present, usually for a short

time, in vesicular fluids and in tissues at the margins of lesions. This virus is free to be transmitted by fomites, such as saline, contaminated food, or milking machines, or by arthropods. Despite the extent of the epithelial damage, healing is usually rapid and complete. Infection results in solid homologous immunity, but there is little heterologous immunity, e.g., between the New Jersey and Indiana strains of vesicular stomatitis virus.

Laboratory Diagnosis

Virus can be recovered from vesicular fluids and tissue scrapings by standard virus isolation techniques in cell culture (or in embryonated eggs or in suckling mice by intracerebral inoculation). Virus isolated in this way is identified by serological means. Because diagnosis may involve differentiation from foot-and-mouth disease, these procedures should be carried out in an authorized reference laboratory.

Epidemiology

Vesicular stomatitis virus can be stable in the environment for days, for example on milking machine parts where transmission results in teat and udder lesions, in cool water, in soil, and on vegetation, where transmission results in mouth lesions. However, the virus appears to be transmitted principally via flying arthropods, but despite research for over 30 years the specific vectors and mode of transmission remain unknown.

Throughout the Americas, vesicular stomatitis of cattle, horses, and swine is a recurring disease problem, which appears annually or at intervals of 2 or 3 years in tropical and subtropical countries, and at intervals of 5 to 10 years in temperate regions of both North and South America. Even though the geographical range of the viruses is large, disease problems are restricted to favorable habitats: for example, in the upper Mississippi valley in the United States, disease appears regularly in aspen parklands, a narrow zone separating hardwood forest from open prairies. In the western mountainous regions of the United States, disease seems to move up and down valleys, rarely reaching higher pastures. Some clinical observers have reported that the disease appears almost simultaneously over large areas, or in multiple spreading foci. These characteristics, together with the seasonal occurrence of the disease, have suggested that the causative viruses might be arthropod-borne. However, over many years few virus isolates have ever been made from arthropods. In a large epizootic of vesicular stomatitis—New Jersey that occurred in the western United States in 1982, many virus

isolates were made from flies, mostly from the common house fly *Musca domestica*, but it is not clear how flies, including biting flies, might fulfill known patterns of virus transmission between herds and between individual animals. The manner by which vesicular stomatitis viruses are transmitted over long distances also remains controversial despite years of study. Arthropod involvement is also suggested here.

Prevention and Control

Outbreaks of disease may be explosive, so avoidance of pasturages known as sites of transmission may help to avoid infection, but in general little is usually done even in the face of an epizootic. In temperate zones epizootics occur at such infrequent intervals that the index of suspicion falls to a low level during interepidemic periods. Both inactivated and attenuated live-virus vaccines are available, but neither is much used.

Human Disease

Vesicular stomatitis viruses are zoonotic, being transmissible to humans (typically, farmers and veterinarians) from vesicular fluids and tissues of infected animals, but there are no practical measures for preventing occupational exposure. The disease in humans resembles influenza, presenting with an acute onset of fever, chills, and muscle pain. It resolves without complications within 7 to 10 days. Human cases are not uncommon during epizootics, but because of lack of awareness few cases are reported.

BOVINE EPHEMERAL FEVER

Bovine ephemeral fever is widespread in the world, spanning tropical and subtropical zones of Africa, Australia, and Asia. From these enzootic sites the disease extends intermittently into temperate zones in major or minor epizootics. The disease has never been reported in North or South America or Europe.

Clinical Features

Clinical features in cattle are characteristic, but all are not seen in an individual animal. Onset is sudden; the disease is marked by a biphasic or polyphasic fever with an immediate drop in milk production. Other clinical signs are associated with the second and later febrile phases: these include depression, muscle stiffness, and lameness, and, less often, nasal and ocular discharges, cessation of rumination, and con-

stipation. Infrequently there is diarrhea and temporary or permanent paresis. Usually, recovery is dramatic and complete in 3 days (range 2–5 days). Mortality rate is 1–2%, but is highest in well-conditioned beef cattle and high-producing dairy cattle. Subclinical cases do occur; but their relative rate is unknown because antibody testing is confounded by intercurrent infections in the same areas by related but nonpathogenic rhabdoviruses.

Pathogenesis and Immunity

The pathogenesis of the disease is poorly understood, but it seems clear that pathophysiological effects of host inflammatory response are involved in the expression of disease. In all cases there is an early neutrophilia with an abnormal level of immature neutrophil polymorphonuclear cells in the circulation ("left shift"). There is a rise in plasma fibrinogen and a significant drop in plasma calcium. Experimentally, there is a dramatic response to nonsteroid antiinflammatory drugs and often to calcium infusion. Infection results in solid homologous immunity, although there may be antigenic variation among isolates, and hence repeat clinical episodes in herds.

Laboratory Diagnosis

Laboratory diagnosis is difficult, requiring virus isolation by blind passage in mosquito (*Aedes albopictus*) cell cultures or suckling mouse brain. Detection of a rise in antibody is the most practical diagnostic technique available; this may be done by the neutralization test, which is virus-specific, or by immunofluorescence or gel precipitin tests, which are cross-reactive with related rhabdoviruses.

Epidemiology and Control

Bovine ephemeral fever virus is apparently transmitted by two types of arthropod vectors: *Culicoides* and mosquitoes (culicine and anopheline mosquitoes). Enzootic and epizootic spread is limited to the distribution of vectors. There is epidemiological evidence that more arthropod vector species remain to be identified. Prevention by vector control is impractical in the areas of the world where this disease is prevalent, but in Japan, South Africa, and Australia inactivated and attenuated live-virus vaccines have been tried on an experimental basis. Problems with vaccines stem from lack of potency—inactivated vaccines probably require more antigenic mass than it has been possible to achieve, and attenuated live-virus vaccines suffer from a loss in immunogenicity linked with the attenuation process.

TABLE 30-4
Diseases Caused by Rhabdoviruses of Fish

Disease	Species affected	Geographical distribution	Growth temperature of virus[a]	
			Range	Optimum
Viral hemorrhagic septicemia	Rainbow trout (*Salmo gairdneri*)	Europe	6°–20°C	14°–15°C
Infectious hematopoietic necrosis	Salmonids	Pacific Northwest of North America	4°–20°C	13°–18°C
Spring viremia of carp	Cyprinids	Europe	4°–32°C	Variable[b]
Pike fry rhabdovirus disease	Northern pike (*Esox lucius*)	Europe	10°–31°C	21°–28°C
Eel rhabdovirus disease	American eel (*Anguilla rostrata*)	America	—	29°C

[a]In cultured piscine cells.
[b]Depends on cell line.

FISH RHABDOVIRUSES

Five serologically distinct rhabdoviruses affecting fish have been recognized as causing economically important diseases (Table 30-4). Morphologically, and in their RNA and protein composition, the virions of fish rhabdoviruses closely resemble those of mammalian rhabdoviruses. The protein profiles of infectious hematopoietic necrosis virus and viral hemorrhagic septicemia viruses resemble those of the member viruses of the genus *Lyssavirus;* those of spring viremia of carp virus and pike fry rhabdovirus resemble those of the member viruses of the genus *Vesiculovirus.* All five fish rhabdoviruses are antigenically distinguishable by neutralization tests. The viruses may be propagated in a variety of piscine cell lines and also in mammalian, avian, and reptilian cells, the optimal growth temperatures and the temperature range for growth differing from one virus to another.

Viral Hemorrhagic Septicemia of Trout

This disease causes considerable losses in European countries, and is most severe when the water temperature falls below 15°C; mortality may reach 80% when the virus is introduced into a new area. The acute

disease is marked by lethargy, darkened pigmentation, hemorrhages into viceral organs, muscle, and swim bladder, and death. The chronic disease may be silent, or there may be continuing mortality. Diagnostic tests (fluorescent antibody, immunodiffusion, or neutralization) are available. Control involves avoidance of contamination of premises (via use of certified viral hemorrhagic septicemia-free stock), eradication by slaughter and disinfection when disease is found, and, in some cases, reduction of losses by control of the water temperature. Vaccine has shown promise in pilot experiments in Denmark and France.

Infectious Hematopoietic Necrosis

This disease of salmonids is caused by a group of serologically related viruses, each infecting particular species of salmon or trout. The disease causes frequent losses in hatcheries along the Pacific coast of North America from Alaska to northern California, and in Japan. Epizootics usually involve juvenile fish at water temperatures below 15°C; mortality may reach 50–90%. Survivors become lifelong carriers and shed large amounts of virus in urine and feces and in ovarian and seminal fluids at spawning. Acutely infected fish are dark in color, lethargic, and show anemia, exophthalmia, distention of the abdomen, and hemorrhages at the base of fins. Diagnosis is made by virus isolation and serology. Control measures center on isolation of premises and are similar to those for viral hemorrhagic septicemia. Elevation of water temperature to at least 18°C is very effective in controlling the disease, but is usually not economically feasible. Inactivated and attenuated live-virus vaccines have been developed, but the most promising control measure for the future is selective breeding for resistance.

Spring Viremia of Carp

This disease occurs in European countries when the water temperature rises in spring. Diseased fish excrete large amounts of virus and the virus is also spread by a bloodsucking ectoparasite, *Argulus*. Infected fish become lethargic and have abdominal swelling indicative of visceral organ edema and hemorrhages. Control is possible only by isolation of premises, although a vaccine has been developed.

Other Rhabdovirus Diseases of Fish

Pike fry rhabdovirus causes a disease similar to spring viremia of carp virus in hatchery-reared pike fry. The disease is a particular problem in the Netherlands, where it is controlled primarily by isolation and by

washing of eggs with iodophor disinfectants. Two related rhabdoviruses have been found in eels which have the potential for causing losses in eel (and trout) farms in Japan and European countries.

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CHAPTER 31

Retroviridae

The veterinarians Ellerman and Bang in Denmark in 1908 and the medical pathologist Rous in the United States in 1911 demonstrated that avian leukosis and avian sarcoma could be transmitted to other chickens by inoculation of cell-free filtrates derived from the respective tumor tissues obtained from diseased birds. The two related viruses—avian leukosis and avian sarcoma viruses—are prototypic of the etiological agents of similar infectious malignant tumors now recognized in many other animal species, including cattle, cats, mice, and primates. These viruses are now classified as members of the family *Retroviridae,* a large family that includes many viruses of veterinary importance (Table 31-1). The name *retro* (= reverse) derives from the reverse transcriptase (RNA-dependent DNA-polymerase) that is found within the virions of all members of the family.

The family *Retroviridae* consists of three subfamilies—*Oncovirinae, Lentivirinae,* and *Spumavirinae*—only the first of which contains tumorigenic viruses, the oncoviruses. The lentiviruses cause important chronic dis-

TABLE 31-1
Diseases of Domestic Animals Caused by Retroviruses

Subfamily	Host	Virus	v-*onc*	Diseases
Oncovirinae	Chicken	Avian leukosis viruses	−	Lymphomas, leukemias, anemia, osteopetrosis
		Avian sarcoma viruses	+	Sarcomas
		Reticuloendotheliosis viruses	−[a]	Lymphomas, anemia, sarcoma
	Cattle	Bovine leukemia virus	−	Leukemia
	Swine	Porcine sarcoma virus	+	Sarcoma
	Cat	Feline leukemia viruses	−	Leukemia
		Feline sarcoma viruses	+	Sarcoma
Lentivirinae	Sheep	Maedi–visna virus	n.a.[b]	Maedi–visna, progressive pneumonia
	Goat	Caprine arthritis–encephalomyelitis virus	n.a.	Arthritis, encephalomyelitis
	Horse	Equine infectious anemia virus	n.a.	Anemia
Spumavirinae	Cattle	Bovine foamy virus	n.a.	None
	Cat	Feline foamy virus	n.a.	

[a]Reticuloendotheliosis virus-T is a recombinant that contains a v-*onc*.
[b]n.a., Not applicable.

eases in sheep, goats, and horses, and this subfamily also includes the etiological agent of human acquired immunodeficiency syndrome. The spumaviruses are not pathogenic and are recognized only when they are found in cultured cells. As explained in Chapter 12, tumor virologists use the vernacular term "retrovirus" to refer to the members of the family *Retroviridae* with which they work. However, all tumor-producing retroviruses belong to the subfamily *Oncovirinae*. Since in this chapter we are concerned with retroviruses belonging to two subfamilies, *Oncovirinae* and *Lentivirinae*, we will use "retrovirus" when referring to the family as a whole and "oncovirus" or "lentivirus" when referring to viruses of these subfamilies.

PROPERTIES OF RETROVIRUSES

Retrovirus virions are spherical, 80–130 nm in diameter, and have a unique three-layered structure. Innermost is the genome–nucleoprotein complex, which includes about 30 molecules of reverse transcriptase,

TABLE 31-2
Properties of Retroviruses

Enveloped spherical virion, 80–130 nm diameter
Helical ribonucleoprotein within icosahedral capsid within envelope
Linear (+) sense ssRNA genome, diploid (inverted dimer), total size variable, about 10 kb; some defective, some may carry oncogene
Reverse transcriptase transcribes DNA from virion RNA; circular dsDNA is formed and integrated into cellular chromosomes
Provirus may or may not be expressed
Virions bud from plasma membrane
Some viruses of subfamily *Oncovirinae* produce tumors, particularly leukemias and sarcomas; viruses of subfamily *Lentivirinae* produce slow demyelinating neurological disease, arthritis, a generalized chronic debilitating disease, or acquired immunodeficiency syndrome

and is thought to have helical symmetry. This structure is enclosed within an icosahedral capsid, which in turn is surrounded by a host cell membrane-derived envelope from which project glycoprotein peplomers (Plate 31-1).

The retroviral genome is diploid, comprising two identical molecules, noncovalently linked at their 5′ ends. Each haploid segment is a linear, single-stranded, (+) sense molecule of about 5 kb, with a 3′ polyadenylated tail and a 5′ cap (see Fig. 31-1). The genome of nondefective retroviruses contains three genes, each coding for two or more polypeptides. The *gag* gene (standing for *g*roup-specific *a*ntigen) encodes the virion core proteins, the *pol* gene encodes the reverse transcriptase (*pol*ymerase), and the *env* gene encodes the virion peplomer proteins (*env*elope).

The RNA of the rapidly transforming oncoviruses contains a fourth gene, the viral oncogene (v-*onc*) (see Chapter 12). The presence of the v-*onc* gene is usually associated with deletion of some of the *env* base sequences, so that most v-*onc*-containing viruses are unable to synthesize a complete envelope and are therefore replication-defective. They are always found associated with other avian leukosis viruses that are replication-competent; these act as helpers for sarcoma virus replication (see below). Rous sarcoma virus is an exception; it contains complete *gag*, *pol*, and *env* genes and is therefore replication-competent, although its genome also contains the viral oncogene v-*src*.

The virion RNAs of different oncoviruses from the same species of animal show extensive homologies; those of different species (e.g., chicken, cow, cat, and mouse) show virtually no homology.

The first systematic classification of oncoviruses was based on their host species and on the appearance of the virions as they matured, as

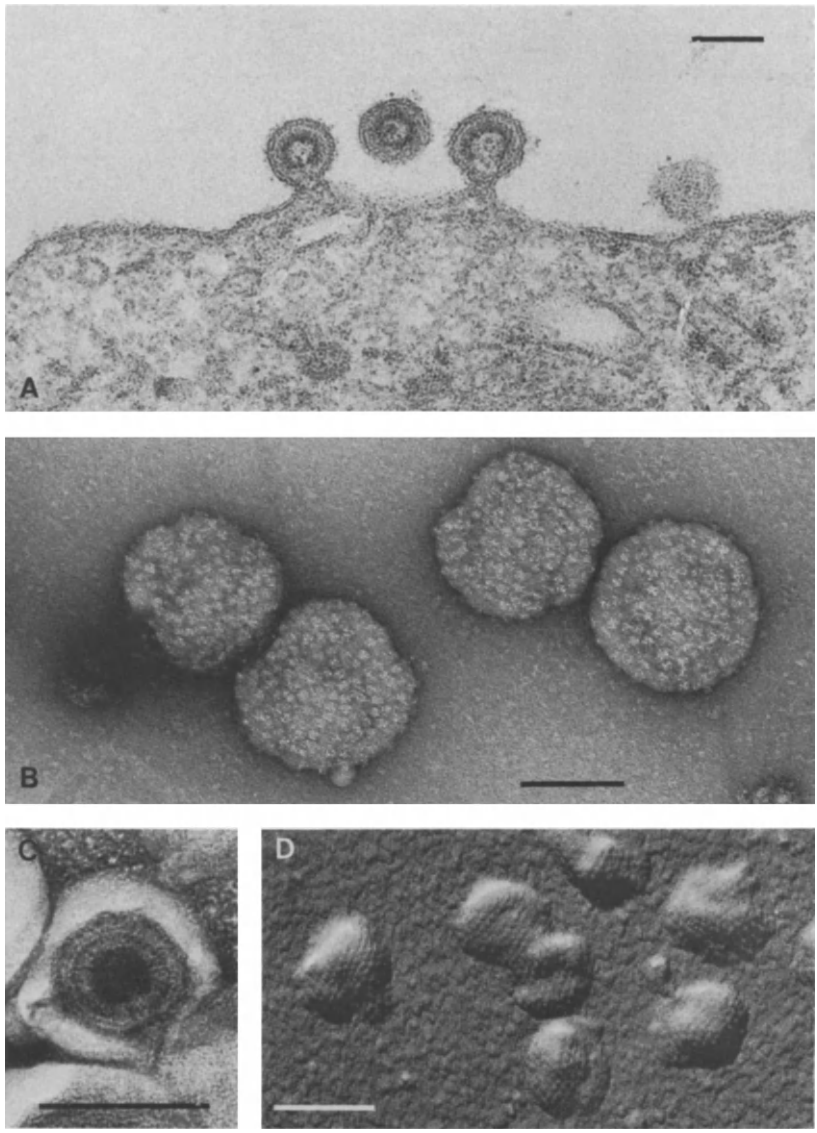

PLATE 31-1. Retroviridae, *subfamily* Oncovirinae, *murine leukemia virus. (A) Budding of virions from a cultured mouse embryo cell. (B) Virions negatively stained with uranyl acetate, showing peplomers on the surface. (C) Virion somewhat damaged and penetrated by uranyl acetate, so that the concentric arrangement of core, shell, and nucleoid becomes visible. (D) Cores isolated by ether treatment of virions, freeze-dried, and shadow-cast. The hexagonal arrangement of the subunits of the shell around the core is recognizable (bars = 100 nm). (Courtesy Drs. H. Frank and W. Schafer.)*

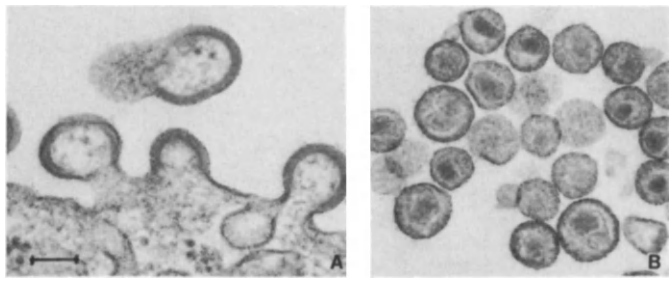

PLATE 31-2. Retroviridae, *subfamily* Lentivirinae, *maedi–visna virus. (A) Budding of virions. (B) Mature extracellular virions. (Courtesy R. J. Munn.)*

seen by thin-section electron microscopy of infected cell cultures. Four major types of oncovirus particles, categorized as A, B, C, and D, were recognized and the viruses subdivided accordingly. All oncoviruses of veterinary importance belong to the type C subgroup.

Morphologically, no viral components can be detected within the cytoplasm of infected cells until characteristic electron-dense, crescent-shaped forms (hence "type C"), the nascent virion nucleocapsids partially surrounded by the envelope, appear at the cell membrane.

Lentiviruses differ from the oncoviruses in details of their maturation and in the appearance of the nucleocapsid in mature virions. The plasma membrane is greatly thickened at the site of budding (Plate 31-2), to an extent not seen with oncoviruses, and in the mature virions the nucleocapsid has the appearance of a dense "band," often with a concentration of electron-dense material at one end.

The antigenic relations between different species of oncovirus are complicated. Antibodies to the envelope glycoproteins react only with the same virus, i.e., they are type specific, while those reacting with the major core proteins specified by the *gag* gene are group specific and cross-react with other viruses from the same animal species. Some antigens are shared by viruses associated with several animal species (interspecies antigens), but distinguish avian from mammalian oncoviruses.

The internal proteins of different species of lentiviruses (the *gag* and *pol* gene-products) show extensive cross-reactivity, but no cross-reactions with the equivalent proteins of the oncoviruses. Envelope antigens show much less cross-reactivity. The nucleic acid homology of the conserved *pol* genes of different species of lentiviruses is over 50%, compared with less than 30% for this gene in lentivirus and oncovirus RNAs.

Antibodies to the envelope glycoproteins of oncoviruses neutralize

viral infectivity. Other antibodies, directed against antigens expressed on the surface of cells during maturation, are cytotoxic and may prevent tumor formation.

Retroviruses are inactivated by lipid solvents and detergents and by heating at 56°C for 30 minutes, but are more resistant than other viruses to UV light and X irradiation, probably because their genome is diploid.

VIRAL REPLICATION

The essential features in the replication cycle of a nondefective retrovirus are shown in Fig. 31-1. The molecular biological aspects of retrovirus replication that relate to the role of the integrated provirus in tumorigenesis have been discussed in Chapter 12. Oncoviruses (but not lentiviruses) replicate only in dividing cells. Following adsorption and penetration, viral RNA is released in the cytoplasm, and is copied to DNA by the virion-associated reverse transcriptase acting as an RNA-dependent DNA polymerase. The ssDNA copy is made double-stranded by the same enzyme, now acting as a DNA-dependent DNA polymerase. The dsDNA enters the nucleus, becomes circularized, and then is integrated into the host cell DNA, the interval between infection and integration being about 24 hours. The integrated DNA (provirus) serves as template for the production of mRNAs which are translated into proteins, or virion RNA, which is encapsidated into progeny virions. The *env* polyprotein becomes associated with the plasma membrane and is then cleaved. The *gag* and *gag–pol* polyproteins together with virion RNA move to a position under the cell membrane, at the site where the envelope proteins are already present in the membrane. The nucleocapsids then assemble by a series of protein cleavages while budding of the virions takes place.

Replication of retroviruses is accompanied by a high rate of mutation, because the reverse transcriptase lacks an "editing" function (see Chapter 5), and also because of a high frequency of recombination with other retrovirus genes and with cellular genes. This variability accounts for the variations found in the types of tumors produced by acutely transforming oncoviruses. It makes classification and definition of viral species difficult, a feature which is compounded by the occurrence of phenotypic mixing of the envelope proteins, to produce what are called *pseudotypes*, which have the genome of one species or subtype and the envelope antigens of another. Pseudotypes have the ability to invade cells according to the receptor specificity of their envelope, but their progeny behave according to their genome specificity.

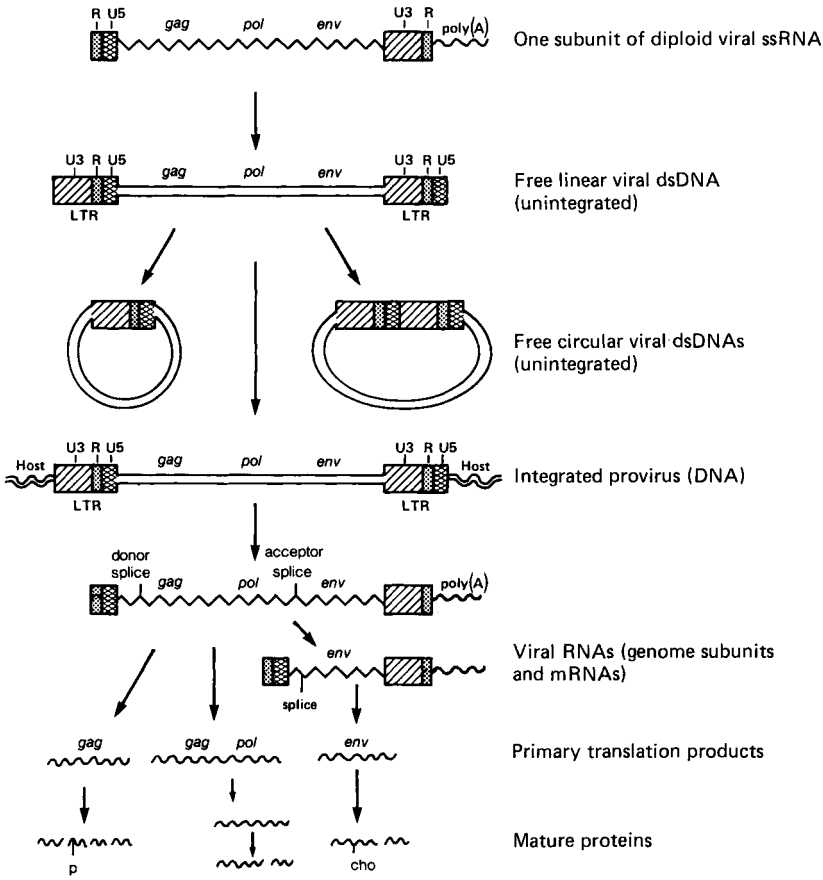

FIG. 31-1. *Replication cycle of avian leukosis virus, an exogenous replication-competent retrovirus. [From D. R. Lowy,* In *"Virology" (B. N. Fields et al.,* eds.), *p. 240. Raven Press, New York, 1985.]*

TRANSMISSION OF RETROVIRUSES

Oncoviruses may be transmitted from one animal to another by three distinct modes: horizontal or vertical—vertical transmission being either as virions or in the germ plasm (Fig. 31-2). For the chicken all three modes of transmission occur, although genetic transmission is restricted to nonpathogenic endogenous oncoviruses. If chickens are infected horizontally when over 5 or 6 days of age, they are unlikely to develop leukemia; instead they develop a transient viremia and become immune by developing virus-neutralizing antibody. If the virus is transmitted

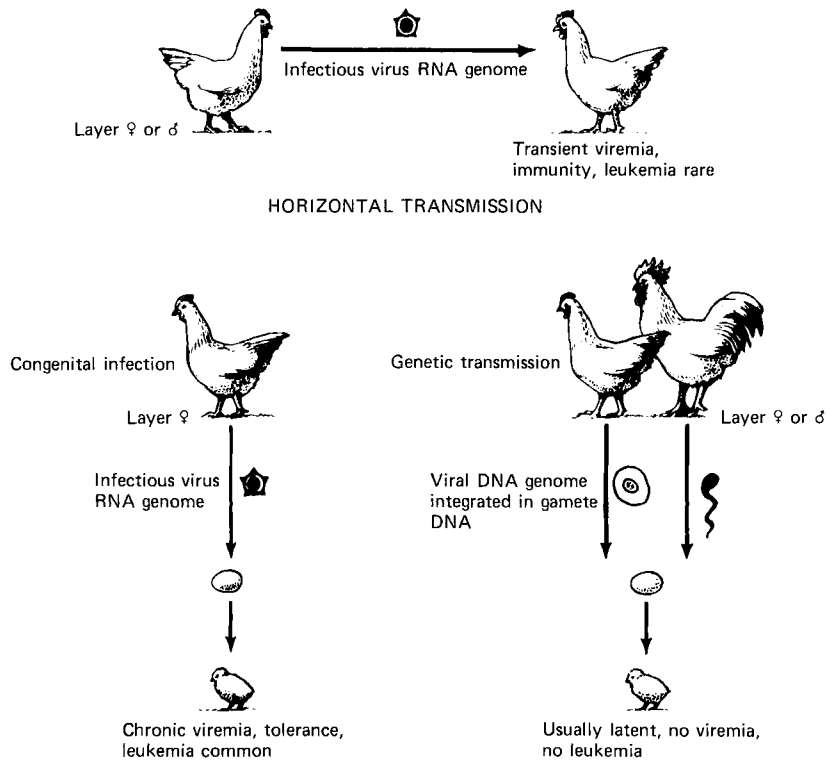

FIG. 31-2. *Horizontal and vertical transmission of avian leukosis viruses. [From R. A. Weiss, In "Virus Persistence," 33rd Symposium Soc. Gen. Microbiol. (B. W. J. Mahy, A. C. Minson, and G. K. Darby, eds.), p. 267. Cambridge University Press, Cambridge, 1982.]*

congenitally via the egg, or in the case of mammalian viruses via the placenta, or within the first few days of life via the milk or by other horizontal modes, the animal becomes viremic and the viremia persist for life, because of the induction of immunological tolerance. Such animals may appear to grow normally but frequently develop leukemia and associated diseases and are a major source of exogenous leukosis virus that is continuously shed and horizontally transmitted. Genetic (germline) transmission occurs when germ cells, ova and sperm, contain endogenous virus. Presumably it may also occur if provirus is acquired by germ cells following horizontal transmission.

Lentiviruses are transmitted horizontally, never in the germ plasm. The caprine lentivirus is often transmitted in colostrum or milk. Little is known of the mode of transmission of spumaviruses.

AVIAN ONCOVIRUS DISEASES

Since much of our understanding of the biology of oncoviruses derives from research on the avian oncoviruses, we will depart from the usual order of animal species and discuss them first. Oncoviruses of chickens fall into two antigenically distinct groups: the avian leukosis viruses and the avian reticuloendotheliosis viruses. Each of these groups of viruses, especially the first, is large and complex, and the diseases they cause are of considerable economic importance.

Avian Leukosis–Sarcoma Viruses

In considering these viruses we need first recall some facets of their biology that were described earlier (Chapter 12), namely, that there are three classes of viruses involved: endogenous, exogenous replication-competent, and exogenous replication-defective. *Endogenous* avian leukosis viruses occur in the genome of every chicken as DNA provirus. They are rarely expressed, but if induced by various manipulations they are nonpathogenic. *Exogenous* avian leukosis viruses are replication-competent and have a standard complement of *gag*, *pol*, and *env* genes. For the most part they are nonpathogenic, but in the course of lifetime infection a small percentage of infected birds develop leukemia or lymphoma. Thirdly, some exogenous viruses acquire a c-*onc* gene and then rapidly induce malignant tumors. The great majority of such rapidly transforming viruses lose part of their genome when they acquire the oncogene, so that they become replication-defective and depend on the helper activity of another oncovirus for productive replication. A small minority, however, like Rous sarcoma virus, have a full complement of viral genes plus a v-*onc* gene, and are then rapidly oncogenic and are also capable of replication without a helper virus.

Assay Methods. These properties lead to the use of special methods for the isolation and assay of these viruses. Rous sarcoma virus and other replication-competent acutely transforming avian oncoviruses are assayed directly by focus formation assays in chick fibroblasts. Since they produce infectious virions, these are readily recovered and can then be studied in detail. Replication-defective rapidly transforming viruses, which carry v-*onc* genes, can also be assayed by focus formation, but virions can only be obtained in cells carrying a replication-competent leukosis virus; the yield always consists of a mixture. The replication-competent leukosis viruses, on the other hand, do not transform cells *in vitro*. Instead, they interfere with transformation by viruses with the same envelope antigen that carry a v-*onc* gene, and assay is carried out by an interference test. Assays of leukosis viruses and of the corre-

sponding antigens can also be carried out by serological methods, using complement fixation, ELISA, or radioimmunoassay.

Clinical and Pathological Features. Avian leukosis viruses are enzootic in virtually all flocks of chickens, and most chickens in a flock will have been infected within a few months of birth. If rapidly transforming viruses are not present, disease occurs sporadically in birds over 14 weeks of age, with an overall incidence of about 3%, but sometimes as much as 20%.

The variety of syndromes produced by avian leukosis–sarcoma viruses is shown in Table 31-3.

Diseases Caused by Exogenous Nondefective Avian Oncoviruses. These viruses cause tumors only in birds that are congenitally infected and have a persistent viremia. Over the course of their life proviral DNA is integrated into many different kinds of cells, sometimes, by chance, in a location where the activity of a c-*onc* gene is disturbed in such a way as to initiate tumor production (see Chapter 12). Since lymphoid cells represent 10% of all the cells in the animal and have a very high rate of cell division, particularly in the early weeks of life, the probability that lymphoid cells are infected and transformed by leukosis viruses is higher than for most other cell types.

Lymphoid Leukosis (synonym, visceral lymphomatosis). This is the commonest form of avian leukosis and occurs in chickens 14–30 weeks of age. Clinical signs are nonspecific. The comb may be pale, shriveled, and occasionally cyanotic. Inappetance, emaciation, and weakness occur and the abdomen may be enlarged. Tumors may be present for some time before clinical illness is recognized, although with the onset of the first signs the course may be rapid. Hematological changes are inconsistent; leukemia is uncommon and lymphoblastoid cells are rarely seen in the circulating blood.

Tumors are usually present in the liver, spleen, and bursa and may occur in other internal organs. Microscopically the lesions are focal, multicentric aggregates of lymphoblasts with B lymphocyte markers. They may secrete large amounts of IgM, but their capacity to differentiate into IgG-, IgA-, or IgE-producing cells is arrested. The primary target cells are poststem cells in the bursa, within which the transformed cells invade blood vessels and metastasize hematogenously. Bursectomy, even up to 5 months of age, abrogates the development of lymphoid leukosis.

Osteopetrosis (synonym, thick leg). In this form of the disease the bones are affected by a uniform or irregular thickening of the diaphyseal or metaphyseal regions. In advanced cases osteoma, osteogenic sarcoma,

TABLE 31-3

Syndromes Produced in Chickens by Avian Leukosis–Sarcoma Viruses

Type of virus[a]	Syndrome	Rate of development	Viral oncogene	Cell first affected	Types of lesions
Replication-competent	Lymphoid leukosis	Slow	—[b]	Lymphoblast	Lymphoid cell infiltrations of various organs
	Osteopetrosis	Slow	—	Osteoclast or osteoblast	Thickened long bones
	Renal tumors	Slow	—	Renal cells	Nephroblastoma, carcinoma
Replication-defective	Erythroblastosis	Rapid	v-*erb*B	Erythroblast	Anemia
	Myeloblastosis	Rapid	v-*myb*	Myeloblast	Anemia, leukemia
	Myelocytomatosis	Rapid	v-*myc*	Myelocyte	Carcinoma, sarcoma
	Hemangioma	Rapid	?	Capillary endothelium	Hemangioma
	Sarcomas	Rapid	v-*fps* v-*yes*	Various mesenchymal cells	Sarcoma, carcinoma
Replication-competent, rapidly transforming	Sarcoma	Very rapid	v-*src*	Various mesenchymal cells	Sarcoma

[a]In addition to these exogenous viruses, all chickens carry endogenous oncoviral DNA as part of their genome, which may code for a normal cell protein.

[b]Not present.

and chondrosarcoma may occur. Lesions are usually most obvious in the long bones of the leg but may also be present in the pelvis, shoulder girdle, and ribs. Birds with osteopetrosis frequently also have anemia and often have lesions of lymphoid leukosis.

Renal Tumors. These are usually found coincidentally at slaughter but may be associated with emaciation and weakness before death. Two forms occur: (1) nephroblastomas, which originate from embryonic rests or nephrogenic buds in the kidneys, and (2) carcinomas. Numerous retrovirus particles can be seen budding from transformed renal cells.

Diseases Caused by Defective Avian Leukemia Viruses. A variety of neoplasms are generated by replication-defective leukemia viruses propagated by coinfection with a nondefective helper, which is usually an exogenous avian leukemia virus. These defective viruses can be divided into three major groups—avian erythroblastosis, avian myeloblastosis, and avian myelocytomatosis—and some minor groups. Their different pathogenic potential is due to the different v-*onc* genes they carry (see Table 31-3).

Erythroblastosis. The incubation period may be as short as 21 days. Two patterns are recognized: (1) a proliferative form characterized by the presence of many erythroblasts in the blood, and (2) an anemic form in which the predominant feature is anemia with few circulating erythroblasts. The primary target cells are erythroblasts, which resemble normal erythroblasts in appearance except that retrovirus particles can be demonstrated either within cytoplasmic vacuoles or budding from the plasma membrane. Lesions are mainly attributable to hemostasis because of the accumulation of erythroblasts in the blood vessels, particularly the capillaries and sinusoids.

Myeloblastosis. The clinical signs are similar to those of erythroblastosis and develop after an incubation period that may be as short as 10 days. The target cell is the myeloblast in the bone marrow.

The pathological features of myeloblastosis and erythroblastosis overlap. In myeloblastosis, leukemia is a major feature; up to 10^9 myeloblasts per milliliter are present in the blood and in an hematocrit there may be more buffy coat than red cells. Bone marrow displacement may result in secondary anemia.

Myelocytomatosis. Signs similar to those seen in erythroblastosis develop after an incubation period of 3 to 11 weeks. The target cells, which are nongranulated myelocytes (morphologically distinct from myeloblasts), proliferate to occupy much of the bone marrow, and tumor growth may extend through the bone and periosteum. The tumors are distinctive and characteristically occur on the surface of bone, in association with the periosteum, and near the cartilage, although any organ or

tissue may be affected. Visceral organs may be infiltrated with myelocytes. Histologically, the tumors consist of compact masses of strikingly uniform myelocytes with very little stroma, similar to normal bone marrow myelocytes.

Hemangioma. After an incubation period of less than 3 weeks, a hemangioma develops, usually as a single tumor in the skin, or on the surface of the viscera, as a "blood blister," which may rupture, and birds may then bleed to death. Their visibility in the skin encourages cannibalism. The target cell is located in the blood vessel wall.

Connective Tissue Tumors. A variety of malignant tumors including fibrosarcoma, fibroma, myxosarcoma, myxoma, histiocytic sarcoma, osteoma, osteogenic sarcoma, and chondrosarcoma are caused by avian oncoviruses containing v-*onc* genes. Most are replication-defective, but they may be replication-competent, e.g., Rous sarcoma virus.

Diagnosis. History, clinical signs, the location of tumors, and gross and histopathological postmortem findings are usually enough to make a diagnosis of avian leukosis. The most important disease, as far as differential diagnosis is concerned, is Marek's disease (see Table 19-4 for differentiating features), a distinction which is important because Marek's disease can be controlled by vaccination. Viral isolation is rarely required in veterinary practice but is used for research purposes.

Epidemiology. Transmission may occur horizontally or vertically (see Fig. 31-2). Horizontal transmission is relatively inefficient, requiring prolonged, close contact, and was not of major significance in the natural transmission of the disease until intensive chicken production began in the 1940s. This led to the appearance of lymphoid leukosis as an economically important disease, since horizontal transmission via saliva led to conditions which promoted egg-borne transmission. Individual infected hens may transmit virus via ova either continuously or intermittently, although some known infected hens do not transmit virus at all, and transmission is less efficient in hens over 18 months old. Congenitally infected chickens may be immunologically tolerant, and their blood may contain up to 10^9 virions per milliliter. They excrete virus in saliva and feces but are otherwise healthy, although some eventually develop leukosis. These birds transmit virus horizontally throughout their lives, although, more importantly, such hens transmit virus via ova, the virus infecting cells of the blastocyst from the eight-cell stage. During embryogenesis the pancreas is particularly favored as a site of replication, and large amounts of virus accumulate in the albumen. At hatching, large amounts of virus are shed in the meconium, resulting in heavy environmental contamination.

Most day-old chicks have maternal antibody titers between 1 and 10%

of those of their dams. Thus the efficiency of passive antibody transfer is low, and the titer declines so that chicks are negative by 4 to 7 weeks of age. Then, if they have not been congenitally infected, they become infected by horizontal transmission, and develop a transient viremia followed by high levels of antibody; virus is usually eliminated and the antibody persists for life. However, some birds remain persistently infected and act as a source of virus for both horizontal and vertical transmission.

Roosters may be involved in the germ-line transmission of endogenous (nonpathogenic) oncoviruses, but play no part in congenital infection.

Control. Eradication of horizontally transmitted virus has been accomplished in experimental flocks and those used as a source of eggs for human, domestic mammal, and particularly avian virus vaccine production. Establishment and maintenance of leukosis-free flocks, which still carry endogenous avian oncovirus genes, is expensive and is not used for commercial flocks.

Hygiene is important in minimizing the level of virus contamination, particularly in the immediate posthatching peirod, when the age, population density, and levels of virus are highly conducive to horizontal transmission. The all-in, all-out principle and thorough cleaning and disinfection of incubators, hatcheries, brooding houses, and equipment constitute standard practice. The risk of introducing additional strains of virus is minimized if stock are obtained from a single source.

The introduction of intensive methods for broiler and egg production in the 1940s was followed by an increased incidence of leukosis, in part related to the unwitting selection of genetically susceptible chicken lines. Most modern commercial flocks have been built up with genetically resistant strains, and accordingly there has been a sharp reduction in the incidence of leukosis. Resistance correlates with viral subgroup and with the absence of receptors for viral envelope glycoproteins, the genes for virus receptors being located on an autosomal chromosome. It is possible to select for genetically resistant birds by challenging chorioallantoic membranes or chick embryo fibroblast cell cultures, derived from leukosis-free birds, with appropriate pseudotypes of Rous sarcoma virus. Failure to produce foci of cell transformation correlates with resistance, and lines of chickens can be bred that are homozygous for the resistance allele. Viral mutants able to bypass resistance frequently emerge, so that in practice genetic resistance as a basic for control requires an ongoing program.

Immunization, using either inactivated and attenuated live-virus vaccines, has met with limited success.

In addition to eliminating the occurrence of tumors, eradication of leukosis viruses has a number of other benefits which include reduced mortality from other causes, improved growth rate, and improved production, quality, fertility, and hatchability of eggs.

Diseases Caused by Avian Reticuloendotheliosis Viruses

Reticuloendotheliosis viruses are pathogenic avian oncoviruses that are antigenically and genetically unrelated to avian or mammalian leukosis–sarcoma oncoviruses. Five member viruses have been recognized. The prototype of the group, reticuloendotheliosis virus-T, was isolated from an adult turkey that died of visceral reticuloendotheliosis and infiltrative nerve lesions. Reticuloendotheliosis virus-T is replication-defective and carries a v-*onc* gene, v-*rel*. The other avian reticuloendotheliosis viruses—reticuloendotheliosis-associated virus, chick infectious anemia virus, Trager duck spleen necrosis virus, and chick syncytial virus—are replication-competent.

When inoculated into day-old chicks, reticuloendotheliosis virus-T produces severe hepatosplenomegaly with either marked necrosis or lymphoproliferative lesions. Reticuloendotheliosis virus-T pseudotypes with avian leukosis virus envelopes are produced in chickens which carry the latter viruses. Some major outbreaks of reticuloendotheliosis virus-T disease, involving the deaths of several million chickens, have occurred as a consequence of contamination of turkey herpesvirus Marek's disease vaccine with reticuloendotheliosis virus-T virus. There is some evidence that the virus may be mechanically transmitted by mosquitoes.

BOVINE LEUKEMIA

Bovine leukemia and lymphosarcoma attracted attention early in this century in several European countries, notably Denmark, where clusters of herds with a high incidence of these diseases suggested a viral etiology. However, bovine leukemia virus was not isolated until 1969. Herds and areas characterized by a high prevalence of bovine leukemia virus are recognized in most countries. Overall prevalence figures for a particular country vary between 4 and 165 per 100,000 cattle per year, reflecting the number of low- and high-prevalence herds.

Pathogenesis

The course of bovine leukemia virus infection suggests a multistage process. The major target cells are B lymphocytes. Infection may be

clinically inapparent or may progress to a persistent lymphocytosis and finally to tumor production, which is marked by enlarged lymph nodes and leukemic infiltrations into a variety of organs and tissues. Some tumors, particularly those from terminal cases, do not contain bovine leukemia virus or viral antigens. However, cocultivation of lymphocytes with susceptible cell cultures, with or without mitogens, results in production of infectious bovine leukemia virus. The range of susceptible cells in culture includes human, canine, and bat cells, in which the virus produces syncytia.

In contrast to the situation with chickens and cats, neither endogenous nor defective (v-*onc*-bearing) bovine oncoviruses have been recognized. Leukemogenesis probably depends on an influence on bovine c-*onc* genes by integrated bovine leukemia virus provirus.

Epidemiology and Control

Virus is shed in the urine and milk, and may also be transmitted by bloodsucking insects or iatrogenically by common equipment. Virus is transmitted horizontally within herds but does not extend readily to neighboring herds, suggesting that close and prolonged direct exposure is required. Congenital infection via the placenta also occurs. The incidence of infection is much higher than that of recognized disease, the occurrence of which is influenced by both genetic and environmental factors.

Agar gel diffusion, ELISA, and syncytium inhibition tests are used for diagnosis. Test and removal programs have been adopted by several European countries, including Denmark and the Federal Republic of Germany, and these countries require that imported cattle be test-negative. In other countries, including the United States and Canada, individual owners have undertaken test and removal programs on a voluntary basis but national programs have not been promulgated. An inactivated vaccine that prevents disease following bovine leukemia virus challenge has been used on an experimental basis.

OVINE ONCOVIRUS DISEASES

Ovine leukemia is associated with an oncovirus closely resembling bovine leukemia virus antigenically, and outbreaks of leukemia–lymphosarcoma, in which there is evidence for both congenital and horizontal transmission, are recognized in sheep. However, little more is known about the ovine virus, and there is no evidence that the virus is naturally transmitted between sheep and cattle.

A more common retroviral disease in sheep, ovine pulmonary ade-

nomatosis, produces signs similar to those of the lentiviral disease, ovine progressive pneumonia (see p. 571). However, the causative agent appears to be an oncovirus, but since it has not yet been cultivated, little is known of its molecular biology. Originally described in South Africa, where it was called jaagsiekte, it occurs widely in the Americas and in some countries in Europe. In Peru it is responsible for about a quarter of the annual mortality in sheep. In affected sheep, pea-sized, nodular lesions are found scattered through the lungs. Histologically these lesions are adenomas and adenocarcinomas, which metastasize to regional lymph nodes.

PORCINE LYMPHOSARCOMA

An oncovirus has been isolated from porcine lymphosarcomas, which are detected in 0.3 to 5 swine per 100,000 at slaughter and account for 25% of all porcine tumors. The tumors are found in swine from 3 to 4 months of age. Most porcine cell lines contain spontaneously produced virions of the porcine oncovirus, which is highly host specific. Viral sequences are present in multiple copies in wild Old World but not in New World species of the family Suidae, suggesting that the virus occurs as an endogenous virus in Old World wild pigs.

FELINE LEUKEMIA AND SARCOMA

Feline oncoviruses may be endogenous, exogenous replication-competent (feline leukemia virus), or exogenous defective (feline sarcoma virus), producing no pathological effects, leukemia and sarcoma, respectively. Neoplastic and nonneoplastic diseases due to feline leukemia virus occur worldwide and are the most common nonaccidental cause of death in cats. In a Californian survey the incidence of feline leukemia virus neoplasms was estimated to be 41.6 per 100,000 cats per year, and it has been estimated that deaths from all feline leukemia virus-related diseases are 250 per 100,000 cats per year. The prevalence of antibody to feline leukemia virus varies from 6% in sparse, isolated populations to 50% in urban and colony cats.

Feline leukemia virus was first recovered in 1964 and a few other isolates have been made, but it has proved impossible to isolate the virus from the majority of tumors that would be expected to contain it. However, the presence of the viral genome is demonstrable by hybridization and transfection. It has therefore been suggested that viral replication and release are not required to produce disease.

Feline sarcoma virus, on the other hand, is known to be defective,

carrying the v-*onc* gene v-*fms* and lacking an *env* gene. All strains that have been recovered from fibrosarcomas are pseudotypes with envelopes provided by feline leukemia virus, and all feline sarcoma virus stocks contain feline leukemia virus. Besides being important as pathogens of cats, several aspects of feline leukemia virus–feline sarcoma virus have attracted the attention of research workers concerned with human medicine. There is no evidence that these viruses can infect humans, but it is prudent to advise that children and women of childbearing age should avoid close contact with cats showing signs of disease associated with feline leukemia virus.

Clinical Signs and Pathological Features

The feline oncoviruses are responsible for a variety of disease syndromes, some neoplastic and others relating to effects on hematopoietic cells and the immune system. Three types of neoplasia are recognized: lymphosarcoma, myeloproliferative disease, and fibrosarcoma. In addition, two types of nonneoplastic diseases are known: anemia and immunopathological disease and its consequences.

Feline Leukemia Virus Lymphosarcoma. This is the most common naturally occurring mammalian lymphosarcoma and accounts for some 30% of all feline tumors. About one-third of all cats with lymphosarcoma have no demonstrable feline leukemia virus antigens (see section on diagnosis, p. 568) and the virus can rarely be isolated. Nevertheless, epidemiological studies support the view that feline leukemia virus causes the vast majority of cases of feline lymphosarcoma.

Four major forms of lymphosarcoma are recognized based on the location of the primary tumor: (1) multicentri, in which tumors occur in various lymphoid and nonlymphoid tissues, (2) a thymic form, occurring particularly in kittens, (3) an alimentary form, usually occurring in older cats in which lymphoid tissues of the gastrointestinal tract and/or mesenteric lymph nodes are affected, and (4) an unclassified form, which is uncommon and in which tumors are found in nonlymphoid tissues such as skin, eyes, and central nervous system. The lymphosarcomas are predominantly T lymphocyte tumors, except the alimentary tract form which is a B lymphocyte tumor.

Feline Leukemia Virus Myeloproliferative Diseases. In this group of diseases transformation of one or a combination of bone marrow cell types is induced by feline leukemia virus. Four types are recognized: (1) erythromyelosis, in which the target is an erythroid cell, (2) granulocytic leukemia in which a granulocytic myeloid cell, usually a neutrophil, is

targeted, (3) erythroleukemia, in which both erythroid and granulocytic myeloid precursors become neoplastic, and (4) myelofibrosis, a proliferation of fibroblasts and cancellous bone resulting in medullary osteosclerosis and myelofibrosis. These diseases, which are similar to those produced by the acutely transforming avian oncoviruses, are characterized by the presence of large numbers of neoplastic cells in the bone marrow, a nonregenerative anemia, and immunosuppression.

Feline Sarcoma Virus Fibrosarcoma. This accounts for 6 to 12% of all feline tumors, usually as solitary tumors in older cats. In young feline leukemia virus-infected kittens, feline sarcoma virus may on rare occasions induce a multifocal subcutaneous fibrosarcoma, which is anaplastic, rapidly growing, and frequently metastatic. One strain of feline sarcoma virus induces melanoma as well as fibrosarcoma. There is no evidence that feline sarcoma virus is transmitted horizontally; the tumors and the virus appear to arise *de novo* following feline leukemia virus infection.

Anemia and Immunopathological Disease. Transformation of erythropoietic cells may produce erythroblastosis, erythroblastopenia, or pancytopenia, all of which are associated with anemia. This group includes both immune complex diseases and immunodeficiency diseases. Sometimes persistent high levels of feline leukemia virus antigens are produced, which when bound in immune complexes produce glomerulonephritis. In other cases lymphoid cells are greatly depleted, in part by antibody-dependent cytotoxicity, feline oncovirus membrane-associated antigens being the target. This leads to a variety of secondary infections, in which the cat fails to thrive, growth is stunted, the hair coat is harsh, there is intercurrent and repeated infection, chronic stomatitis and gingivitus, nonhealing skin lesions, subcutaneous abscesses, chronic respiratory disease, and a high incidence of feline infectious peritonitis (see Chapter 28). Toxoplasmosis and infection with *Hemobartonella felis* are much more common in feline leukemia virus-infected cats than in normal cats. Poor productive performance, including infertility, fetal deaths, and abortions, is also attributed to feline leukemia virus infection.

Pathogenesis and Immunity

There are three antigenic types of feline leukemia virus—A, B, and C—based on differences in the envelope antigens. Cells transformed by either feline leukemia virus or feline sarcoma virus, unlike infected, nontransformed cells, express a novel viral antigen in their plasma membrane (feline oncovirus membrane-associated antigen; FOCMA),

antibodies to which, like antibodies to the envelope antigens, protect cats against disease. The cytoplasm of feline leukemia virus-infected cells contains the envelope protein and several internal proteins. Antibodies to the internal proteins and the reverse transcriptase are not protective but may be involved in immune complex disease.

Within 6 weeks of infection with feline leukemia virus, one of two host–virus relationships develops: persistent active infection, or a self-limiting infection. Persistent active infection is recognized by the presence of persistent viremia. The serum of persistently infected cats lacks both neutralizing and FOCMA antibodies. Viremia persists for months and is usually terminated by feline leukemia virus-related disease. Persistently infected cats shed virus in secretions and represent the most important source for the dissemination of feline leukemia virus. Immunosuppression is the most common sequel to persistent feline leukemia virus viremia and accounts for most feline leukemia virus-related deaths. Viremic cats have suppressed blastogenic responses to T cell mitogens, suppressed antibody responses, prolonged allograft rejection times, hypocomplementemia, thymic atrophy, depletion of paracortical zones of lymph nodes, and an almost total failure of interferon production. Age appears to have some influence on the disease pattern, perhaps because of an association between the virus and dividing cells.

The vast majority of cats exposed to infection with feline leukemia virus develop a self-limiting infection. They remain nonviremic, develop neutralizing and FOCMA-related antibodies, do not shed virus, and do not develop feline leukemia virus-related disease. Sometimes there is a transient viremia, which disappears with the development of neutralizing and FOCMA-related antibodies.

Finally, in some cats persistent viremia is initially accompanied by persistent high FOCMA-related antibody. This is an unstable condition; such cats either develop neutralizing antibody, or the FOCMA-related antibody declines and the cats develop feline leukemia virus-related disease.

Diagnosis

Viral isolation is rarely possible, but a number of diagnostic tests have been developed based on detecting viral proteins in cells in the blood, by either indirect immunofluorescence or ELISA. The indirect-immunofluorescence test is performed on blood smears on glass slides, which may be mailed to an appropriate laboratory. ELISA is performed on plasma or buffy coat cells, and is available commercially in kit form. Both tests detect a group-specific antigen within the core.

Epidemiology

Only the nonpathogenic endogenous type of feline leukemia virus is transmitted vertically, via the germ plasm. Although many cats are exposed to horizontally transmitted, pathogenic feline leukemia virus, relatively few become infected, in spite of the fact that the saliva of persistently infected and viremic cats may contain 10^6 infectious virions per milliliter. Prolonged, direct exposure is usually required for transmission, which may occur by mutual grooming or exchange of fleas. Circumstantial evidence suggests that biting, such as occurs during fighting, is probably the most important method of transmission, which may also occur iatrogenically via blood transfusion, multiple-used syringes, and surgical instruments.

The prevalence of feline leukemia virus infection and disease parallels the opportunities for exposure. The prevalance in single, confined, household cats is about 1%; infection rates progressively rise if cats also go outside particularly to shows, live in a multiple-cat household, or live in breeding colonies. Infection rates may be as high as 33% in colonies in which the virus is enzootic.

Control and Treatment

Using immunufluorescence or ELISA procedures for detecting viral antigens, it is possible with a test and removal program to establish feline leukemia virus-free cat colonies. Such programs may be undertaken by large catteries, particularly where the incidence of infection is high and there is clinical evidence of disease due to feline leukemia virus. The laboratory tests aid in identifying preclinical or subclinical infections and confirming clinical diagnosis.

There is a potentially large market for an efficacious feline leukemia virus subunit vaccine. In 1984 an inactivated vaccine based on the cell surface antigen (FOCMA) was licensed in the United States. Clinical trials suggest that this vaccine reduces the incidence of disease by 70%. Various cytotoxic drugs used in cancer therapy in humans have been tried in particularly valuable cats, without success. Immunological procedures designed to provide passive protection or to remove immune complexes may temporarily alleviate clinical signs but are not widely used.

DISEASES CAUSED BY LENTIVIRUSES

In 1933, 20 karakul sheep were imported into Iceland from Germany, and within 2 years two diseases, called maedi (= dyspnea) and visna (=

wasting) emerged, which in the following years were responsible for the deaths of 105,000 sheep. A further 600,000 sheep, encompassing the rest of the Icelandic sheep population, were slaughtered through 1965, when the diseases were declared eradicated. These diseases have an incubation period of over 2 years, an insidious onset, and a protracted clinical course, lasting 6 months to several years, unless terminated by intercurrent disease. Sigurdsson, who demonstrated that both Icelandic diseases were transmissible wiih cell-free filtrates, described the diseases as "slow-virus infections," thus introducing into virology a term that has been widely used for other infections as well (see Chapter 11). Maedi and visna appear to have been caused by the same or very closely related lentiviruses.

Visna, in which the lesions occur in the central nervous system, has rarely been recorded in sheep outside Iceland, although a few cases have been described in the Netherlands. However, a disease very like maedi occurs in several countries in Europe and in the United States, where it is called ovine progressive pneumonia, but it does not occur in Australia or New Zealand, which have very large sheep populations.

Maedilike and visnalike diseases occur in goats, particularly in India and less commonly in Germany and the Netherlands. However, a much more important lentivirus infection of goats was recognized in the United States in 1974, which is now known to occur worldwide: caprine arthritis–encephalomyelitis. The internal antigens of the caprine and ovine lentiviruses cross-react; the virions are closely related but distinguishable.

Equine infectious anemia is a naturally occurring disease affecting all members of the family Equidae. It occurs worldwide and has a protean clinical course, with lifelong persistence of the virus. Infections are characterized by anemia, immune complex disease, bone marrow depression, and recurrent episodes of fever. Although its causative agent was once thought to belong to the subfamily *Oncovirinae*, the virus is now classified as a lentivirus.

Properties of Lentiviruses

The lentiviruses are typical retroviruses but differ from the oncoviruses in details of their morphology (see Plate 31-2) and chemical composition. In suitable cell cultures (sheep chorioid plexus cells for maedi–visna viruses; caprine fetal synovial membrane cells for caprine arthritis–encephalomyelitis virus), some strains produce cytopathic changes consisting of syncytia and foci of degeneration; other strains are noncytocidal and establish persistent infections. Equine infectious ane-

mia virus can be grown in primary horse peripheral blood leukocyte cultures and in an equine dermal cell line, in which the presence of virus is recognized by complement fixation, immunofluorescence, or gel diffusion tests.

The replication of lentiviruses is similar to that of oncoviruses, and involves a dsDNA intermediate which establishes an integrated provirus state. The slowness of lentivirus infections refers to the progression of the disease, not to the rate of replication of the virus, which is completed in permissive cells in 72 hours. In contrast to oncoviruses, lentiviruses do not require dividing cells to support their replication. Factors that influence the levels of viral mRNA, protein, and infectious virus production are incompletely understood, but may be influenced by the number of copies of viral DNA per cell. Initially a cell may contain only one or a few copies of integrated provirus, but the number is progressively amplified to about 300, most of which remain nonintegrated. Transcription and translation occurs from nonintegrated DNA. These gene dosage effects may regulate the level of virus production and independently the degree and rate of consequent cell and tissue damage *in vivo*.

Lentivirus DNA sequences are not detectable in normal ovine cell DNA, suggesting that endogenous lentiviruses do not exist. There is no evidence that lentiviruses can acquire *onc* genes.

Ovine Lentivirus Diseases

Visna. It is not known whether the virus strain, sheep age, or route of infection determined whether Icelandic sheep developed visna or maedi. In Iceland visna was recognized in sheep 2 years of age or older, but in the same population of sheep maedi was not seen before 3 years of age. This observation suggests either a shorter incubation period, infection at an earlier age, or a more efficient mode of infection for visna. Following experimental infection, the incubation period varies from a few months to 9 years. The onset of clinical signs is insidious and usually begins with slight weakness of the hind legs. Affected sheep may straggle the flock and stumble and fall for no apparent reason. There is progressive weight loss, and trembling of facial muscles and lips may occur. The paresis eventually leads to paraplegia. There is no fever, the appetite is maintained, and sheep remain alert. The clinical course may last several years, with periods of remission. The cerebrospinal fluid contains up to 200 mononuclear cells per milliliter (normal, 50 cells/ml).

Maedi–Progressive Pneumonia. The onset of clinical signs in the pulmonary lentivirus diseases of sheep is insidious and is seldom detected

in sheep under 3 years old. Incubation periods of up to 8 years have been recorded. There is progressive weight loss, and dyspnea, initially detectable only after exercise, becomes progressively more apparent. Affected sheep straggle when the flock is driven. The head may jerk rhythmically with each inspiration, nostrils are flared, there may be a slight nasal discharge and a cough. Severely dyspneic sheep spend much time lying down. The clinical course may last 3–8 months; it may be prolonged by careful nursing or shortened by pregnancy, stress such as occasioned by inclement weather or poor nutrition, or intercurrent disease, particularly pneumonia due to *Pasteurella* spp. Pregnant ewes may abort or deliver weak lambs.

Arthritis and lymphoid tumors of the mammary gland in addition to pneumonia have been observed in sheep following experimental infection with strains of virus recovered in the United States.

Pathogenesis and Pathology. Prior to 1933, Icelandic sheep were genetically isolated for 1000 years and it has been suggested, but unproven either for them or other breeds of sheep, that there may be a genetic predisposition to lentivirus disease, especially visna. The ovine lentiviruses are probably most commonly acquired by droplet infection via the respiratory tract. Experimentally, visna can be produced by intracerebral inoculation of sheep. A lymphocyte-associated viremia occurs, in which about one in every 10^6 peripheral blood leukocytes is infected.

Lentiviruses are exceptionally resistant to interferon, and despite a diverse range of immune responses, including the production of neutralizing antibodies and a cell-mediated immune response, neither virus nor infected cells are eliminated. Immune suppression abrogates or delays the progress of degenerative changes, indicating that immune mechanisms are significant in the inflammatory changes in both lung and central nervous system. In an infected sheep, antigenic variation in the envelope antigens (see Chapter 11) may be an important mechanism for circumventing viral elimination.

Apart from neurogenic muscle atrophy, no gross lesions are found in visna, and histologically observed lesions are usually confined to the central nervous system; occasionally slight lung abnormality may be present. The characteristic lesion of the central nervous system is a demyelinating leukoencephalomyelitis. The meninges and subependymal spaces are infiltrated with mononuclear cells, mainly lymphocytes with some plasma cells and macrophages. There is perivascular cuffing, neuronal necrosis, malacia, and demyelination scattered patchily throughout the central nervous system.

Gross findings in maedi and ovine progressive pneumonia are re-

stricted to the lungs and associated lymph nodes. The lungs show extensive consolidation and do not collapse when the thoracic cavity is opened. Bronchial and mediastinal lymph nodes are greatly enlarged. Histological examination reveals hyperplasia of the fibrous tissue and muscle of the alveolar septa, and a mononuclear cell inflammatory infiltration.

Epidemiology and Control. Droplet transmission is facilitated by housing and close confinement and was important in Iceland, where sheep were housed for 6 months during the winter. Transmission is usually direct, although infection via drinking water or from fecal or urine contamination may occur. Asymptomatic sheep are rarely a source of virus for infection of other sheep, with the possible exception of ewe to lamb transmission via milk. Evidence for transplacental infection and infection via semen is conflicting. Biting arthropods (lice, fleas) and surgical equipment could readily transmit virus mechanically from viremic sheep.

Visna–maedi was eradicated from Iceland by a drastic slaughter policy before the availability of any diagnostic test. Test and removal programs are used in Norway and the Netherlands. Gel diffusion, complement fixation, ELISA, and a syncytial plaque reduction assay are used for antibody detection.

Caprine Arthritis–Encephalomyelitis

Two syndromes are recognized: encephalomyelitis in kids 2–4 months old and, more commonly, arthritis in goats from about 12 months of age onward. In the United States, caprine arthritis–encephalomyelitis is now the most important disease of goats; infection rates as high as 80% have been reported in some herds, and the economic loss from the disease is substantial. The virus is not known to be naturally transmitted to other animal species. Experimentally, the virus infects sheep and causes arthritis. However, despite the high incidence of infection of goats in Australia and New Zealand, infection of sheep has not been reported in these countries.

Clinical Features and Pathology. The central nervous system disease usually occurs in kids, occasionally in adult goats. It is a progressive leukoencephalomyelitis associated with ascending paralysis. Affected goats also show progressive wasting and trembling and the hair coat is dull, but they remain afebrile and alert, and usually maintain good appetite and sight (Plate 31-3). Terminally there is paralysis, deviation of the head and neck, and paddling. At autopsy, lesions may be visible as

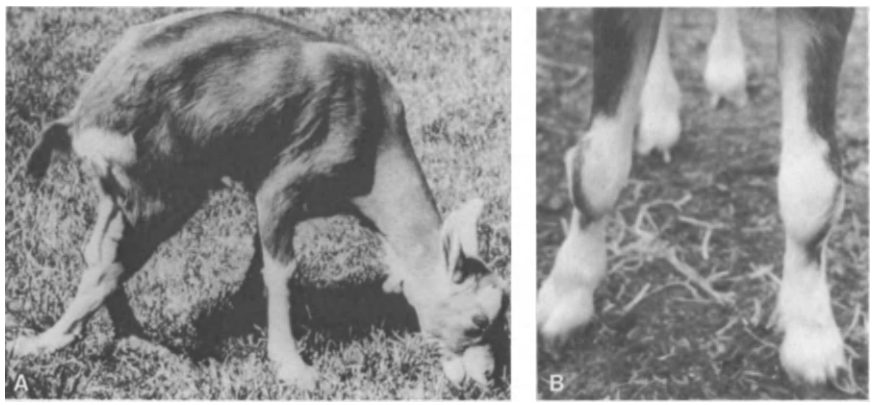

PLATE 31-3. *Caprine arthritis–encephalomyelitis. (A) Kid goat with paralyzed hindquarters, but alert and attempting to graze. (B) Enlarged carpal joints in a 5-year-old goat. (Courtesy L. Cook.)*

focal malacia in white matter, but are more reliably identified microscopically as foci of mononuclear cell inflammation and demyelination.

The onset of arthritis is usually insidious and progresses slowly over months to years, but in some cases disease may appear suddenly and remain static. The joints are swollen and painful, particularly the carpal joints (Plate 31-3), but also hock, stifle, shoulder, fetlock, and vertebral joints. Cold weather exacerbates the signs. Bursae, particularly the atlantooccipital, and tendon sheaths are thickened and distended with fluid. Thickening of the joint capsules results in restricted movement and flexion contracture. The basic lesion is a proliferative synovitis of joints, tendon sheaths, and bursae characterized by villous hypertrophy, synovial cell hyperplasia, and infiltration with lymphocytes, plasma cells, and macrophages. Progression is accompanied by degenerative changes including fibrosis, necrosis, mineralization of synovial membranes, and osteoporosis. Mild interstitial pneumonia and hyperplasia of pulmonary lymphoid tissue may be seen at postmortem.

Epidemiology and Control. Virus is acquired during the neonatal period, via colostrum or milk; the cycle of infection can be broken if kids are delivered by cesarian section and fed cow's milk, or milk from caprine arthritis–encephalomyelitis virus-free does.

Antibodies can be detected by gel diffusion, indirectly immunofluorescence, or ELISA tests, and form the basis of voluntary control programs based on test and removal.

Equine Infectious Anemia

Equine infectious anemia is an important chronic disease of horses that occurs worldwide.

Clinical Signs and Pathogenesis. Following primary infection, most horses develop fever after an incubation period of 7 to 21 days. The disease is recognized as four interchanging, overlapping syndromes. In acute equine infectious anemia there is a marked fever, weakness, severe anemia, jaundice, blood-stained feces, tachypnea, and petechial hemorrhages of the mucosae. Perhaps as many as 80% of acute cases end in death; others pass into the subacute form, in which continuing moderate fever is followed by recovery. Recovery from either the acute or subacute disease is followed by lifelong persistent infection. Recovered viremic horses may appear and perform well, but some experience recurrent episodes of disease while others develop chronic disease that varies from mild signs of illness and failure to thrive to episodic or persistent fever, cachexia, and ventral edema.

The disease is due to infection of lymphocytes, in which degenerative or proliferative responses may occur. Lifelong, cell-associated viremia develops in all infected horses. It is uncertain whether anemia develops as a consequence of bone marrow suppression, increased clearance of red cells from the circulation, or autoimmune destruction of erythrocytes. Vasculitis, including glomerulonephritis, is mediated by immune complexes.

Epidemiology and Control. Tabanid flies and stable flies (*Stomoxys* spp.), mosquitoes, and possibly midges can serve as mechanical vectors for equine infectious anemia virus. Transmission occurs particularly in the summer months in low-lying, humid, swamp areas such as occur in the Mississippi delta region of the United States and in parts of South and Central America, South Africa and northern Australia. National prevalence figures are uneven geographically and reflect the importance of insect transmission. On farms on which infection has been enzootic for many years the prevalence may be as high as 70%. Iatrogenic transmission by the use of nonsterile equipment such as rasps, gags, bridles, nose twitches, stomach tubes, or saliva collecting equipment has been responsible for some major outbreaks. Transplacental infection has been recognized; colostrum and milk, saliva, urine, and semen are other unproven but possible modes of transmission.

In enzootic areas the rate of transmission may be reduced by insect-proof stabling of horses during those times of the year (summer) and

that time of the day (dusk) when insects are most active. Iatrogenic transmission is avoided by careful hygiene.

The development in 1970 of a gel diffusion test for detecting antibodies to equine infectious anemia virus was followed in the United States by regulations, promulgated by federal and state agencies and some breed societies, to limit the movement of seropositive horses. In some instances a negative test has been required as a condition of entry to racetracks, saleyards, and shows. Buyers of horses also increasingly sought negative test certification. The United States Department of Agriculture introduced regulations relating to the licensing and operation of laboratories authorized to conduct the gel diffusion (Coggins) test, and horses imported into the United States and some other countries are required to have a negative test certificate. For horses remaining within a state, testing is not compulsory, nor is it compulsory for an owner to destroy a horse giving a positive test.

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CHAPTER 32

Reoviridae

The name "reovirus" (*r*espiratory *e*nteric *o*rphan virus) was proposed in 1959 for a group of viruses previously classified as picornaviruses, which were typically recovered from the respiratory and gastrointestinal tracts but were not associated with any disease. A few years later it was shown that their genome consisted of dsRNA and then that it was segmented. In the early 1970s the orbiviruses were separated from the unclassified arboviruses, because, unlike other arboviruses, they lacked an envelope and had a segmented dsRNA genome. Then in the mid-1970s it was recognized that a large group of viruses that had been observed in the feces of animals and humans with diarrhea, named rotaviruses because of their distinctive morphology, also had a segmented dsRNA genome. These three groups now constitute three genera within the family *Reoviridae: Orthoreovirus,** *Orbivirus*, and *Rotavirus*. Three other genera within this family infect plants and insects, and are not discussed further.

Though ubiquitous, most orthoreoviruses are nonpathogenic, but the

*The official name of the genus is *Reovirus*, but this name leads to ambiguities when using the vernacular "reoviruses" for both family and genus; hence we will use the term *Orthoreovirus*.

TABLE 32-1
Diseases Caused by Viruses of the Family Reoviridae

Genus	Viruses	Principal species affected	Disease
Orthoreovirus	Mammalian orthoreovirus 1–3	Isolated from many species of mammals and birds	Hepatoencephalomyelitis in mice
	Avian orthoreovirus 1–11	Chickens, turkeys, and geese	Arthritis, nephrosis, enteritis, chronic respiratory disease, myocarditis
Orbivirus[a]	Bluetongue virus 1–24	Sheep, cattle, and deer	Bluetongue
	Ibaraki virus	Cattle	Acute febrile disease similar to bluetongue
	Epizootic hemorrhagic disease of deer virus 1–7	Deer	Epizootic hemorrhagic disease
	African horse sickness virus 1–9	Horses, donkeys, mules, and zebras	African horse sickness
	Equine encephalosis virus 1–5	Horses	Abortion and encephalitis
Rotavirus	Rotavirus: many types, often host-specific	Most animals	Enteritis

[a]Besides those listed, there are seven other serogroups within the *Orbivirus* genus, none of which is known to cause diseases of domestic animals.

genera *Orbivirus* and *Rotavirus* contain several important pathogens (Table 32-1).

PROPERTIES OF REOVIRUSES

All members of the family *Reoviridae* share certain properties (Table 32-2), but the three genera differ in several ways, including the number and size of segments of dsRNA in the genome and the structure of the inner and outer capsid. All reoviruses have nonenveloped spherical virions about 70 nm in diameter, which consist of an inner and outer capsid. In all genera the inner capsid is a stable structure consisting of 32

TABLE 32-2
Properties of Reoviruses

Nonenveloped spherical virion, diameter 70 nm
Two concentric capsids, the inner capsid being icosahedral
dsRNA genome, segmented, 10–11 molecules, total size 18–23 kb: *Orthoreovirus* (10 segments, 23 kb); *Orbivirus* (10 segments, 23 kb); *Rotavirus* (11 segments, 18 kb)
Five enzymes including transcriptase in core
Cytoplasmic replication
Genetic reassortment occurs between species within each genus

capsomers arranged in icosahedral symmetry, but there are striking differences in the outer capsid structure in the three genera (Plate 32-1). The orthoreoviruses have a well-defined outer capsid with 92 capsomers comprising hexamer and pentamer subunits and consisting mainly of two proteins, with two molecules of a third protein near each icosahedral vertex (see Fig. 5-4). Orbivirus inner capsids are surrounded by a diffuse layer formed by two proteins but lacking any clear morphological subunits, which is readily dissociated from the inner capsid. The rotavirus outer capsid also lacks visible subunit structure, but in the electron microscope it appears more sharply defined than that of the orbiviruses.

The RNA of orthoreoviruses consists of three size classes, which can be further differentiated by polyacrylamide gel electrophoresis into 10 discrete segments (Fig. 32-1). These code for 10 species of proteins, one of which is cleaved once during translation.

The genome of the orbiviruses is also in 10 segments, with a different size distribution from those of orthoreoviruses. *In vitro* translation experiments have allowed the assignment of a distinct polypeptide to each

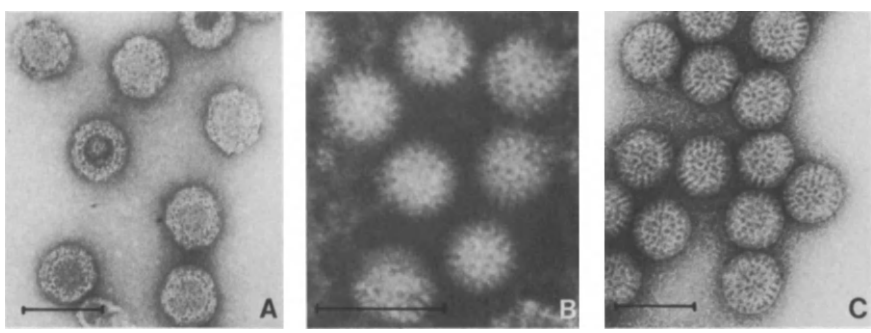

PLATE 32-1. Reoviridae. *(A)* Orthoreovirus, *(B)* Orbivirus *(bluetongue virus), and (C)* Rotavirus *(bars = 100 nm). (Courtesy Dr. E. L. Palmer.)*

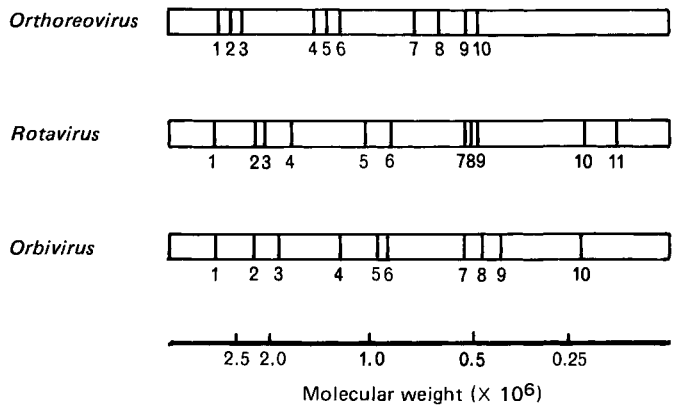

FIG. 32-1. *Schematic diagram of the dsRNA genome segments of an orthoreovirus, an orbivirus, and a rotavirus separated by polyacrylamide gel electrophoresis.*

RNA segment (Fig. 32-2). Seven structural proteins and three nonstructural proteins have been identified, but the functions of two additional viral proteins, translated from segment 10, are unknown.

The genome of rotaviruses comprises 11 segments (Fig. 32-1), which code for at least 12 proteins. In contrast to orthoreoviruses, the major

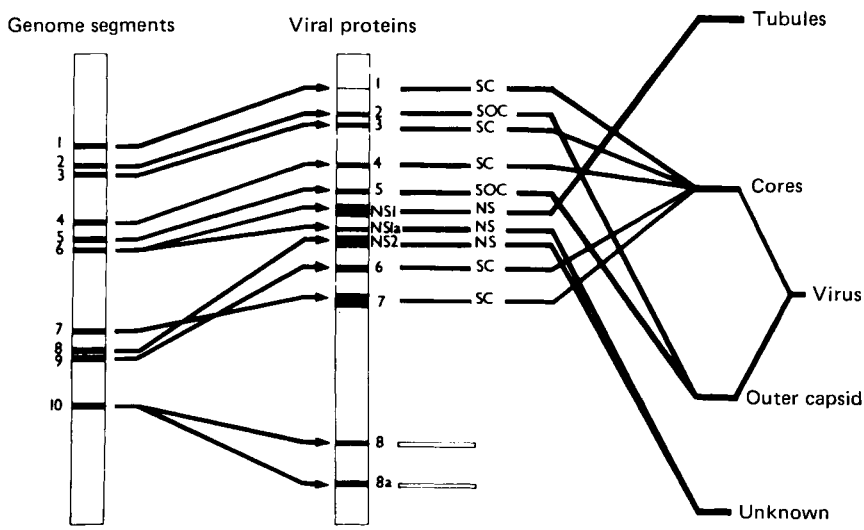

FIG. 32-2. *Coding assignments for the genome segments of bluetongue virus 1. The core proteins (SC), outer capsid proteins (SOC), and nonstructural proteins (NS) are indicated. [From P. P. C. Mertens, F. Brown, and D. V. Sangar,* Virology **135,** *207 (1984).]*

component of the outer capsid is a glycoprotein. Until recently, all rotaviruses were thought to share a common inner capsid antigen that could be detected by immunofluorescence or complement fixation. However, some atypical rotaviruses ("pararotaviruses") lack the common antigen and have genome segments 5, 7, and 9 which differ from those of other rotaviruses.

Orthoreoviruses and rotaviruses are resistant to lipid solvents and are stable over a wide pH range, but orbiviruses have a narrow zone of pH stability (pH 6-8), and lose some infectivity upon exposure to ether. Proteolytic enzymes, in general, increase the infectivity of orthoreoviruses and rotaviruses, e.g., chymotrypsin, which is found in the small intestine, leads to loss of the outer capsid, with enhanced infectivity.

In the presence of protein, orbiviruses are remarkably stable, e.g., bluetongue virus has been reisolated from blood held for 25 years at room temperature. Not all disinfectants are active against rotaviruses; although iodophores and phenolic compounds inactivate the virus, hypochlorite is ineffective. Ethanol (95%) is an efficient disinfectant for laboratory use.

VIRAL REPLICATION

Reovirus replication occurs in the cytoplasm. The endocytosed virion is uncoated only partially, by lysosomal hydrolases, to a "subviral particle" (see Plate 4-2). This activates the virion-associated transcriptase and capping enzymes to transcribe 5′-capped mRNA molecules which, uniquely, are not polyadenylated at their 3′ termini. Only certain genes (segments) are transcribed initially; the others are derepressed following the synthesis of an early viral protein. Protein associates with each mRNA molecule and (−) sense RNA strands are synthesized, producing dsRNA molecules. These in turn serve as templates for the transcription of more mRNA, which this time is uncapped. By a mechanism that is unclear, these uncapped reovirus mRNA molecules are then translated preferentially to yield a large pool of viral structural proteins. Finally, the subviral particles associate with additional proteins to complete the maturation of new virions.

The maturation of rotaviruses involves an unusual type of budding of single-capsid particles into vesicles of rough endoplasmic reticulum; the pseudoenvelope thus acquired is subsequently removed and the outer capsid is added in the vesicles, since the major outer capsid protein is glycosylated and its synthesis can be completed only as it traverses the endoplasmic reticulum membrane.

DISEASES CAUSED BY ORTHOREOVIRUSES

Orthoreoviruses are generally considered to be nonpathogenic, the important exceptions being infections of rodents and poultry. There are three serotypes of mammalian *Orthoreovirus*, all of which infect a wide range of species and are found worldwide. In mouse colonies, *Orthoreovirus* 3 can cause natural disease, sometimes called hepatoencephalomyelitis, which is characterized by jaundice, ataxia, oily hair, and growth retardation.

Avian orthoreoviruses, of which 11 serotypes have been identified, cause disease in some flocks but are associated with only subclinical infection in others. They cause chronic respiratory disease in chickens, turkeys, and geese. They have also been associated with tenosynovitis or arthritis, usually in meat-producing birds over 5 weeks old. Bilateral swelling of the digital flexor and the tarsometatarsal extensor tendons produces lameness and can lead to rupture of the gastrocnemius tendon. Articular erosions may be recognized in chronic cases. Morbidity is often 100% with mortality less than 2%, although in some outbreaks the mortality has been higher.

Avian orthoreoviruses are most easily isolated in avian cell cultures and produce vacuoles in cells prior to syncytium formation. Identification of an isolate as being an orthoreovirus can be made by fluorescent-antibody techniques; a serum neutralization test is used for typing.

DISEASES CAUSED BY ORBIVIRUSES

Bluetongue and African horse sickness have been diseases of economic importance in South Africa since the early days of European settlement. The importance of these two diseases in Africa, and their ability to cause periodic but extensive epizootics in the Middle East and parts of Europe, stimulated scientific inquiry into similar viruses in other parts of the world. It has now emerged that many orbiviruses exist that are nonpathogenic for domestic animals. Isolates have been made from many different families of arthropods (mosquitoes, *Culicoides*, sandflies, and ticks), and from birds and terrestrial mammals. All orbiviruses so far discovered appear to be arthropod-borne.

The orbiviruses are differentiated into 11 serogroups, of which 5 include viruses that produce disease in domestic animals (Table 32-1). No genus-specific antigen has been detected, but viruses within a serogroup have a common antigen demonstrable by immunofluorescence, immunodiffusion, and complement fixation tests. Low-level cross-reactions among individual viruses, normally considered mem-

bers of distinct serogroups, have been reported, causing some uncertainty in serological classification. Reassortment of genome segments between serologically related viruses has been described for the bluetongue and other serogroups, and a classification system based on genetically interacting groups has also been advanced.

Bluetongue

Bluetongue is an arthropod-borne virus disease of ruminants characterized by congestion, edema, and hemorrhage. The disease is of most importance in sheep, but its severity in this species varies from subclinical to severe depending on the strain of virus, the breed of sheep, and the local ecology. Economic losses result from death and loss of condition in sheep that survive. Convalescence may be protracted and wool growth may be impaired, leading to a "break" in the fiber. Infection of cattle and goats is often inapparent but disease can be severe in some wildlife species, particularly white-tailed deer (*Odocoileus virginianus*) in North America. The virus can cause congenital infection in cattle and sheep, resulting in abortions and fetal abnormalities.

Until the 1940s, bluetongue was recognized only in Africa, then its presence was confirmed in the countries of the eastern Mediterranean. In 1956–1957 a major epizootic occurred in Portugal and Spain in which hundreds of thousands of sheep were affected. This epizootic generated a worldwide escalation in the recognition of bluetongue as an "emerging disease" likely to cause severe economic losses. The virus was first isolated in the Western Hemisphere from sheep in California in 1952 and from cattle in Oregon in 1959.

By the late 1970s, it was realized that bluetongue virus was more widely distributed in the countries of the tropics and subtropics than had previously been thought (Table 32-3). The virus has been isolated in several countries without clinical disease being recognized in domestic livestock. Today, it is probable that some livestock in most, if not all,

TABLE 32-3
Serotypes of Bluetongue Virus in Various Regions of the World

Region	Serotypes
Southern and western Africa	1–15, 18, 19, 22, 24
Middle East	1, 3, 4, 10, 12, 16
Iberian Peninsula	10
United States of America	2, 10, 11, 13, 17
Australia	1, 20, 21, 23
South America (Brazil)	4

countries in the tropics and subtropics are infected with bluetongue or closely related viruses.

The inclusion of a virus within the bluetongue group is based on the detection of a group antigen by the complement fixation test. As mentioned earlier, orbiviruses do not always fit neatly into serogroups and the terms "bluetonguelike virus" and "bluetongue-related virus" have emerged to confuse the nomenclature. Classification of viruses may appear to be a topic of esoteric interest, but in fact a diagnosis of "bluetongue" has a profound effect on international livestock trade. Veterinarians responsible for international disease control usually err on the side of caution when they become aware of "bluetongue-related" or "bluetonguelike" viruses in the exporting country.

Studies in the 1940s on the antigenic types of bluetongue virus causing disease in sheep in South Africa led to the recognition of 10 serotypes, each of which produced solid homologous immunity but a variable degree of protection to heterologous challenge. Subsequent classification of new serotypes has relied on RNA hybridization and comparisons by serum neutralization tests in cell culture, and 24 bluetongue virus types are currently recognized.

Clinical Features. In sheep, the disease is characterized by fever which may last several days before hyperemia, excess salivation, and frothing at the mouth are noticed; a nasal discharge, initially serous but becoming mucopurulent and specked with blood, is common. The tongue may become cyanosed—hence, "bluetongue" (Plate 32-2B). There is a marked loss of condition and the sheep may die, often through aspiration pneumonia. The coronary bands of the feet exhibit hyperemia and are painful. Edema of the head and neck is not uncommon; animals with coronitis are often reluctant to walk (Plate 32-2C) and tend to be recumbent. Hyperemia of the skin may occur, leading to "wool break" some weeks later. Muscle degeneration occurs and in many animals convalescence is protracted. Morbidity may be as high as 80% and mortality 50%. The disease in deer is similar. In contrast, the disease in cattle is usually inapparent and rarely acute.

Bluetongue may cause abortion and congenital abnormalities (Plate 32-2D). In calves infected *in utero* the virus infection may be persistent for several months to years after birth. A similar observation has been made with lambs, but the duration of the persistent viremia (2 months) is shorter.

Pathogenesis. Bluetongue virus replicates in hematopoietic cells and endothelial cells of the blood vessels. Adult sheep sometimes remain viremic for more than 28 days, but the virus has been reported to persist in cattle for longer periods than in sheep.

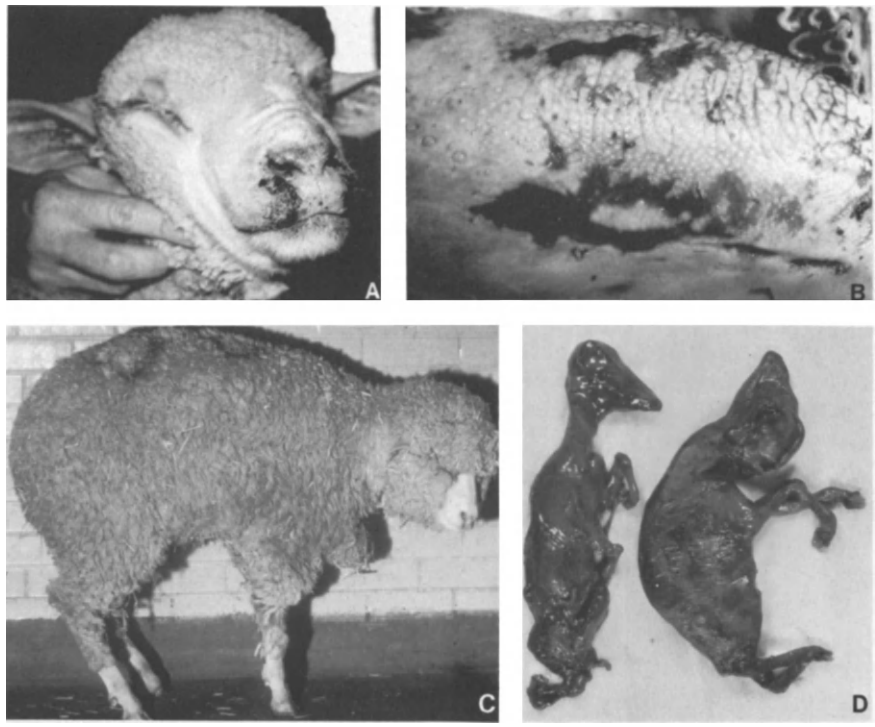

PLATE 32-2. *Bluetongue in sheep. (A) The muzzle is swollen and has erosions. (B) Erosion of the lateral margins of the tongue, which is greatly swollen and cyanosed. (C) Lameness due to hyperemia of the coronary band of the hoofs. (D) Mummified fetal lambs aborted at 135 days gestation. The ewe was infected about day 60 of gestation. (A and B, courtesy Dr. B. Erasmus; C, courtesy United States Department of Agriculture.)*

Rarely, and only when the bull is viremic, bluetongue virus may be recovered from semen. To the extent that this might lead to the transmission of virus to cows and their offspring, this route offers an alternative to arthropod transmission for the perpetuation of the virus.

Laboratory Diagnosis. Clinical diagnosis of overt disease in sheep should not present a problem, but the diagnosis in cattle is more difficult. Bluetongue can be confused with the vesicular diseases, bovine virus diarrhea–mucosal disease, mild cases of rinderpest, infectious bovine rhinotracheitis, and malignant catarrh. In both cattle and sheep, photosensitization should be excluded from the diagnosis. Postmortem, apart from hemorrhage at the base of the pulmonary artery, there are no pathognomonic gross pathological signs.

Bluetongue virus is often difficult to isolate in the laboratory. The chances of virus isolation are enhanced if blood is collected from animals

showing early clinical signs or a pronounced pyrexia, and is most likely to be successful if the buffy coat is inoculated intravenously into 10- or 11-day-old chick embryos. Virus can be adapted to cell culture, but this system is generally considered insensitive for primary isolation. A range of serological techniques are used for diagnosis. Cloned genome segments of bluetongue virus are now available for use as diagnostic probes, but their usefulness has yet to be evaluated.

Epidemiology. Bluetongue virus is transmitted by arthropods. Transplacental transmission may occur, but the virus is not transmitted by contact or through infected animal products. The epidemiology of bluetongue depends on interactions of host, vector, climate, and virus. It occurs most commonly in late summer, when the vectors, *Culicoides* spp. ("no-see-ums" or biting midges), are most numerous. *Culicoides* breed in many habitats, particularly damp muddy areas and cow dung. Moisture is important for their life cycles, but some species may be found in apparently arid areas and others can breed in highly saline water.

Female *Culicoides* take a blood meal every 3–4 days until the end of their life, which can be as long as 70 days. If the blood contains virus, it infects the cells in the hemocoel and salivary glands of the vector. After an extrinsic incubation period of 7 to 10 days, the virus is excreted in the saliva and can be transmitted. There is no evidence of transovarial transmission in arthropods. Not all species of *Culicoides* are vectors, and different species constitute the principal vectors in different parts of the world. Long-range, windborne dispersal of infected *Culicoides* may sometimes occur and constitute a mechanism by which bluetongue virus can be introduced to a distant area.

Prevention and Control. Bluetongue viruses are now recognized to infect ruminants in every continent where livestock are reared. Geography and climate apparently predispose certain areas to epizootics of bluetongue, depending on the temporary introduction of efficient insect vectors into an area where livestock are susceptible. When the climate changes, the vector is no longer able to survive and virus "dies out."

The attenuated live-virus vaccines available for the control of bluetongue in South Africa have several disadvantages: (1) live-virus vaccines have been associated with fetal death and cerebral abnormalities in sheep in the United States, (2) the use of multivalent live vaccines may lead to the emergence of genetic reassortants, and (3) transmission of attenuated vaccine virus by the vector can occur, with possible reversion to virulence. Research into the development of inactivated vaccines has progressed to a stage which suggests that a safe inactivated vaccine that will protect sheep and cattle from infection can be developed.

Control by vaccination is necessary where virulent bluetongue viruses are enzootic. However, it is important to minimize the possibility of their introduction into new areas. In view of the widespread distribution of bluetongue viruses in the tropics and subtropics, the control of its movement between countries by examination and testing and certification of livestock and germ plasm may not appear, at first glance, to have been effective. However, this impression is probably erroneous. In retrospect, most of the geographical expansion of bluetongue probably occurred before animals were examined for that infection by other than clinical examination. The limited number of types of bluetongue virus in Australia and the United States, countries that until recently have had very restrictive attitudes to the importation of livestock from countries infected with bluetongue virus, testifies to the probable success of the policy.

Ibaraki and Epizootic Hemorrhagic Disease

Two viruses closely related to bluetongue virus,Ibaraki virus and epizootic hemorrhagic disease of deer virus, belonging to the epizootic hemorrhagic disease virus group of the orbiviruses, have been isolated from cattle and deer, respectively, affected with a disease clinically indistinguishable from bluetongue. Epidemic hemorrhagic disease virus has also been isolated from cattle, which appear to be its reservoir host. Ibaraki disease was first recorded as an acute, febrile disease of cattle in Japan in 1959, and the virus is present in many parts of Southeast Asia. Epizootic hemorrhage disease of deer was first associated with a virus in 1955 in the United States. In 1964, the disease was seen in Alberta, Canada, and the virus has been isolated from cattle and arthropods in the United States and arthropods in Africa. Similar viruses exist in Australia. Outbreaks of bluetongue and epidemic hemorrhagic disease are uncommon, but are regarded as the most important diseases of Cervidae in North America.

African Horse Sickness

African horse sickness virus causes disease in horses, mules, and donkeys with up to 95% mortality. Apart from Venezuelan equine encephalitis, it is the most important viral disease capable of causing widespread mortality in horses. Epizootics of African horse sickness have been recognized in South Africa since 1780. The virus is considered enzootic only in sub-Saharan Africa, but on several occasions it has caused devastating outbreaks of disease in horses outside Africa. Since World War II, major epizootics have occurred in the Middle East and Indian subcontinent in 1959–1961 and in North Africa and southern

Spain in 1965–1966. In the former epidemic, over 300,000 horses, donkeys, and mules were reported to have died. African horse sickness has never been recognized in the Western Hemisphere or Australia.

Nine serotypes of African horse sickness virus are recognized in South Africa, and additional serotypes are suspected to occur elsewhere in Africa. There is no significant serological or genetic relationship between African horse sickness and other orbiviruses of veterinary importance.

Clinical Features. The severity of clinical disease in horses, donkeys, and mules varies with the strain of virus. Horses are generally the most susceptible, with high morbidity and mortality rates; there is also high morbidity in mules but mortality is low, while donkeys are the least sensitive, usually developing only a mild febrile response. In acute cases, the disease is characterized by severe and progressive respiratory disease leading to death. After an incubation period of 3 to 5 days, the animal develops fever for 1 or 2 days (40°–41°C); the breathing rate then increases, often to 70 per minute, and the affected animal stands with its forelegs apart, head extended, and nostrils dilated. Spasmodic coughing may occur terminally, accompanied by profuse sweating and discharge of frothy fluid from the nostrils. This pulmonary form is most commonly seen in completely susceptible horses infected with a highly virulent strain of the virus.

In contrast, some cases are mild and easily overlooked. Apart from fever, which may last for 5 to 8 days, other clinical signs are unusual, although the conjunctiva may be slightly congested. This type of disease is most commonly seen in donkeys and vaccinated horses infected with a heterologous virus type. Disease of intermediate severity is seen in some animals. The incubation period is 7–14 days, followed by fever,which persists for 3 to 6 days. As the temperature falls, characteristic edema appears involving the supraorbital fossae and eyelids (Plate 32-3). Subsequently the edema extends to affect the lips, tongue, intermandibular space, and laryngeal region. Subcutaneous edema may also track down the neck toward the chest. Mortality rates for such cases may be as high as 50%; death occurs within 6 to 8 days of onset of fever. Terminally, the affected animal has signs of colic. This syndrome is sometimes referred to as the cardiac form and is usually associated with virus strains of low virulence or is seen in vaccinated horses exposed to a heterologous type. Excess pericardial and pleural fluid may be found at autopsy.

Pathogenesis. The clinical signs of African horse sickness and bluetongue in sheep have many similarities, and it may be assumed that the pathogenesis is similar. After the bite of an infective arthropod, the virus replicates in the local lymph node before producing a transient primary

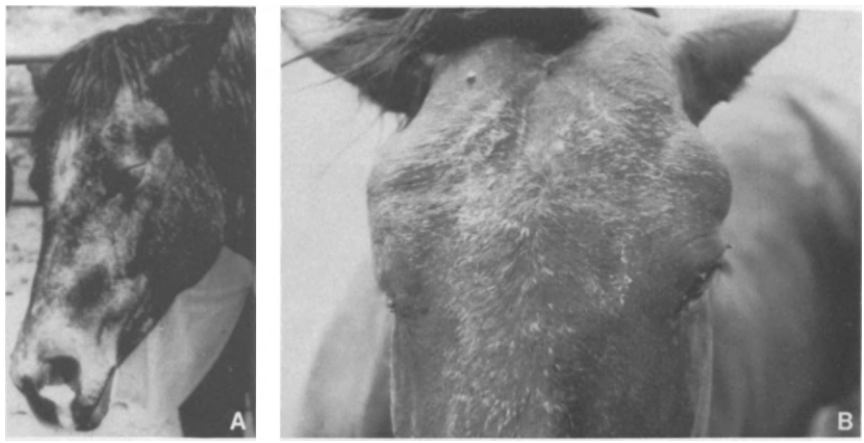

PLATE 32-3. *African horse sickness. (A) Respiratory form, with profuse frothy nasal discharge. (B) Characteristic edema of the supraorbital fossae and eyelids. (Courtesy Dr. B. Erasmus.)*

viremia, which leads to infection of other tissues and organs in the reticuloendothelial system and then a secondary viremia. As with bluetongue, the mechanisms by which the virus causes disease are unknown, but may involve vasculitis of small and medium-sized blood vessels.

Laboratory Diagnosis. African horse sickness is considered an exotic disease outside Africa; suspicion of the disease in any country outside Africa should be reported immediately to regulatory veterinary authorities. Clinical diagnosis of the pulmonary and cardiac forms is not difficult; the edema of the supraorbital fossa is characteristic of the disease. Excess pleural and pericardial fluid at postmortem provides a further reason to suspect the disease, especially in enzootic areas and in the appropriate season.

The virus is most easily isolated by intracerebral inoculation of 2- to 6-day-old mice with blood or spleen suspension, using washed cell fractions. The serotype of virus isolates is determined by serum neutralization assays in mice or cell cultures.

Epidemiology. African horse sickness is usually seasonal, occurring in the late summer on swampy low-lying farms and affecting especially horses that are not stabled at night. This indicates that crepusculid and night-flying insects are the vectors. The virus infects mosquitoes, but *Culicoides* spp. are thought to be the principal vectors.

The host range of African horse sickness virus includes zebra, but clinical disease in this species is unusual. Virus persists in the Gray's

zebra longer than in horses, suggesting that it may be the original reservoir host.

Despite the extensive epizootics of African horse sickness in North Africa, the Middle East, and the Indian subcontinent, the disease has always died out there. Possible explanations include the absence of a suitable reservoir mammalian host such as the zebra or lack of persistence of a vector temporarily introduced into these areas.

Prevention and Control. Attenuated live-virus vaccines have been used in South Africa for many years. The polyvalent vaccine containing all nine serotypes is generally unsatisfactory, since it fails to protect all horses and can cause neurological disease. However, no satisfactory inactivated vaccine is available.

Vigilance in monitoring the worldwide incidence of African horse sickness is important. The explosive epizootics of the disease outside continental Africa in the 1960s has demonstrated its invasive potential. Recognition that the related virus of bluetongue has established enzootic infection in the Americas, Asia, and Australia serves as a reminder that, contrary to previous experience in the Middle East and Mediterranean areas, African horse sickness virus could become enzootic if introduced to a new area such as the Americas.

Equine Encephalosis

Prior to 1967, African horse sickness virus was the only orbivirus known to cause clinical disease in horses. In that year, sporadic cases of peracute deaths, preceded by alternating periods of hyperexcitement and depression—hence the name encephalosis—occurred in horses in various parts of South Africa. At autopsy, general venous congestion, fatty liver degeneration, brain edema, and catarrhal enteritis were observed. Isolations of an orbivirus were made in BHK-21 cell cultures from various organs and blood collected from affected horses.

Serum neutralization tests indicated at least five serotypes; serological surveys have revealed a high incidence of infection with each of them. Further work is needed to define the origin and veterinary importance of the encephalosis viruses, which have been recognized only in South Africa.

DISEASES CAUSED BY ROTAVIRUSES

Rotaviruses are a major cause of diarrhea in intensively reared farm animals throughout the world. Rotaviruses infections range from subclinical, through enteritis of varying severity to death. The clinical signs,

diagnosis, and epidemiology of disease are similar in all species. Disease is usually seen only in young animals, 1–8 weeks old, but rarely during the first week after birth.

Clinical Features

Rotaviruses are a main cause of a widespread and common type of diarrhea in young animals (especially calves, piglets, foals, and lambs) referred to as "white scours" or "milk scours." The incubation period is 16–24 hours. Ingestion of a large volume of milk may be a contributory factor if there is a concurrent reduction in the production of lactase caused by rotavirus infection. Other factors, particularly reduced colostrum intake, but also pathogenic *E. coli*, poor hygiene, chilling, and overcrowding may contribute to the severity of the disease. Young animals may die as a result of dehydration or secondary bacterial infection but most recover within 3 or 4 days.

Pathogenesis

Rotaviruses infect the epithelial cells at the apices of the villi of the small intestine, causing atrophy (see Chapter 10). The villi become shortened and covered with cuboidal epithelial cells from the crypts, which have reduced levels of disaccharidases such as lactase, and impaired glucose-coupled sodium transport. Undigested lactose in the milk promotes bacterial growth and exerts an osmotic effect; both features exacerbate the damage to villi caused by the virus and lead to diarrhea.

Laboratory Diagnosis

Demonstration of the virus particles in feces by negative staining and electron microscopy is the most widely used diagnostic technique. Negative-contrast electron microscopy has several advantages for diagnosing rotavirus and other enteric infections. It detects a number of different viruses that are difficult to isolate and also demonstrates combined viral infections, but the major advantage is simplicity and speed: a diagnosis can be reached within 10 minutes of receipt of the sample in the laboratory. The main disadvantage is that a high concentration of viral particles is required (at least 10^5 per gram of feces), but this can be offset by using immunoelectron microscopy (see Chapter 13).

Not all diagnostic laboratories have immediate access to an electron microscope. A range of serological procedures for examination of feces employing antigen-capture techniques, such as the ELISA, are available.

Rotavirus infection can also be diagnosed rapidly and simply by polyacrylamide gel electrophoresis, which separates the dsRNA genome segments. The method utilizes silver staining of subnanogram amounts of nucleic acid in feces and is as sensitive as electron microscopy and ELISA.

Bovine, porcine, and avian rotaviruses are not cytopathogenic for cultured cells initially, but can be serially passaged if grown in the presence of low concentrations of trypsin, which cleaves one of the proteins of the outer capsid, promoting uncoating in the cell.

Epidemiology

Rotaviruses are excreted in the feces of infected animals in high titer (10^{11} viral particles per gram); maximum shedding occurs on the third and fourth days. Rotaviruses survive in feces for several months so gross contamination of the rearing pens can build up, which explains why intensively reared animals are more commonly affected. Some rotaviruses are highly resistant to chlorination and can survive for long periods in water supplies, so that waterborne transmission is also possible. Rotavirus groups can be distinguished by serological tests and polyacrylamide gel electrophoretic analysis of genome segments, but meaningful correlations of such groupings with pathogenicity have yet to be developed.

Prevention and Control

Although the management of intensive rearing units can be improved to reduce the incidence of disease, there is little likelihood that improved hygiene alone will cumpletely control rotavirus infections. Local immunity of the small intestine is more important than circulating antibody in providing resistance to infection. In domesticated mammals, rotavirus antibodies present in the colostrum are particularly important in protecting neonatal animals. Although much of the colostral antibody enters the circulation, serum antibody levels are not critical for protection; far more important is the presence of antibody in the gut lumen. Ingestion of large volumes of colostrum for a short period gives protection for only 48 hours after suckling ceases, whereas continuous feeding of smaller amounts of colostrum can provide protection for as long as it is available. Inoculation of the dam with inactivated rotavirus vaccine promotes higher levels of antibody in the colostrum and milk and a longer period of antibody secretion, with a corresponding decrease in the incidence of disease in neonates.

Recovery in affected calves can be helped by feeding them water

instead of milk for 30 hours at ihe onset of diarrhea. Antibiotic cover to control the secondary bacterial diarrhea and oral electrolyte solutions containing glucose to offset dehydration may also be useful.

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CHAPTER 33

Birnaviridae

Infectious bursal disease, also known as avian infectious bursitis, was recognized as a disease of chickens in 1957 and a virus was identified as the causal agent in 1962. The viral etiology of infectious pancreatic necrosis of fish was recognized in 1960. In 1973 it was noted that these two viruses had a similar and distinctive morphology. Their allocation to a new family was clinched by the recognition in the early 1970s that the genome of each consisted of two pieces of dsRNA, but it was not until 1984 that this family was officially designated *Birnaviridae*. Other members of the family affect insects and molluscs.

Infectious bursal disease occurs worldwide, and few commercial flocks are free of the virus. It is of considerable economic importance and is of scientific interest because of the nature of the virus and its special affinity for pre-B lymphocytes of the bursa of Fabricius, leading to acquired B cell deficiency in affected birds.

PROPERTIES OF BIRNAVIRUSES

The nonenveloped, icosahedral virions are 60 nm in diameter, with 32 capsomers (Plate 33-1). They are relatively heat stable, and their infectivity is resistant to exposure at pH 3 and to ether and chloroform. There

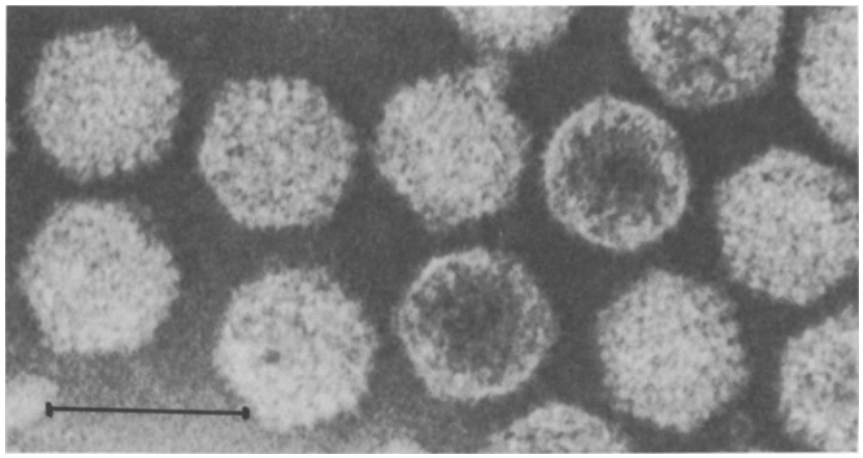

PLATE 33-1. *Negatively stained virions of infectious bursal disease virus (bar = 100 nm). (Courtesy Dr. E. L. Palmer.)*

are four structural polypeptides, none of which is glycosylated, and probably a virion-associated transcriptase, although this has not yet been demonstrated. The genome consists of two segments of dsRNA, 3.3 and 3.8 kbp, respectively (Table 33-1).

There are two serotypes (I and II) of infectious bursal disease virus, which show minimal cross-protection, and three serotypes of infectious pancreatic necrosis virus.

VIRAL REPLICATION

Infectious bursal disease virus replicates in both chicken and mammalian cells; infectious pancreatic necrosis virus replicates in fish cell lines incubated below 24°C. Both produce cytopathic effect 3–4 days after inoculation.

TABLE 33-1
Properties of Birnaviruses

Nonenveloped icosahedral virion, 32 capsomers, 60 nm diameter
dsRNA genome, segmented, two molecules (3.3 and 3.8 kbp)
Cytoplasmic replication
Occur in chickens (infectious bursal disease virus), fish (infectious pancreatic necrosis virus), molluscs, and insects

Birnaviruses replicate in the cytoplasm, without greatly depressing cellular RNA or protein synthesis. Few details are available concerning the molecular biology of replication, but it is presumed that mRNA is transcribed by a viral transcriptase. One of the two major capsid polypeptides cannot be found in infected cells; it is produced during viral maturation by cleavage of a larger precursor protein. The latter is found in trace quantities in normal (infectious) virions but is a major structural component of defective interfering particles.

INFECTIOUS BURSAL DISEASE OF CHICKENS

Clinical Features

When infectious bursal disease virus is newly introduced into a flock, morbidity approaches 100% and mortality may reach 30%. Disease is most severe in chicks 3–5 weeks old; neither laying hens nor chicks 1–14 days old (which are protected by maternal antibody) show signs of disease, although they produce antibodies to the virus. After an incubation period of 2 or 3 days, chicks show depression, ruffled feathers, anorexia, diarrhea, trembling, and dehydration, and 20–30% die. The clinical disease lasts for 3 or 4 days, then surviving birds recover rapidly.

Naturally occurring strains of infectious bursal disease virus vary in virulence, and an attenuated live-virus vaccine has been developed by growth of the virus in Vero cells. Infectious bursal disease virus serotype II causes inapparent infections in chickens and turkey poults.

Pathogenesis and Immunity

The most striking feature of the pathogenesis and pathology is the selective replication of infectious bursal disease virus in the bursa Fabricius, which is enlarged up to five times its normal size, edematous, hyperemic, and cream-colored, with prominent longitudinal striations (Plate 33-2B). Hemorrhages occur beneath the serosa and there are necrotic foci throughout the bursal parenchyma. At the time of death the bursa may be atrophied and gray and the kidneys are usually enlarged, with accumulation of urates due to the dehydration and possibly with immune complexes in the glomeruli. Histological findings include severe depletion of lymphocytes from the bursal follicles. B lymphocyte lysis also occurs, to a lesser extent, in other lymphoid tissues such as the spleen.

Following oral infection virus replicates in gut-associated macrophages and lymphoid cells from which it enters the portal circulation,

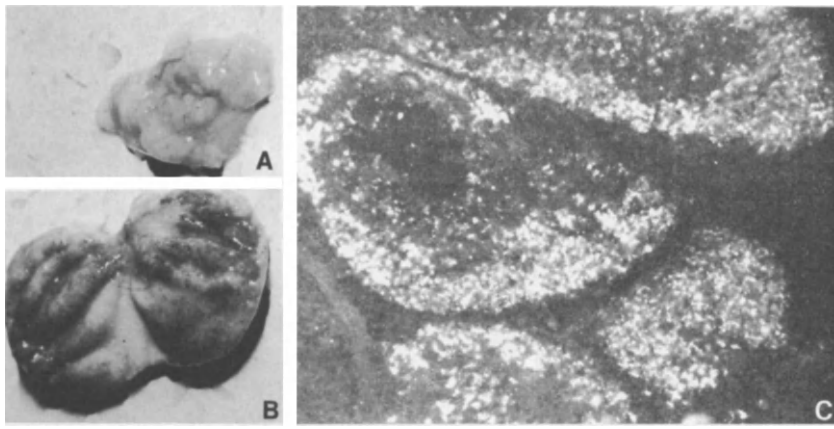

PLATE 33-2. *Infectious bursal disease. (A) Normal bursa of Fabricius. (B) Enlarged, hemorrhagic bursa of a diseased chick. (C) Specific immunofluorescence in the follicles of the bursa of Fabricius in a chick infected with infectious bursal disease virus 24 hours earlier. (C, Courtesy Dr. H. Becht.)*

leading to primary viremia. Within 22 hours of infection, viral antigen is detectable within the bursal lymphoid cells, but not in lymphoid cells of other tissues. Large amounts of virus released from the bursa produce a secondary viremia resulting in localization in other tissues. Chicks that are surgically bursectomized before infection with a normally lethal dose of virus exhibit no clinical signs but produce high levels of neutralizing antibody, whereas nonbursectomized chicks die within 3 or 4 days. Recovered birds develop high levels of antibody to infectious bursal disease virus because their mature peripheral B lymphocytes are still functional. *In vitro* studies confirm the results of fluorescent-antibody staining of tissues of affected chicks (Plate 33-2C). Virus replicates to a high titer in suspensions of pre-B lymphocytes from the bursa, but poorly in lymphocyte suspensions from spleen, lymph nodes, or thymus.

The predilection of the virus for bursal lymphocytes leads to an important immunopathological result in birds that recover from the infection. The occurrence of what has been called "viral bursectomy" results in a diminished antibody response and increased susceptibility to a wide range of infectious agents, including several such as *Salmonella* spp. and *E. coli* which are not highly pathogenic in normal chicks. The immunosuppression leads to a variety of intercurrent infections which are most obvious in the weeks immediately following recovery from infection with the virus. There is a correlation between the variety and sever-

ity of intercurrent infections and the age of the bird; the younger birds are at the time of infection (within the period of high susceptibility between 3 and 6 weeks of age), the wider the range of intercurrent infections. In addition, the immunosuppression leads to diminished antibody production after vaccination. Aplastic anemia has been recognized in birds that have recovered from infectious bursal disease.

Laboratory Diagnosis

Immunofluorescence of impression smears of bursal tissue, gel diffusion tests with infected bursal tissue as the antigen, electron microscopy of bursal specimens, and viral isolation in embryonated eggs are all useful in confirming the clinical diagnosis. The presence of virus or viral antigen can be detected in bursal tissue by immunofluorescence for 3 to 4 days after infection, for 5 to 6 days by immunodiffusion, and for up to 14 days by viral isolation.

Epidemiology

Infectious bursal dissease virus is excreted in the feces for 2 to 14 days. It is highly contagious, and transmission occurs directly through contact and oral uptake. The virus is extremely stable, and persists months in pens and for weeks in feed. The usual cleaning and disinfection measures often do not lead to its elimination from contaminated premises, hence indirect transmission via contaminated feed, water, dust, litter, and clothing or mechanical spread through insects further spread the virus. Vertical transmission probably occurs via the egg.

The disease is most severe when the virus is introduced into a "clean" flock. If the disease then becomes enzootic, the course is much milder and spread occurs more slowly.

Control

No fully satisfactory regimen of vaccination is yet available. Currently available live-virus vaccines are not sufficiently attenuated for use in the vaccination of chicks, so breeding stock is vaccinated by adding vaccine virus to drinking water, in the hope that passively transferred maternal antibody will prevent infection of the newly hatched chicks at the time of their maximum susceptibility. An increasingly common practice is to follow oral live-virus vaccination of breeding stock, after they have reached the age of about 18 weeks, with an injection of inactivated vaccine in oil adjuvant just before they begin laying. Vaccination is repeated a year later. This results in a well-maintained high level of

neutralizing antibody throughout the laying life of the birds. Maternal antibody provides effective protection for chicks for between 4 and 7 weeks after hatching.

INFECTIOUS PANCREATIC NECROSIS VIRUS OF FISH

Infectious pancreatic necrosis is a highly contagious and lethal disease of salmonid fish reared in hatcheries. The virus produces a subclinical infection in pike, carp, and barbels. First recognized in North America in 1941, it now occurs in many countries, probably because of worldwide shipment of eggs and live fish.

Disease is usually observed in trout fingerlings shortly after they commence to feed. With increasing age, the infection becomes subclinical. Affected fish are dark in color, with a swollen abdomen, exophthalmos, and cutaneous hemorrhages, and are described as frantically whirling on their long axis and then lying quietly on the bottom. The mortality varies between 10 and 90%. Histologically, pancreatic necrosis involving both ascinar and islet cells is a constant finding.

Surviving fish become lifelong carriers of the virus, which they shed in feces, eggs, and sperm. Since no effective vaccine has been developed, efforts at control are based on hygiene, water disinfection, and, if an outbreak occurs, complete destocking.

Because international commerce in live fish and eggs is an important mode of spread of infection, the Code Zoosanitaire International has established guidelines for export which specify freedom from clinical disease or pathological changes in the farm of origin for at least 12 months, and negative results from attempts to isolate infectious pancreatic necrosis virus from pond water, eggs, sperm, and fish.

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CHAPTER 34

Other Viruses

In the foregoing 17 chapters we have described diseases of veterinary importance caused by viruses belonging to 18 families. There are a few other families of viruses no members of which have as yet been incriminated as the cause of diseases of veterinary importance, and a few other viruses and viruslike infectious agents whose taxonomic status is as yet uncertain. These are briefly described in this chapter.

HEPADNAVIRIDAE

Although the prototype virus of this family, hepatitis B virus, is of major importance in human medicine, these viruses are of limited veterinary importance. However, closely related viruses have been discovered in Pekin ducks, woodchucks (*Marmata monax*), and ground squirrels (*Spermophilus beecheyi*), and there is a suspicion that a form of hepatitis in dogs that is associated with hepatocellular carcinoma may be caused by a hepadnavirus. Although no hepadnavirus has yet been

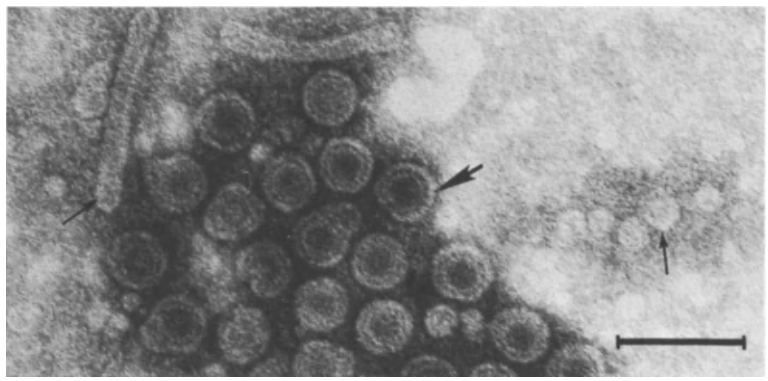

PLATE 34-1. Hepadnaviridae. *Negatively stained preparation of hepatitis B virions (Dane particles, large arrow) and accompanying HBsAg particles (small arrows) (bar = 100 nm). (Courtesy Dr. I. D. Gust and J. Marshall.)*

propagated in tissue culture, much is known of their structure and something of their mode of replication.

The icosahedral virion of hepatitis B virus, sometimes called the Dane particle, is 43 nm in diameter and is composed of two concentric layers: an outer capsid and an inner core (Plate 34-1). The virions of the animal hepadnaviruses are slightly larger (47 nm) and those of duck hepatitis virus more pleomorphic.

The genome consists of a molecule of circular, partially double-stranded DNA. The complete strand is 3.2 kb in size; the other strand varies between 1.7 and 2.8 kb, leaving 15–50% of the molecule single-stranded. This gap is repaired *in situ* in the virion by a DNA polymerase carried in the core. The long strand contains a discontinuity ("nick") at a unique site, and has a protein molecule covalently attached to its 5′ end. Table 34-1 summarizes the properties of hepadnaviruses.

TABLE 34-1
Properties of Hepadnaviruses

Spherical virion, 42–47 nm
Outer icosahedral shell and inner core
Partially double-stranded DNA genome; circular; (−) sense strand 3.2 kb, nicked; (+) sense strand variable size, but smaller
DNA polymerase, protein kinase, and reverse transcriptase in virion
DNA replicates via an RNA intermediate
Integration of viral DNA into host cell DNA occurs, but is probably not necessary for replication

The capsomers of the outer capsid of the hepatitis B virion are dimers consisting of one molecule each of the glycosylated and nonglycosylated forms of the major polypeptide of the virion. This is known as the hepatitis B surface antigen (HBsAg). The core of the virion is made up of an antigenically distinct protein (HBcAg), which is phosphorylated by a protein kinase present in the core. A third antigen, also detectable in the core, is known as HBeAg; it shares at least part of the amino acid sequence of HbcAg and is thouglt to be a monomeric form or breakdown product of HBcAg. Each of these protein antigens is used in diagnostic tests and in tests to judge the status of human patients in regard to persistent virus carriage and chronic disease potential.

Virions present in the serum of chronic carriers of the hepadnaviruses of humans and animals are accompanied by a large excess—up to 10^{13} particles per milliliter—of smaller, noninfectious particles of HBsAg (Plate 34-1). These are spheres or filiments, 22 nm in diameter, consisting solely of HBsAg that makes up the outer capsid of the virion (together with some adsorbed albumin and other serum proteins). Though noninfectious, HBsAg particles are important for two reasons: (1) they serve as a diagnostic marker of infection, and (2) they can be harvested for use as a vaccine for human hepatitis B.

Studies of livers infected with the duck hepadnavirus suggest that these viruses have a unique mode of replication. The complete (−) sense viral DNA strand appears to be transcribed to give a full-length (+) sense RNA copy. A reverse transcriptase, probably utilizing a protein primer, then transcribes a (−) sense DNA strand from the (+) sense RNA template, the latter being simultaneously degraded. The viral DNA polymerase then utilizes the (−) DNA strand as a template for the transcription of (+) sense DNA. Newly synthesized DNA is packaged into virions before this last step is complete, so viral DNA is only partially double-stranded. Integration of viral DNA into cellular DNA is a regular occurrence and may lead to persistent infection and sometimes to primary liver cancer, in humans, ducks, and woodchucks.

The hepadnaviruses were once thought to affect liver cells only, but high concentrations of hepadnavirus DNA have been found in the pancreatic cells of infected ducks, and viral antigens have been found in bone marrow and pancreatic cells of human patients. Hepatitis B in humans causes acute hepatitis, chronic hepatitis, liver cirrhosis, and primary hepatocellular carcinoma. Hepatic carcinoma is found in hepadnavirus-infected Pekin ducks; infected woodchucks develop acute hepatitis and, commonly, hepatic carcinoma, but not cirrhosis. Infected ground squirrels rarely exhibit hepatitis.

ARENAVIRIDAE

The prototype arenavirus, lymphocytic choriomeningitis virus, which produces a clinically inapparent lifelong infection in mice, has been known for over 50 years and has provided an important model for studies of persistent infections (see Chapter 11) and acquired immunological tolerance. More recently, three arenaviruses have been discovered in Africa and nine of the Americas, mainly in South America.

The family *Arenaviridae* derives its name from the presence within virions of host ribosomes, which in electron micrographs resemble grains of sand (Plate 34-2B). The ribosomes are not functional but are incorporated into virions coincidentally during budding. Arenaviruses are pleomorphic, 110–130 nm (rarely up to 300 nm) in diameter, and are composed of a lipid bilayer envelope covered with glycoprotein peplomers surrounding two fine helical circular nucleocapsids. The genome comprises two linear segments of ssRNA, 7.8 and 4.2 kb in size, each formed into a circle by hydrogen bonding of the termini. Most of the genome is of (−) sense, but the 5′ half of the smaller genome segment is of (+) sense: the term ambisense has been coined to describe this and the similar genome of bunyaviruses (see Chapters 4 and 29). Arenaviruses replicate to high titer in cell cultures, e.g., in the E-6 cloned line of Vero (African green monkey) cells. The viruses replicate in the cytoplasm and mature by budding from the plasma membrane.

Each arenavirus is maintained in nature by one or two rodent species

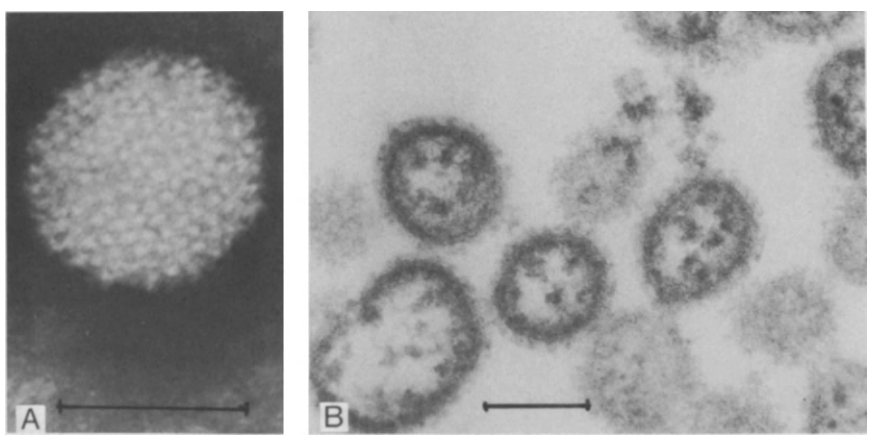

PLATE 34-2. Arenaviridae. *(A) Negative stain, Tacaribe virus. (B) Thin section of cell infected with Lassa virus (bars = 100 nm).*

in which persistent infection and chronic virus shedding in urine and oral secretions accounts for transmission to new generations, transmission to humans, and, in the case of lymphocytic choriomeningitis virus, transmission to colonies of laboratory rodents. Despite the fact that the infections they cause in their reservoir host species are subclinical, 4 of the 12 known arenaviruses are pathogenic for humans: Lassa virus (the etiological agent of Lassa fever), Machupo virus (Bolivian hemorrhagic fever), Junin virus (Argentine hemorrhagic fever), and lymphocytic choriomeningitis virus. In each case the natural history of the human disease is determined by the pathogenicity of the virus, the geographical distribution and habits and habitat of the rodent reservoir host, and the nature of the human–rodent interaction. In general, these factors limit the incidence of human disease episodes, but because of their serious nature such episodes can be of great concern. The diseases in humans caused by Lassa, Machupo, and Junin viruses start with fever, headache, malaise, myalgia, and pharyngitis, progressing in some patients to frank hepatitis, interstitial hemorrhages in many sites, hypotension, pulmonary edema, shock, and death.

Lymphocytic choriomeningitis virus is widely distributed throughout Europe and the Americas in the common house mouse (*Mus musculus*). The virus may be present in other parts of the world, but confirmatory evidence is lacking. In regions where the virus is known to exist, it is highly focal in mouse populations. For this reason the distribution of human cases is also focal; in addition, the distribution of human cases is seasonal, probably because mice move into closer contact with humans in houses and barns in cold weather. The same circumstances introduce lymphocytic choriomeningitis virus into laboratory and commercial mouse and hamster colonies. Such silent introductions of the virus have often caused significant problems, including compromised diagnostic reagents, failed research protocols, and clinical disease in laboratory and animal care personnel. Nevertheless, in many colonies in many countries wild mouse entry is still not effectively prevented.

In the United States and Europe, lymphocytic choriomeningitis virus has also been introduced sporadically into commercial hamster colonies; the increasing popularity of hamsters as pets has resulted in many human disease episodes, some involving hundreds of cases. Human infection with lymphocytic choriomeningitis virus manifests as any of three syndromes: (1) most commonly as an influenzalike illness with fever, headache, myalgia, and malaise, (2) less often as an aseptic meningitis with severe headache, papilledema, and elevated cerebrospinal fluid pressure, or, (3) rarely, as an encephalomyelitis with depression, coma, and disturbed nerve function.

Past infection in humans is usually diagnosed serologically, by indirect immunofluorescence, using inactivated-cell culture "spotslides" as antigen subsltrate. Such tests may be set up to measure IgM antibody so as to indicate recent infection. Lymphocytic choriomeningitis in rodent colonies is diagnosed serologically, with confirmation by virus isolation in cell culture. Lassa, Machupo, and Junin viruses are classified as biosafety level 4 pathogens, and must be handled only in maximum security laboratories. Lymphocytic choriomeningitis virus isolates from nature, including from laboratory rodent colonies, also require adequate containment facilities, usually at biosafety level 3. Specimen transport must be arranged in keeping with national and international regulations.

FILOVIRIDAE

In 1967 a previously unknown hemorrhagic fever occurred in Germany and Yugoslavia among laboratory workers and veterinarians engaged in processing kidneys from African green monkeys (*Cercopithecus aethiops*) that had been imported from Uganda for poliovirus vaccine production; there were seven deaths among 31 cases. Many of the monkeys from the same shipments died of a similar hemorrhagic disease. A virus isolated from patients, named Marburg virus, was found to be morphologically unique, antigenically unrelated to any known human or animal virus, and uniformly lethal when inoculated into monkeys. In 1976, epidemics of hemorrhagic fever occurred in Zaire and the Sudan; there were more than 500 cases and 430 deaths. A virus, named Ebola virus, was isolated and found to be morphologically identical but antigenically completely distinct from Marburg virus. Recently, the two viruses have been placed in a new family, *Filoviridae.*

Since the initial outbreaks, sporadic human cases of Marburg and Ebola hemorrhagic fever have been recognized in eastern and southern Africa. Investigations failed to identify the source of the virus in these episodes, but serosurveys revealed antibodies in a small percentage of people living in certain rural areas in several African countries. In parallel serosurveys, no antibodies have been found in animals, including monkeys, with the exception that Ebola antibodies have been found sporadically in domesticated guinea pigs raised for food in central Africa. Although it is presumed that Marburg and Ebola viruses are zoonotic, transmitted to humans from an ongoing life cycle in animals (and/or arthropods), there is no indication that guinea pigs are a reservoir host; rather, it is thought that both guinea pigs, monkeys, and

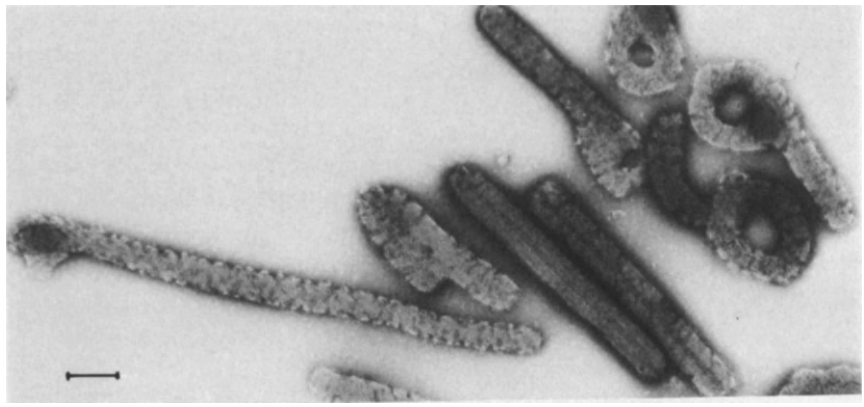

PLATE 34-3. Filoviridae. *Negatively stained preparation of virions of Ebola virus (bar = 100 nm).*

humans are infected incidentally from a still-unknown reservoir host cycle. The large outbreaks of Ebola virus infection in the Sudan and Zaire were due to nosocomial infections, enhanced by the use of contaminated needles and syringes.

In order to avoid risks associated with the importation of monkeys, most countries have improved and extended quarantine procedures. In this context it is relevant that wild-caught monkeys are no longer being widely used for vaccine production because of export prohibitions established by source countries for conservation purposes.

The filoviruses are surely the most bizarre of all viruses; they appear as very long filamentous rods or as more compact convoluted forms, each composed of a lipid bilayer envelope covered with peplomers surrounding a helically wound nucleocapsid (Plate 34-3). The viruses are 80 nm in diameter and have a median length of 800 to 1000 nm, but particles as long as 14,000 nm have been seen. The genome is a single molecule of (−) sense ssRNA, 12.7 kb in size. Marburg and Ebola viruses are distinguishable by small differences in genome size and protein profile, as well as by the absence of antigenic cross-reactivity. The viruses replicate well in cell cultures, e.g., in Vero (African green monkey) cells, as well as in guinea pigs, hamsters, and monkeys. Viral replication in the cytoplasm of host cells is marked by the formation of large inclusion bodies, and maturation occurs by budding from the plasma membrane.

Clinical features of Marburg and Ebola hemorrhagic fevers are similar in humans and monkeys; there is fever, subcutaneous and intestinal hemorrhage, intractable diarrhea, severe pharyngitis and conjunctivitis,

necrotizing hepatitis, prostration, shock, and, in 60 to 90% of human cases and virtually 100% of monkeys, death in 6 to 9 days.

Because of the extreme danger involved in all diagnostic procedures, including necropsy, great care must be taken when these viruses are suspected as the cause of disease, either in African monkeys or in humans who have traveled in central Africa within the previous 10 days and have been exposed to monkeys. Consultation and laboratory diagnosis is available from the maximum containment laboratories that operate in several countries. Specimen transport must be arranged to conform with national and international regulations.

ASTROVIRUSES

Astroviruses were first described in 1975, when they were found by electron microscopy in the feces of children with diarrhea. They have now been identified in the feces of calves, lambs, piglets, and kittens, often as dual infections with other viruses such as rotaviruses. Their distinctive stellate morphology (Plate 34-4) led to the claim that they constituted a new viral group. Subsequent studies showed that their genome resembled that of the picornaviruses, but their taxonomic status remains undetermined.

They can be cultivated in primary embryonic kidney cell cultures if trypsin is added to the growth medium, but are noncytopathogenic.

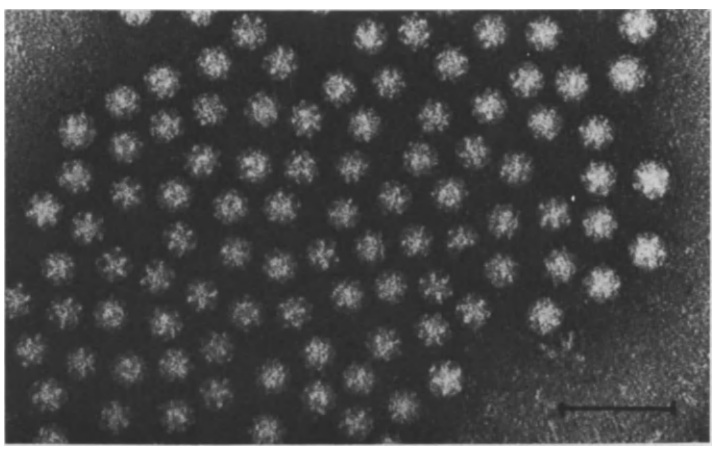

PLATE 34-4. *Astrovirus. Negatively stained preparation (bar = 100 nm). [From D. R. Snodgrass and E. W. Gray,* Arch. Virol. **55,** *287 (1977); courtesy Dr. D. R. Snodgrass.]*

There are no antigenic cross-reactions between isolates from different animal species.

The astroviruses mainly affect the villi of the small intestine, where they replicate in the mature epithelial cells of the apical half of the villus and also the subepithelial macrophages, producing partial villus atrophy. Lactase levels in the small intestine are reduced. Apart from abnormally colored and soft feces, most animals remain normal; few clinical signs are seen in experimentally infected gnotobiotic animals, hence astroviruses, though often associated with diarrhea, are considered relatively unimportant pathogens.

TOROVIRUSES

The name *Toroviridae* has been proposed fur a group of previously unclassified RNA viruses. The prototype members are: (1) Berne virus, recovered in 1972 from a horse in Switzerland, and shown by a serological survey to occur among horses and cattle in that country, and (2) Breda virus, recovered from the feces of diarrheic calves in the United States. Serum neutralization tests have revealed antibodies in ungulates, lagomorphs, and wild rodents, and typical virions have been seen in stools of human beings with gastroenteritis. The Berne virus grows in secondary horse kidney cells.

The morphology of the Berne and Breda viruses is similar and characteristic. The virions are enveloped and contain an elongated nucleocapsid of presumed helical symmetry. The capsid may be bent into an open torus, conferring a disk- or kidney-shaped morphology to the virion (largest diameter, 120–140 nm) or straight, resulting in a rod-shaped particle (35 × 170 nm) (Plate 34-5). The genome consists of a single molecule of (+) sense ssRNA, 20.6 kb in size. The lipoprotein membrane carries glycoprotein peplomers, which are responsible for hemagglutinating activity. Major structural proteins of 22K and 20K have been identified, the latter occurring in the capsid. Replication is dependent on some nuclear function of the host cell.

SUBACUTE SPONGIFORM ENCEPHALOPATHIES

Scrapie is the prototype of a group of diseases called the "subacute spongiform encephalopathies," which includes the human diseases kuru and Creutzfeld–Jakob disease, mink encephalopathy, and "wasting disease" of mule deer and elk.

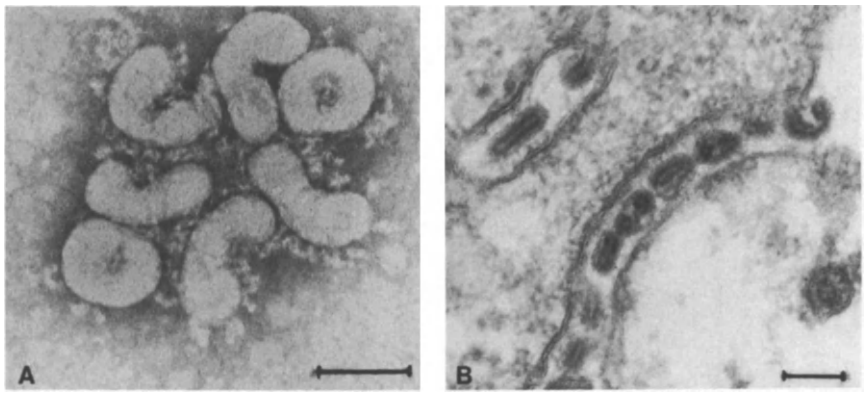

PLATE 34-5. *Torovirus. (A) Negatively stained preparation of virions of Berne virus, showing characteristic morphology. (B) Thin section showing accumulation of virions at a membrane in the Golgi complex (bars = 100 nm). (Courtesy Dr. M. Weiss.)*

Scrapie

Scrapie is a chronic fatal ataxic disease of sheep and occasionally of goats, which is widely distributed in Europe and North America and occurs in a few countries in Africa and Asia. Affected sheep scrape on posts and other objects to relieve the intense pruritus associated with the disease, hence the name. It has been known for some 50 years that the disease was transmissible from sheep to sheep, with an incubation period of several years, but research on the nature of the etiological agent lagged until it was shown about 25 years ago that it could be transmitted to mice. Even so, the very long incubation period greatly hampers research.

What makes scrapie and the other subacute spongiform encephalopathies different from all other infectious diseases is the nature of the infectious agent. Filtration suggests a diameter of 30 to 50 nm for the infective particles; yet electron microscopy of fractions of high infectivity obtained from scrapie-infected brains (10^7–10^8 LD_{50} per milliliter) reveal no structures resembling virions. Further, the infectivity associated with such preparations shows an unusual resistance to inactivation by heating and by UV irradiation. Initially, analogies were sought with the "viroids" found in certain infectious diseases of plants, which have been shown to be small circular RNA molecules with no structural protein. However, the infectious agent of scrapie differs in many respects from viriods, not least in the fact that all attempts to demonstrate a nonhost nucleic acid by DNA homology, transfection, and nuclease inactivation have been negative.

The key unresolved question is whether the scrapie agent contains a small nucleic acid moiety, not yet identified. One view is that the demonstrated hydrophobicity of the scrapie particle, its very strong tendency to aggregate and to associate with fragments of plasma membrane, and the inaccuracy of available assays, which depend on the production of disease after long incubation periods, tend to obscure the fact that the infectious agent is indeed a very small but otherwise conventional virus; the very small target size measured by radiation inactivation and other methods of inactivation may reflect a highly resistant, highly aggregated fraction. Another view (the "virino" model) is that the nucleic acid is too small to code for any protein but has a regulatory function and that the agent's protein component is host-derived. A third view is that the scrapie-associated fibrils found in infected tissues represent aggregated "infectious protein," for which the name "prions" has been suggested. Needless to say, the notion of infectivity and specificity in the absence of nucleic acid is highly heretical.

The incubation period of scrapie in naturally infected sheep is 1–4 years. The earliest clinical sign is pruritus, manifested by rubbing the affected parts against objects and biting the flanks. Tremors of the muscles, which may be elicited by rubbing the skin over the flanks, led to the French name for the disease: "tremblant du mouton." Later motor disturbances develop, with a weaving gait, staring eyes, and eventually

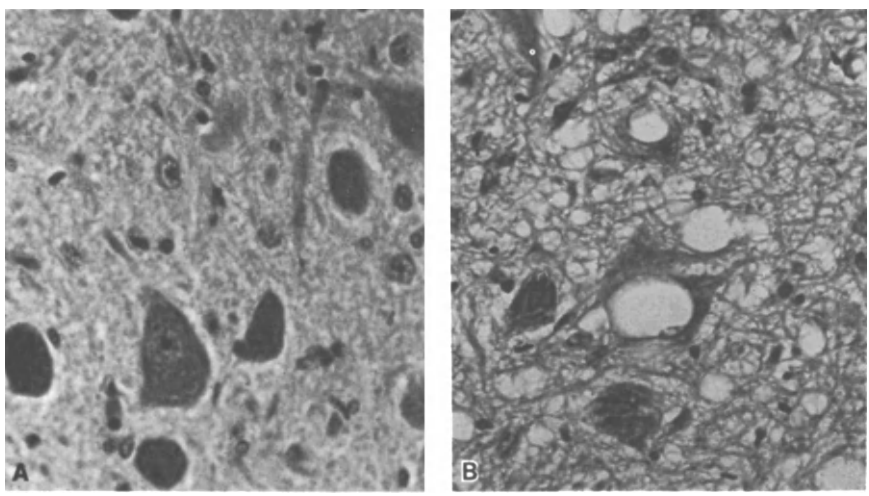

PLATE 34-6. *Scrapie. (A) Section of brain of a normal sheep. (B) Section of brain of sheep that had died of scrapie. Note extensive spongiform changes, but no evidence of inflammatory reaction or immune response (H and E stain; ×290). (Courtesy J. D. Foster.)*

hindquarter paralysis. There is never any fever, but affected sheep rapidly lose weight and die, usually within 4 to 6 weeks of developing signs. There is a strong genetic element in the susceptibility of sheep, but not among goats.

The infectious agent has been transmitted to mice, hamsters, ferrets, and monkeys; most experimental study is based on the use of mice, in which there is genetic control of the length of the incubation period and the distribution of lesions, which is also dependent on the strain of scrapie agent used. The only pathological lesions occur in the central nervous system, in which there is hypertrophy of astrocytes, vacuolation of the neurons, and neuronal degeneration, but a complete absence of any signs of an inflammatory reaction or an immune response (Plate 34-6B).

The mechanism of spread among sheep remains uncertain. The agent may spread horizontally from naturally infected sheep to uninfected sheep or goats, but this has not been observed among experimentally infected animals. Experimentally, all susceptible species have been infected by the oral route. The scrapie agent appears to pass from ewes to lambs even without suckling, but it is not yet certain whether this is due to transplacental or perinatal infection. The first appearance of the agent in naturally infected lambs occurs in the tonsils and suprapharyngeal and intestinal lymph nodes, suggesting infection by the oral route. Susceptible sheep have developed the disease in pastures previously occupied by scrapie-affected sheep.

In Europe, where the disease has long existed, no rational control measures are taken. Successful efforts have been made to exclude the disease from countries with large sheep populations (Australia, New Zealand) by strict control of importations and slaughter of any imported animals that may develop disease during a long period of quarantine. The disease was first recognized in the United States in 1947 and subsequently in 1952, all introductions being traced to Canada and before that to importations from the United Kingdom. Attempts have been made to eradicate the disease from the United States, infected flocks being slaughtered and the owners indemnified from public funds. However, the long incubation period of the disease and extensive interstate movement of sheep makes eradication problematic. Quarantine restrictions on importations from the United Kingdom have prevented the establishment of new foci.

Transmissible Mink Encephalopathy

This disease was first recognized on mink farms in Wisconsin in 1947, carcasses of scrapie-infected sheep having been fed to the mink. The

pathological signs and pathogenesis of the disease are similar to those of scrapie. There seems no doubt that it is due to infection of mink with the scrapie agent. It spreads between mink by fighting and cannibalism; transplacental or perinatal infection from the mother does not occur.

Wasting Disease of Deer and Elk

Workers in the United States have described a chronic wasting disease in mule deer and elk in Colorado and Wyoming, with behavioral changes and progressive weight loss over a period of weeks or months. The histopathological changes are identical with those seen in scrapie.

BORNA DISEASE

Borna disease is a rare progressive encephalopathy of horses, occasionally of sheep, that has been recognized for over a century and is characterized by incoordination, followed by paralysis and death. It occurs sporadically in Germany and more recently in Switzerland, but has not been positively diagnosed elsewhere.

Passage and filtration experiments in animals in 1927 established its viral etiology, but in spite of the fact that the infectious agent has been passaged by intracerebral inoculation in rabbits, rats, and other animals, and that it grows in a variety of cell cultures, viral particles have never been visualized and the nature of the virus remains obscure, although filtration experiments suggest a size between 85 and 125 nm. Infectivity can be passaged indefinitely by intracerebral inoculation of rabbits or rats; there is an incubation period of 3 to 4 weeks before encephalitis occurs, usually leading to death within 2 to 3 weeks. Rats sometimes exhibit chronic encephalitis associated with abnormal behavior, which is due to immunopathological targeting of cells in the limbic system and retinal neurons.

In cultured cells the infectious agent produces a persistent, slowly progressive infection, the virus being noncytopathogenic and remaining cell-associated. Specific staining of infected cells by immunofluorescence is used for diagnosis, and specific immunoglobulins can be found in the serum and cerebrospinal fluid of affected horses.

Unlike other forms of viral encephalitis in horses, the incubation period is relatively long (3–4 weeks) and the natural disease shows no seasonal incidence. Both the signs and the course of disease in horses vary from one case to another. Usually low fever is followed by difficulty in swallowing, hyperesthesia, and other signs of cerebral irritation, followed by lethargy and paralysis. Ninety percent of affected animals die 1–3 weeks after the onset of signs. Histopathological lesions include

perivarscular cuffing and degeneration of ganglion cells, as seen in other forms of viral encephalitis. Pathognomonic of Borna disease are round or oval intranuclear inclusion bodies in ganglionic neurons of the hippocampus and olfactory lobes, readily visualized after staining with Giemsa stain.

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CHAPTER 35

Viral Diseases by Domestic Animal Species

In the preceding chapters the contribution of individual viruses to the broad spectrum of veterinary disease has been examined in the context of the families of viruses. In this final chapter the opposite perspective is taken: the most important diseases and syndromes caused by viruses in each domestic animal species are listed together with their etiological associations. This listing is not meant to substitute for a textbook of veterinary medicine or infectious diseases, nor is it intended to be used for differential diagnosis, where viral and other etiologies must be considered together. The aim is to provide a "bird's eye" view of the commonest viral diseases and syndromes in each animal species. A practicable way of doing this in a concise form is to provide one or two tables for each major domestic animal species.

EXPLANATION OF THE TABLES

Such a tabulation inevitably involves oversimplification, but page numbers have been provided to direct the reader to appropriate pages for detailed coverage of each of these viral infections. Nevertheless, in these tables we have attempted to ascribe to each disease a measure of its importance (+ to ++++), as viewed from the perspective of veterinary medicine in countries with modern agricultural practices. When considering diseases that have been eradicated from these countries, we have incorporated into our assessment the relative risk of these exotic diseases should they be reintroduced. For example, foot-and-mouth disease, which is given a ++++ rating in cattle, is an important disease even in countries in which it does not currently occur, because of its potential impact on beef exports and the rapidity with which this virus can spread from herd to herd. In contrast, rinderpest, though one of the most important diseases of ruminants in countries where it has not been eradicated, is given only a ++ rating, because there is little chance of the virus being reintroduced in countries with modern veterinary services and, even if it were to be introduced in these countries, its eradication would present few difficulties. Had these tables been assembled from the perspective of the developing countries, then some diseases considered important in areas where intensive husbandry systems predominate would have been rated as inconsequential. For example, infectious bovine rhinotracheitis deserves a high rating in the intensive cattle production environment (dairy or beef) but is less important in developing countries.

There is an arbitrary element in relation to the group of diseases (generalized, respiratory, etc.) to which certain infections are allocated. For example, most generalized skin diseases result from generalized, bloodborne infection, but they have been listed as skin diseases. Where viruses cause generalized signs and also signs of particular importance in some system, or in the newborn, they have been entered in both categories. The other headings in the tables have been selected to draw attention to the classification of the causal virus and its persistence, the geographical distribution of the disease, and the availability of vaccines.

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Tables 35-1 through 35-11 follow.

TABLE 35-1
Generalized and Respiratory Diseases of Cattle

Disease	Importance	Virus family (genus or subfamily)	Number of serotypes	Geographical distribution	Vaccines	Persistence	Pages
Generalized Diseases (including central nervous system involvement)							
Bluetongue	++	*Reoviridae (Orbivirus)*	24	Tropics and subtropics; temperate areas of North America	Attenuated but used only in sheep	±	583
Bovine ephemeral fever	++	*Rhabdoviridae* (unnamed)	1	Africa, Asia, Australia	Inactivated and attenuated	−	544
Bovine leukemia	++	*Retroviridae (Oncovirinae)*	1	Worldwide; control and eradication programs in Europe	None	+	563
Bovine virus diarrhea	++	*Togaviridae (Pestivirus)*	1	Worldwide	Inactivated and attenuated	+	462
Foot-and-mouth disease	++++	*Picornaviridae (Aphthovirus)*	7	Eradicated in North and Central America, Australia, Japan;	Inactivated	+	427

				controlled in European countries; common elsewhere			
Malignant catarrhal fever	+	*Herpesviridae (?Gammaherpesvirinae)*	1	Worldwide	None	+	366
Rabies	+	*Rhabdoviridae (Lyssavirus)*	1	Central and South America	Inactivated and attenuated	+	534
Rift Valley fever	++	*Bunyaviridae (Phlebovirus)*	1	Africa	Attenuated and inactivated	−	524
Rinderpest	++	*Paramyxoviridae (Morbillivirus)*	1	Eradicated except in Africa and parts of Asia	Attenuated	−	496
Respiratory Diseases[a]							
Bovine respiratory syncytial virus infections	+++	*Paramyxoviridae (Pneumovirus)*	1	Worldwide	Attenuated	−	501
Infectious bovine rhino-tracheitis[b]	+++	*Herpesviridae (Alphaherpesvirinae)*	1	Worldwide	Attenuated	+	347
Malignant catarrhal fever	+	*Herpesviridae (?Gammaherpesvirinae)*	1	Worldwide	None	+	366
Parainfluenza-virus 3 infection	+	*Paramyxoviridae (Paramyxovirus)*	1	Worldwide	Inactivated and attenuated	−	492

[a]Rhinoviruses (p. 442) and adenoviruses (p. 330) have been associated with respiratory disease in cattle but are of little clinical importance.
[b]Caused by same virus (bovine herpesvirus 1) as infectious pustular vulvovaginitis.

TABLE 35-2

Diseases of Cattle Affecting the Intestinal Tract, Reproductive System, and Skin

Disease	Importance	Virus family (genus or subfamily)	Number of serotypes	Geographical distribution	Vaccines	Persistence	Pages
Enteric Diseases[a]							
Bovine coronavirus diarrhea	+	*Coronaviridae (Coronavirus)*	1	Worldwide	Inactivated, given to dam	–	508
Bovine rotavirus diarrhea	++	*Reoviridae (Rotavirus)*	Several	Worldwide	Attenuated, given to dam	–	590
Bovine virus diarrhea	++	*Togaviridae (Pestivirus)*	1	Worldwide	Inactivated and attenuated	+	462
Reproductive and Neonatal Diseases							
Akabane disease	+	*Bunyaviridae (Bunyavirus)*	1	Africa, Asia, Australia	Inactivated	–	527
Bluetongue	++	*Reoviridae (Orbivirus)*	24	Tropics, subtropics, and temperate areas of North America	Attenuated but used only in sheep	+	583
Bovine virus diarrhea	++	*Togaviridae (Pestivirus)*	1	Worldwide	Inactivated and attenuated	+	462
Infectious pustular	++	*Herpesviridae (Alphaherpesvirinae)*	1	Worldwide	Attenuated	+	347

vulvo-vaginitis[b]							
Skin Diseases (including stomatitis)							
Bovine herpes mammillitis and pseudo-lumpyskin disease	+	*Herpesviridae (Alphaherpesvirinae)*	1	Worldwide	None	+	351
Bovine papillomatosis	+	*Papovaviridae (Papillomavirus)*	6	Worldwide	Autogenous	+	325
Cowpox	+	*Poxviridae (Orthopoxvirus)*	1	Europe	None in use	−	393
Lumpyskin disease	+	*Poxviridae (Capripoxvirus)*	1	Africa	Attenuated	−	400
Mucosal disease[c]	++	*Togaviridae (Pestivirus)*	1	Worldwide	Inactivated and attenuated	+	462
Pseudocowpox	+	*Poxviridae (Parapoxvirus)*	1	Worldwide	None	−	396
Bovine papular stomatitis	+	*Poxviridae (Parapoxvirus)*	1	Worldwide	None	−	397
Vesicular stomatitis	+	*Rhabdoviridae (Vesiculovirus)*	7	Americas	Inactivated and attenuated	−	541

[a]Astroviruses (p. 608) and toroviruses (p. 609) have been associated with diarrhea in cattle but are of little clinical importance.
[b]Caused by the same virus as infectious bovine rhinotracheitis.
[c]Caused by the same virus as bovine virus diarrhea.

TABLE 35-3
Generalized and Respiratory Diseases of Sheep and Goats

Disease	Importance	Virus family (genus or subfamily)	Number of serotypes	Geographical distribution	Vaccines	Persistence	Pages
Generalized Diseases (including central nervous system involvement)							
Bluetongue	++	*Reoviridae (Orbivirus)*	24	Tropics, subtropics, and temperate areas of North America	Attenuated	±	583
Caprine arthritis–encephalomyelitis	++	*Retroviridae (Lentivirinae)*	1	Most countries	None	+	573
Foot-and-mouth disease	+	*Picornaviridae (Aphthovirus)*	7	Eradicated in North and Central America, Australia, Japan; controlled in European countries; common elsewhere	Inactivated	+	427

Goatpox	+	*Poxviridae (Capripoxvirus)*	1	Africa and Asia	Inactivated and attenuated	–	398
Louping ill	+	*Flaviviridae (Flavivirus)*	1	United Kingdom	Inactivated	–	469
Nairobi sheep disease		*Bunyaviridae (Nairovirus)*	1	Africa	None used	–	528
Peste des petits ruminants	++	*Paramyxoviridae (Morbillivirus)*	1	Africa and Middle East	Attenuated	–	498
Rift Valley fever	++	*Bunyaviridae (Phlebovirus)*	1	Africa	Attenuated and inactivated	–	524
Scrapie	+	Unclassified (spongiform encephalopathy)	Unknown	Most countries	None	+	610
Sheeppox	++	*Poxviridae (Capripoxvirus)*	1	Africa and Asia	Inactivated and attenuated	–	398
Respiratory Diseases[a]							
Ovine pulmonary adenomatosis	++	*Retroviridae* (? *Oncovirinae*)	1	Most countries	None	+	564
Parainfluenza-virus 3 infection	+	*Paramyxoviridae (Paramyxovirus)*	1	Worldwide	Inactivated and attenuated	–	492
Ovine progressive pneumonia (visna–maedi)	+	*Retroviridae (Lentivirinae)*	1	Most countries	None	+	571

[a]Adenoviruses (p. 330), and respiratory syncytial viruses (p. 501) have been associated with respiratory disease in sheep and goats but are of little clinical importance.

TABLE 35-4
Diseases of Sheep and Goats Affecting Intestinal Tract, Reproductive System, and Skin

Disease	Importance	Virus family (genus or subfamily)	Number of serotypes	Geographical distribution	Vaccines	Persistence	Pages
Enteric Diseases[a]							
Nairobi sheep disease[b]	+	*Bunyaviridae (Nairovirus)*	1	Africa	None in commercial use	–	528
Peste des petits ruminants[b]	++	*Paramyxoviridae (Morbillivirus)*	1	Africa and the Middle East	Attenuated	–	498
Reproductive and Neonatal Diseases							
Akabane disease	+	*Bunyaviridae (Bunyavirus)*	1	Africa, Asia, Australia	Inactivated	–	527
Bluetongue	++	*Reoviridae (Orbivirus)*	24	Tropics, subtropics, and temperate areas of North	Attenuated	±	583

				America			
Border disease[c]	+	*Togaviridae (Pestivirus)*	1	Worldwide	None in use	+	466
Rift Valley fever	++	*Bunyaviridae (Phlebovirus)*	1	Africa	Attenuated and inactivated	−	524
Wesselsbron disease	+	*Flaviviridae (Flavivirus)*	1	Africa	Attenuated	−	470
Skin Diseases							
Goatpox	+	*Poxviridae (Capripoxvirus)*	1	Africa and Asia	Inactivated and attenuated	−	398
Orf (contagious pustular dermatitis)	++	*(Poxviridae (Parapoxvirus)*	1	Worldwide	Wild-type virus, atypical site	−	397
Sheeppox	++	*Poxviridae (Capripoxvirus)*	1	Africa and Asia	Inactivated and attenuated	−	398

[a]Rotaviruses (p. 590), astroviruses (p. 608) and adenoviruses (p. 330) have been associated with enteric disease in sheep and goats but are of little clinical importance.

[b]Two generalized diseases which often present as enteric diseases.

[c]Caused by same virus as bovine virus diarrhea.

TABLE 35-5
Generalized and Respiratory Diseases of Swine

Disease	Importance	Virus family (genus or subfamily)	Number of serotypes	Geographical distribution	Vaccines	Persistence	Pages
Generalized Diseases (including central nervous system involvement)							
African swine fever	++++	Unclassified	Unknown	Sub-Saharan Africa, Spain, and Portugal	None	+	378
Foot-and-mouth disease	++++	*Picornaviridae (Aphthovirus)*	7	Eradicated in North and Central America, Australia, Japan; controlled in European countries, common elsewhere	Inactivated	–	427
Encephalomyocarditis	+	*Picornaviridae (Cardiovirus)*	1	Worldwide	None	–	441
Hog cholera	++++	*Togaviridae (Pestivirus)*	1	Eradicated in North and Central America, Australia, Japan; controlled in European	Attenuated	+	466

				countries, common elsewhere			
Porcine hemagglutinating virus encephalomyelitis	+	*Coronaviridae (Coronavirus)*	1	Worldwide	None	−	510
Porcine lymphosarcoma	+	*Retroviridae (Oncovirinae)*	1	Worldwide	None	+	565
Porcine polioencephalomyelitis	+	*Picornaviridae (Enterovirus)*	1	Worldwide	Inactivated, used in eastern Europe	−	438
Pseudorabies	+++	*Herpesviridae (Alphaherpesvirinae)*	1	Worldwide, except Australia and Japan	Inactivated and attenuated	+	353
Swine vesicular disease	+	*Picornaviridae (Enterovirus)*	1	Sporadic in Asia and Europe	None used	−	436
Vesicular exanthema of swine	+	*Caliciviridae (Calicivirus)*	Many	Eradicated in swine worldwide	None used	−	447
Vesicular stomatitis	+	*Rhabdoviridae (Vesiculovirus)*	7	Sporadic in the Americas	Inactivated	−	541
Respiratory Diseases							
Cytomegalic inclusion body disease of swine	+	*Herpesviridae (Betaherpesvirinae)*	1	Worldwide	None	+	365
Swine influenza	+	*Orthomyxoviridae (Influenzavirus)*	Several	Worldwide—sporadic	None in commercial use	−	478

TABLE 35-6
Diseases of Swine Affecting the Intestinal Tract, Reproductive System, and Skin

Disease	Importance	Virus family (genus or subfamily)	Number of serotypes	Geographical distribution	Vaccines	Persistence	Pages
Enteric Diseases							
Rotavirus gastroenteritis	+++	*Reoviridae (Rotavirus)*	Many	Worldwide	Attenuated, given to dam	–	590
Transmissible gastroenteritis	++	*Coronaviridae (Coronavirus)*	1	Worldwide	Attenuated, given to dam	+	509
Reproductive and Neonatal Diseases							
Hog cholera	++++	*Togaviridae (Pestivirus)*	1	Eradicated in North and Central America, Australia, Japan; controlled in European	Attenuated	+	466

				countries, common elsewhere			
Japanese encephalitis virus infection	++	*Flaviviridae (Flavivirus)*	1	Asia	Inactivated and attenuated	−	470
Parvovirus disease	++	*Parvoviridae (Parvovirus)*	1	Worldwide	Inactivated	+	411
Pseudorabies	+++	*Herpesviridae (Alphaherpesvirinae)*	1	Worldwide, except Australia and Japan	Inactivated and attenuated	+	353
Skin Diseases (including stomatitis)							
Swinepox	+	*Poxviridae (Suipoxvirus)*	1	Worldwide	None used	−	401
Swine vesicular disease	+	*Picornaviridae (Enterovirus)*	1	Sporadic in Asia and Europe	None used	−	436
Vesicular exanthema of swine	+	*Caliciviridae (Calicivirus)*	Many	Eradicated in swine worldwide	None used	−	447
Vesicular stomatitis	+	*Rhabdoviridae (Vesiculovirus)*	7	Sporadic in the Americas	Inactivated	−	541

TABLE 35-7
Diseases of Dogs

Disease	Importance	Virus family (genus or subfamily)	Number of serotypes	Geographical distribution	Vaccines	Persistence	Pages
Generalized Diseases (including central nervous system involvement)							
Canine distemper	++++	*Paramyxoviridae (Morbillivirus)*	1	Worldwide	Attenuated	+ (sometimes)	499
Canine parvovirus infection	++++	*Parvoviridae (Parvovirus)*	1	Worldwide	Attenuated and inactivated	+	415
Infectious canine hepatitis	++	*Adenoviridae (Mastadenovirus)*	1	Worldwide	Attenuated and inactivated	+	335
Rabies	+	*Rhabdoviridae (Lyssavirus)*	1	Worldwide except some island countries, Scandinavia, and Australia	Inactivated and attenuated	+	534

Respiratory Diseases							
Canine laryngo-tracheitis	++	*Adenoviridae (Mastadenovirus)*	1	Worldwide	Attenuated	−	334
Parainfluenza-virus 2 infection	+	*Paramyxoviridae (Paramyxovirus)*	1	Worldwide	None	−	491
Enteric Diseases							
Canine coronavirus infection	++	*Coronaviridae (Coronavirus)*	1	Worldwide	Inactivated	?	511
Canine parvovirus infection	++++	*Parvoviridae (Parvovirus)*	1	Worldwide	Attenuated and inactivated	+	415
Reproductive and Neonatal Diseases							
Hemorrhagic disease of pups	++	*Herpesviridae (Alphaherpesvirinae)*	1	Worldwide	None	+	359
Skin Diseases							
Canine papillomatosis	+	*Papovaviridae (Papillomavirus)*	Several	Worldwide	None	+	328

TABLE 35-8
Diseases of Cats

Disease	Importance	Virus family (genus or subfamily)	Number of serotypes	Geographical distribution	Vaccines	Persistence	Pages
Generalized Diseases (including central nervous system involvement)							
Feline infectious peritonitis	+++	*Coronaviridae (Coronavirus)*	1	Worldwide	None	+	512
Feline leukemia	++++	*Retroviridae (Oncovirinae)*	1	Worldwide	Inactivated subunit	+	565
Feline panleukopenia	+++	*Parvoviridae (Parvovirus)*	1	Worldwide	Attenuated and inactivated	+	412
Rabies	+	*Rhabdoviridae (Lyssavirus)*	1	Worldwide except some island countries, Scandinavia, and Australia	Inactivated and attenuated	+	534

Respiratory Diseases							
Feline calicivirus infection	+++	*Caliciviridae (Calicivirus)*	1	Worldwide	Attenuated and inactivated	+	448
Feline rhinotracheitis	+++	*Herpesviridae (Alphaherpesvirinae)*	1	Worldwide	Attenuated and inactivated	+	360
Enteric Diseases							
Feline panleukopenia	+++	*Parvoviridae (Parvovirus)*	1	Worldwide	Attenuated and inactivated	+	412
Feline rotavirus infection	+	*Reoviridae (Rotavirus)*	1	Worldwide	None	−	590
Reproductive and Neonatal Diseases							
Feline panleukopenia	+++	*Parvoviridae (Parvovirus)*	1	Worldwide	Attenuated and inactivated	−	412
Skin Diseases							
Cowpox virus infection	+	*Poxviridae (Orthopoxvirus)*	1	Sporadic in Europe	None used	−	393

TABLE 35-9
Diseases of Poultry and Other Avian Species[a]

Disease	Species	Importance	Virus family (genus or subfamily)	Number of serotypes	Vaccines	Persistence	Pages
Generalized Diseases (including central nervous system involvement)							
Adenovirus infection (egg drop syndrome)	Chickens, ducks, and turkeys	+	*Adenoviridae (Aviadenovirus)*	Unknown	Inactivated	+	336
Avian encephalomyelitis	Chickens, ducks, and turkeys	+	*Picornaviridae (Enterovirus)*	1	Attenuated	–	439
Avian influenza	Chickens, turkeys	++	*Orthomyxoviridae (Influenzavirus)*	Many	Inactivated	–	481
Avian leukosis	Chickens	++	*Retroviridae (Oncovirinae)*	1	Inactivated and attenuated	+	557
Eastern equine encephalitis	Pheasants	+	*Togaviridae (Alphavirus)*	1	Inactivated	–	462
Infectious bursal disease	Chickens	++	*Birnaviridae (Birnavirus)*	1	Inactivated	–	597

Marek's disease	Chickens	+++	*Herpesviridae (Gammaherpesvirinae)*	1	Attenuated	+	368
Newcastle disease	Chickens, pigeons, other avian species	++++	*Paramyxoviridae (Paramyxovirus)*	1	Attenuated and inactivated	–	493
Respiratory Diseases							
Avian reovirus infections	Chickens and other avian species	+	*Reoviridae (Reovirus)*	11	None used	?	581
Avian infectious bronchitis	Chickens	+++	*Coronaviridae (Coronavirus)*	Several	Attenuated	+	514
Infectious larnygotracheitis	Chickens	+++	*Herpesviridae (Alphaherpesvirinae)*	1	Attenuated	+	361
Enteric Diseases							
Duck hepatitis	Ducks and turkeys	+	*Picornaviridae (Enterovirus)*	1	Attenuated	?	441
Duck plague	Ducks	+	*Herpesviridae (Alphaherpesvirinae)*	1	None used	+	363
Skin Diseases							
Avian pox	All species	++	*Poxviridae (Avipoxvirus)*	1	Attenuated	–	403

[a]All diseases of poultry except eastern equine encephalitis (Americas) have a worldwide distribution, but fowl plague (avian influenza) and velogenic Newcastle disease are regarded as exotic viruses in most developed countries.

TABLE 35-10
Generalized and Respiratory Diseases of Horses

Disease	Importance	Virus family (genus or subfamily)	Number of serotypes	Geographical distribution	Vaccines	Persistence	Pages
Generalized Diseases (including central nervous system involvement)							
African horse sickness	++	*Reoviridae (Orbivirus)*	9	Africa	Attenuated	–	587
Borna disease	+	Unclassified	1	Europe	None	+	613
Eastern equine encephalitis	++	*Togaviridae (Alphavirus)*	1	Americas	Inactivated	–	458
Equine arteritis	+	*Togaviridae (Arterivirus)*	1	Worldwide	Attenuated	+	469
Equine encephalosis	+	*Reoviridae (Orbivirus)*	Several	South Africa	None	–	590
Equine infectious anemia	+	*Retroviridae (Lentivirinae)*	1	Worldwide	None used	+	575
Venezuelan equine encephalitis	+	*Togaviridae (Alphavirus)*	7 (1 epizootic type)	South and Central America	Inactivated and attenuated	–	458
Western equine encephalitis	+	*Togaviridae (Alphavirus)*	1	Americas	Inactivated	–	458
Respiratory Diseases[a]							
Adenovirus pneumonia	+	*Adenoviridae (Mastadenovirus)*	Unknown	Worldwide	None	–	334
Equine rhino-pneumonitis	+++	*Herpesviridae (Alphaherpesvirinae)*	2	Worldwide	Inactivated and attenuated	+	357
Equine influenza	+++	*Orthomyxoviridae (Influenzavirus)*	2	Worldwide	Inactivated	–	479

[a]Rhinoviruses (p. 442), equine herpesvirus 2 (p. 365), and parainfluenza type 3 virus (p. 492) have been isolated from horses with respiratory disease but are of little clinical importance.

TABLE 35-11

Diseases of Horses Affecting the Intestinal Tract, Reproductive System, and Skin

Disease	Importance	Virus family (genus or subfamily)	Number of serotypes	Geographical distribution	Vaccines	Persistence	Pages
Enteric Disease[a]							
Reproductive and Neonatal Diseases							
Equine abortion	+++	*Herpesviridae (Alphaherpesvirinae)*	1	Worldwide	Inactivated and attenuated	+	357
Equine arteritis	+	*Togaviridae (Arterivirus)*	1	Worldwide	Attenuated	+	469
Equine coital exanthema	+	*Herpesviridae (Alphaherpesvirinae)*	1	Worldwide	None	+	358
Skin Diseases (including stomatitis)							
Equine papillomatosis and sarcoids	+	*Papovaviridae (Papillomavirus)*	Several	Worldwide	None	+	327
Vesicular stomatitis	+	*Rhabdoviridae (Vesiculovirus)*	7	Americas	None used	–	541

[a]Rotaviruses (p. 590), toroviruses (p. 609), and coronaviruses (p. 511) have been associated with enteric disease but are of minor clinical importance.

Glossary*

abortive infection Viral infection in which no infectious virus is produced, although some viral genes may be expressed.

active immunization Specific acquired immunity resulting from immunization with viruses or viral proteins.

adjuvant Substance administered with antigen than enhances the immune response.

adsorption *See* attachment.

affinity Thermodynamic measure of the strength of binding of an antigenic determinant with the corresponding antibody binding site.

airborne transmission Method of spread of infection by droplet nuclei or dust.

alternate complement pathway Pathway of complement activation initiated via C3 without previous activation of C1, C4, and C2, as in the classical pathway; does not require antibody.

ambisense (applied to ssRNA genome) Part of the nucleotide sequence is of (+) sense, part is of (−) sense.

amphotropic retrovirus A retrovirus that will replicate in the cells of one or more species in addition to those of the original host.

anamnestic (secondary) response Rapid rise in antibody or cell-mediated immunity following second or subsequent exposure to antigen.

anchorage independence Ability of a cell transformed by a tumorigenic virus to grow in suspension in semisolid agar medium.

antibody (immunoglobulin) Specialized serum protein produced in response to an antigen, which has the ability to combine specifically with that antigen.

antibody-dependent cell-mediated cytotoxicity Lysis of target cells that express viral antigen on their surface, to which specific antibody binds; immunologically nonspecific cytotoxic leukocytes bind via Fc receptors to the antibody and mediate lysis.

antigen Substance that can induce an immune response when introduced into an animal and which binds to the corresponding antibody *in vitro.*

antigenic determinant (epitope) Region of an antigen that binds antibody.

antigenic drift Point mutation(s) in gene(s) specifying the surface protein(s) of a virus, resulting in antigenic change.

*For a more comprehensive list and more detailed definitions, the reader is referred to "A Dictionary of Virology" by K. E. K. Rowson, T. A. L. Rees, and B. W. J. Mahy, Blackwell Scientific Publications (1981).

antigenic shift Genetic reassortment between two subtypes of a viral species that has a segmented genome, resulting in the emergence of a new subtype with a completely different surface protein.

antigen-presenting cell Dendritic cell, Langerhans cell, or macrophage which processes and presents antigen to lymphocytes.

antiseptic Chemical germicide for use on skin or mucous membranes.

arbovirus *Ar*thropod-*bo*rne; a virus that replicates in an arthropod and is transmitted by bite to a vertebrate host in which it also replicates.

attachment Specific adsorption of virus to its receptor on the plasma membrane of the host cell.

attenuated Reduced in virulence.

avidity Measure of the firmness of the binding of antigen to antibody; influenced by affinity and valency.

B lymphocyte (B cell) Lymphocyte derived from the bursa of Fabricius in birds or its equivalent in mammals, which differentiates into an antibody-producing plasma cell.

bacteriophage A virus that replicates in a bacterium.

benign tumor A circumscribed tumor (lump) produced by excessive proliferation of cells, without any tendency for invasiveness or metastasis. (*See* malignant tumor.)

biological transmission Transmission by an arthropod after replication in the vector.

booster Second or subsequent dose of vaccine given to enhance the immune response.

bursa of Fabricius Hindgut organ in the cloaca of birds that controls the ontogeny of B lymphocytes.

cancer Vernacular term covering all types of malignant tumor.

cap 7-methylguanosine added to the 5′ terminus of RNA transcript, as part of the processing of a primary RNA transcript into mRNA.

capsid Protein shell which surrounds the viral nucleic acid.

capsomer Morphological units visible with the electron microscope, of which the capsid is constructed.

carcinogenesis *See* tumorigenesis.

carcinoma Malignant tumor of epithelial origin.

carrier An individual (often asymptomatic) carrying and often shedding infectious virus.

case–control study Attempt to identify the cause of a disease by comparing cases with matched controls.

cell-mediated immunity Immunity effected predominantly by T lymphocytes (and accessory cells) rather than by antibody.

chronic infection Infection characterized by continued presence of virus, with or without continuing signs of disease.

classical complement pathway Series of sequential enzyme–substrate interactions activated by antigen–antibody complexes and involving all C components.

clone A population of cells or viral particles derived from a single precursor cell or viral particle and thus having essentially the same genetic constitution.

cloning (molecular) Term denoting the isolation and propagation of foreign genes in prokaryotic or eukaryotic cells by recombinant DNA technology.

cloning vector Plasmid or viral DNA into which foreign DNA may be inserted to be propagated using recombinant DNA techniques.

coated pits Clathrin-coated depression in plasma membrane which constitutes the receptor for hormones, viruses, etc.; receptor-mediated endocytosis follows.

coding redundancy Coding of the same amino acid following substitution of one of the four nucleotides for another as the third nucleotide of the triplet (codon).

codon The triplet of nucleotides that codes for a single amino acid.

cohort study Attempt to identify the cause of a disease by comparing exposed and control populations in a prospective study.

cold-adapted mutant Mutant that replicates satisfactorily at temperatures lower than the host body.

complementation Occurs in doubly infected cells and consists in one of the viruses providing a gene-product which the other requires but cannot make.

complement system Series of serum proteins, the first of which binds to any antigen–antibody complex, triggering a cascade reaction; the later components in the complement cascade exert a variety of effects, including lysis of microorganisms and infected cells, phagocytosis, chemotaxis, and inflammation.

conditional lethal mutants Mutants which will not replicate under conditions in which the wild-type virus replicates, but will replicate under permissive conditions, such as a different temperature or another cell line.

copy DNA DNA copied *in vitro* from RNA by reverse transcriptase.

critical population size Minimum size of population needed to ensure the endemic (enzootic) status of a disease.

cuffing Perivascular infiltration of lymphocytes around blood vessels in brain, in response to certain viral infections.

cup probang Cup-like large thimble on end of flexible rod, used for obtaining samples of pharyngeal fluid from large animals.

cytocidal infection Virus infection resulting in death of infected cell.

cytopathic effect Morphological changes in cells resulting from viral infection.

cytopathogenic Virus which causes a cytopathic effect.

cytotoxic T cell (T_c) Subset of T cells capable of antigen-specific lysis of virus-infected cells that express viral antigen on their surface in association with a major histocompatibility antigen.

defective interfering particle Defective virus which interferes with the replication of homologous complete virus.

defective virus A virus that cannot replicate because it is defective in some way. Some defective viruses can replicate in mixed infections with a helper virus.

delayed hypersensitivity T cell-mediated, antibody-independent, antigen-specific inflammatory reaction.

demyelination Destruction of the myelin from the medullary sheath of Schwann cells in the peripheral nerves or oligodendria in the brain.

dendritic cell *See* antigen-presenting cell.

diploid A genome containing two copies of each gene.

disinfectant Chemical germicide for use on inanimate surfaces.

double-stranded (ds) Nucleic acid that occurs as a two-strand helix. Abbreviation (ds) used throughout book.

early viral genes Genes that are transcribed before viral nucleic acid replication occurs.

eclipse period Interval between viral penetration and production of the first progeny virions.

ecotropic viruses Retroviruses that replicate only in cells from the host species from which they were originally isolated.

endemic, enzootic (disease) Disease that is continuously present in a particular human population (endemic) or animal population (enzootic).

endogenous retroviruses Viruses whose genome occurs as a provirus integrated into the host cell DNA and is thus transmissible from parent to daughter cells during normal cell division.

enhancer Upstream nucleotide regulatory sequence which enhances the expression of genes under its control.

envelope Lipoprotein outer covering of virions of some viruses, derived from cellular membranes but containing virus-specific proteins, usually glycoprotein peplomers.

epidemic, epizootic (disease) Disease occurring in an unusually high number of humans or animals in a population at the same time.

epidemiology Science of the study of disease in populations.

episome Autonomous extrachromosomal genetic element that may become integrated with the chromosome.

epitope *See* antigenic determinant.

exogenous retroviruses Horizontally transmitted retroviruses.

exon Coding sequence of genome that is not deleted by splicing of the primary RNA transcript, but appears in mRNA and is translated into protein.

exotic virus Virus not normally occurring in a particular country.

extrinsic incubation period Interval between infective feed of an arthropod and development of infectivity of that arthropod for a vertebrate.

Fab fragment Fab = "fragment antigen-binding." Portion of an IgG molecule produced by papain digestion and comprising the variable domains of light and heavy chains; carries the antigen-binding site.

Fc fragment Fc = "fragment crystallizable." Portion of an IgG molecule produced by papain digestion and comprising the constant domain of both heavy chains; does not bind to antigen but is responsible for effector functions.

Fc receptor Receptor present on various leukocytes which binds immunoglobulin via the Fc part of the molecule.

fomites Inanimate objects that may be contaminated with viruses and transmit infection.

genome Complete set of genes of an organism.

haploid Genome contains one copy of each gene.

helical symmetry Configuration of nucleocapsid in which the nucleic acid and protein capsomers are arranged as a helix.

helper T cells (T_h) T cells, which when stimulated by antigen in association with class II MHC molecules are able to enhance the function of other lymphocytes.

helper virus A virus which, in a mixed infection with a defective virus, provides some factor(s) which enables the defective virus to replicate.

hemadsorption Adsorption of erythrocytes to the surface of virus-infected cells.

hemagglutination Agglutination of red blood cells.

hemagglutinin Viral surface glycoprotein that binds to red blood cells.

hexamer (hexon) In an icosahedral capsid, those capsomers having six neighboring capsomers.

horizontal transmission Transfer of infectious virus from one individual to another by means other than vertical transmission.

host range Range of species of animals (and cells derived therefrom) susceptible to a particular virus.

humoral immunity Immunity mediated by antibodies.

hybridoma Antibody-secreting cell line formed by the fusion of a myeloma tumor cell with a particular clone of antigen-primed B lymphocytes.

iatrogenic Caused directly by human (medical, veterinary) intervention.

icosahedral symmetry Configuration of nucleocapsid in which protein capsomers are assembled into a symmetrical polyhedron having 20 equilateral triangular faces and 12 vertices.

idiotype The antigenic determinant represented by the unique variable region of a specific antibody molecule.

immune complexes Antigen–antibody complexes.

immune response (*Ir*) genes Genes which influence the immune response to a given antigen and map within the MHC genetic locus.

immunogen *See* antigen.

immunological memory Capacity of an animal which has been exposed to a particular antigen to respond more rapidly and effectively on reexposure to that antigen.

immunological tolerance Specific unresponsiveness to a particular antigen.

inapparent infection Subclinical infection.

incidence (of disease) Proportion of a population contracting that disease during a specified period.

inclusion body Area with altered cytochemical staining properties in the nucleus and/or cytoplasm of an infected cell.

incubation period Interval between the time of infection and the onset of clinical signs.

infectious dose 50 (ID_{50}) Dose of virus required to infect 50% of inoculated hosts.

interference Prevention of the repliction of one virus by another.

interferons Family of cellular proteins produced and secreted in response to foreign nucleic acid (especially viral) that protect other cells against viral infection.

interleukins Soluble substances produced by leukocytes that stimulate the growth or activities of other leukocytes ("acting between leukocytes").

interleukin-1 Substance produced by macrophages that stimulates proliferation of lymphocytes.

interleukin-2 Factor produced by sensitized T cells that stimulates proliferation of other T cells.

intron (intervening sequence) Sequence of DNA that has no coding function, and is excised in processing of RNA transcript to produce mRNA.

isometric particle Particles that are symmetrical in three dimensions; they appear spherical, but their capsids have icosahedral symmetry.

Killer (K) cell "Null" (non-B, non-T) lymphocyte responsible for antibody-dependent cellular cytotoxicity.

latent infection Persistent infection in which little or no infectious virus is detectable, despite the continued presence of the viral genome.

late viral genes Genes transcribed after viral nucleic acid replication.

lethal dose 50 (LD_{50}) Dose of virus required to kill 50% of inoculated animals.

leukemia Tumor of leukocytes.

leukosis Neoplastic proliferation of cells of one or more of the leukopoietic tissues (bone marrow, reticuloendothelial, and lymphoid tissues).

liposome Artificially constructed lipid vesicle into which viral proteins or other substances may be incorporated.

long terminal repeat (LTR) Identical sequences some hundreds of nucleotides long at the termini of proviral DNA of oncoviruses, each comprising sequences from both termini of the viral RNA and including promoter and enhancer sequences.

lymphokine Soluble mediator produced by lymphocytes that influences the function of other cells.

lymphoma Tumor of lymphoid tissue.

lysosome Cytoplasmic organelles containing hydrolytic enzymes.

lytic infection *See* cytocidal infection.

major histocompatibility complex (MHC) Chromosomal region containing the genes for histocompatibility antigens and the genes involved in the immune response.

malignant tumor Invasive tumor resulting from uncontrolled proliferation of abnormal (transformed) cells.
maternal immunity Transfer of maternal antibody to fetus, newborn, or very young offspring.
matrix protein Protein lining the inner surface of the envelope of many enveloped viruses.
maturation phase Period toward the end of the viral replication cycle during which progeny virions are being assembled.
mechanical transmission Mechanical transmission of a virus by an arthropod, without replication in the vector.
messenger RNA (mRNA) RNA complementary to DNA which is formed by transcription and, after processing, is translated into proteins.
metastasis Spread of cells of a malignant tumor to other parts of the body.
MHC restriction The recognition of foreign (viral) antigen by T cells occurs only when that antigen is presented on a cell surface in association with "self" MHC antigen.
mitogen Substance that stimulates proliferation of lymphocytes.
molecular cloning *See* cloning.
monocistronic Corresponding to a single gene (cistron).
monoclonal antibody Antibody produced by a single clone of B cells, with a single specificity for a particular epitope. (*See* hybridoma.)
monokine Interleukin or cytokine produced by macrophages.
multiplicity of infection Measure of the number of virus particles inoculated per cell.
mutation Heritable change in the nucleotide sequence of the genome of an organism.
natural killer (NK) cell Immunologically nonspecific lymphocyte with capacity to kill virus-infected or tumor cells in absence of antibody.
natural passive immunity *See* maternal immunity.
negative stain Chemical that stains the background, outlining the object, e.g., potassium phosphotungstate for electron microscopy.
negative (−) or minus sense *See* sense.
nonsense mutation Mutation which produces one of the three stop codons, resulting in premature termination of the growing polypeptide chain.
nonstructural protein Virus-coded protein found in infected cells but not in the virion.
nosocomial Hospital-acquired.
nucleocapsid Viral nucleic acid surrounded by its protein capsid.
nude mouse Hairless mouse that is congenitally without the thymus gland.
Okazaki fragments Short fragments of DNA synthesized as intermediates in the discontinuous replication of DNA.
oncogene (cellular) Cellular gene that may lead to production of a malignant tumor when mutated or if expressed in an unregulated way.
oncogene (viral) Viral gene that is responsible for the rapid tumorigenicity of some oncogenic viruses.
palindrome Sequence in dsDNA with adjacent reverse repeats; the sequence of one strand read left to right is the same as that of the other strand read right to left.
panzootic (pandemic) Worldwide epizootic (epidemic).
passive immunization Transfer of antibodies from an immune individual to a nonimmune individual, either by maternal transfer or artificial inoculation.
pathogenicity Ability to cause disease.
penetration Entry of a virus particle into a host cell.
pentamer (penton) The 12 capsomers located at the vertices of the virions of an icosahedral virus. Each has 5 neighboring capsomers.

peplomer (spike) Oligomer of viral glycoprotein projecting from the viral envelope.
permissive (temperature or cell) The temperature or cell type that permits the replication of a conditional lethal viral mutant.
persistent infection In animals, infection that persists for longer than 1 month after the primary infection.
phenotype Appearance (characteristics) of a virus or organism, resulting from the expression of its genotype.
plaque Localized region of cell lysis resulting from cell-to-cell spread of virus replicating in a cell monolayer, usually under agar.
plaque assay Assay based on the number of plaques produced when a standard volume of a viral suspension is inoculated on a cell monolayer.
plasma cell Antibody-secreting cell derived as the end stage of differentiation of a B lymphocyte.
plasmid Self-replicating extrachromosomal circular DNA molecule.
plating efficiency Proportion of a population of virions that successfully infects a particular cell culture, usually detected by plaquing.
point mutation Alteration in a single base in the nucleic acid of a genome.
polarity *See* sense.
poly(A) tail Sequence of 50 to 200 adenylate residues added to the 3′ terminus of an RNA transcript, in the process of formation of mRNA.
polycistronic Representing several genes.
polykaryocyte Cell with multiple nuclei. (*See also* syncytium.)
polyploid More than one copy of the genome present (e.g., in a virion).
positive (+) or plus sense *See* sense.
prevalence (of disease) Proportion of a population infected (or sick, or immune) at a specified point in time.
primary immune response Immune response following the first contact of an individual with an antigen.
processing (1) Of RNA transcripts; series of posttranscriptional alterations to primary RNA transcripts, which lead to the formation of mRNA. (2) Of proteins; changes subsequent to translation of polypeptide, such as cleavage, glycosylation, or phosphorylation.
productive (or nonproductive) infection Infection of a permissive (or nonpermissive) cell by a virus resulting in the production (or failure of production) of infectious progeny virions.
prokaryote Unicellular organism (bacterium) lacking a nucleus.
promoter Region of DNA molecule to which RNA polymerase binds in order to initiate transcription.
protomer Identical protein units comprising the helical capsid of a virus.
protooncogene *See* oncogene (cellular).
provirus DNA copy of viral genome covalently integrated into a host cell chromosome, and thus transmissible from a cell to its daughter cells.
pseudotype Genome derived from the replication of one parent virus, enclosed within a capsid specified by a second coinfecting parent virus.
radioimmunoassay Assay for antigen or antibody in which one of the reactants is labeled with a radioisotope (e.g., ^{125}I).
reassortment Recombination between viruses with segmented genomes whereby some progeny of a doubly infected cell acquire genome segments from another virus.
reactivation (cross, multiplicity) Recovery of viable virus following coinfection of a cell with two inactivated parent viruses with lesions in different genes.

receptor-mediated endocytosis Uptake of virion (or hormone) following attachment to a specific receptor on the plasma membrane.

recombination (intramolecular) Exchange of nucleic acid segments between molecules derived from different parents, giving rise to progeny with a different genotype.

repeat (reiterated) sequence Nucleotide sequence present in more than one copy (usually many copies).

replicative intermediate Intermediate in the replication of viral nucleic acid, consisting of one complete (template) strand on which one or several nascent strands of opposite sense are replicating simultaneously, with a polymerase molecule at each growing point.

reservoir host Host species constituting the major source of virus in nature.

restriction endonuclease Enzyme (bacterial in origin) capable of cleaving double-stranded DNA at specific palindromes.

reverse transcriptase Enzyme carried by retroviruses that transcribes DNA from viral RNA.

sarcoma Malignant tumor of cells of mesenchymal origin.

secondary immune response *See* anamnestic response.

secular distribution Occurrence of disease over a prolonged period of time (years).

sense The polarity of a single-stranded nucleic acid; positive (+) sense is that found in mRNA; negative (−) sense is complementary to mRNA.

sentinel study Investigation of the circulation of arboviruses by testing for specific antibodies in exposed ("sentinel") animals.

sigla (acronym) Name formed from a few or initial letters of descriptive words (e.g., *Reoviridae* = *r*espiratory, *e*nteric, *o*rphan viruses).

signal sequence Hydrophobic leader sequence at the N-terminal end of a preprotein directing membrane insertion; subsequently cleaved off.

single-stranded (ss) Nucleic acid that occurs as a single strand. Abbreviation (ss) used throughout book.

site-directed mutagenesis Introduction of a particular point mutation or deletion at a predetermined position.

slow infection Infection with a prolonged preclinical phase (incubation period), which is followed by slowly progressive disease.

splicing Process of excision of introns and linking of exons from RNA transcripts, to form mRNA.

steady-state infection Persistent infection in which all cells of a culture are infected and produce noncytocidal virus.

structural proteins Virus-coded proteins found in virions.

subclinical infection Infection without signs of disease.

suppressor mutation Compensating mutation, generally in another gene, that restores wild-type phenotype without affecting the mutant gene.

suppressor T cell (T_s) Subclass of T lymphocytes that exerts an inhibitory control on B cells, helper T cells, or effector T cells.

surveillance The collection, collation, and analysis of data on disease incidence and its dissemination.

surveillance and containment Strategy for control of an infectious disease by which active cases are searched for and isolated, their source determined, and their contracts vaccinated.

susceptibility Measure of sensitivity of a host cell or organism to infection.

syncytium Polykaryocyte (multinucleated giant cell) formed by fusion of adjacent cells.

T cell Thymus-derived lymphocyte responsible for cell-mediated immunity or for immune regulation.

temperature-sensitive mutation Mutation resulting in a gene-product that is nonfunctional at a certain ("nonpermissive") temperature but functional at the permissive temperature.

tissue culture infectious dose 50 ($TCID_{50}$) Dose of inoculum required to infect 50% of inoculated cell cultures; infection is usually determined by cytopathological changes.

transcriptase RNA polymerase responsible for transcription.

transcription unit Region of genome extending from transcription initiation site to transcription termination site, including all introns and exons.

transduction Transfer by a virus of cellular genes from one organism or cell to another.

transfection Introduction of foreign DNA into a cell.

transformation (1) Transfer of genetic information into a cell via free DNA. (2) Infectious process in which a virus does not kill the host cell but induces morphological, biochemical, and biological changes that may lead to tumor formation.

translocation Movement of immunoglobulin across the wall of the intestinal tract in newborn animals.

translocation cutoff Time at which translocation ceases.

transovarial transmission Transmission from one generation to the next through the egg.

transposition Movement of genes from one chromosome site to another, or to an extrachromosomal genetic element, usually mediated by another genetic element called a transposon.

transstadial transmission Transmission from one developmental stage (instar) of an arthropod to the next stage.

tumor (T) antigens Virus-specific proteins found in transformed cells; some are required to maintain transformation, others are not.

tumor-associated transplantation antigen Antigens found on the surface of tumor cells that are undetectable on normal cells of adult individuals and that act as transplantation antigens.

tumorigenesis (carcinogenesis, oncogenesis) The process of development of a malignant tumor.

tumorigenic virus Virus able to transform cells and to induce tumors when inoculated into certain laboratory animals.

uncoating An early step in the viral replication cycle involving the removal of some or all of the viral coat, thus freeing the viral genome for expression of its functions.

vector (1) Intermediate host (e.g., arthropod) that transmits the causative agent of disease from infected to noninfected hosts. (2) Plasmid or viral DNA employed in recombinant DNA technology to clone a foreign gene in prokaryotic or eukaryotic cells.

vertical transmission Transmission of virus from parent to progeny through the genome, sperm, or ovum, or extracellularly (e.g., through milk or across the placenta).

viral core Viral nucleic acid and associated basic proteins.

viremia Presence of virions in the bloodstream, either free (plasma viremia) or in infected leukocytes (cell-associated viremia).

virgin-soil epizootic (epidemic) Epizootic (epidemic) occurring in a totally nonimmune population.

virion Complete virus particle.

virulence Measure of the ability of an infectious agent to inflict damage on a host.

wild type The original strain of virus, from which mutants may have arisen.

xenotropic viruses Retroviruses that replicate only in cells from animals other than the species from which they were derived.

zoonosis Infectious disease transferred from animals to humans.

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