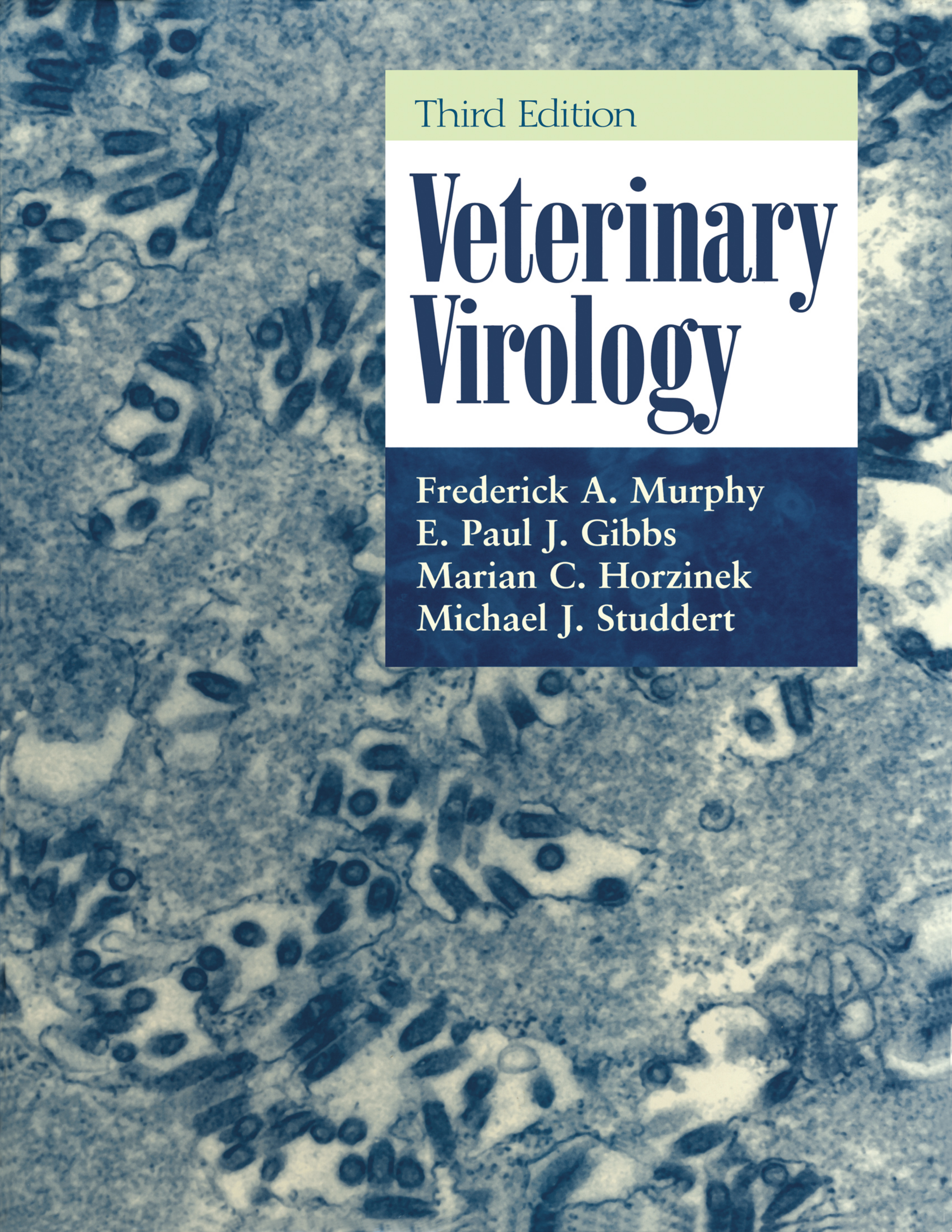

Third Edition

Veterinary Virology

Frederick A. Murphy
E. Paul J. Gibbs
Marian C. Horzinek
Michael J. Studdert

VETERINARY VIROLOGY

Third Edition

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Third Edition

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Cover illustration: Rabies encephalitis. Two hallmarks of street rabies virus infection are minimal cytopathology of infected neurons and minimal inflammatory infiltration into the neuropil. Here, in a thin section of part of the cytoplasm of a neuron in the hippocampus of a hamster inoculated intracerebrally 6 days earlier with a street rabies virus, the architecture of cellular organelles appears normal, but some of the cytosol has been displaced by aggregated viral nucleocapsids (smoothly granular areas; seen by light microscopy as Negri bodies). Large numbers of bullet-shaped virions are evident budding from adjacent endoplasmic reticulum membranes. Thin-section electron microscopy; uranyl acetate and lead citrate stain; magnification X 25,000.

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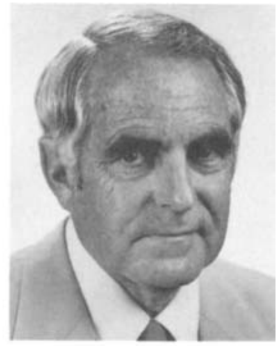
FRANK J. FENNER



DAVID O. WHITE

This book is dedicated to our dear friends, Frank J. Fenner and David O. White, the founders of the series of books that now includes three editions of this book and four editions of "Medical Virology." They set a standard of scholarship that is impossible to match and a *joie de vivre* that made the writing and editing almost fun.

They taught us that the subject of virology must be seen within the context of society as a whole as well as within the context of science. They envisioned virology as being so broad as to extend from its roots as a microbiological science, a molecular and cell biological science, an infectious disease science, to become a major contributor to the overall advance of human and animal well-being. All this as a single seamless cloth. We hope our students will come to understand the "big picture" of veterinary and medical virology as well as Frank and David have throughout their amazing careers.

We would also like to dedicate this book to our families, our teachers and mentors, and our students, all of whom have shaped our thinking and provided our inspiration over the years in so many ways.

Frederick A. Murphy

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Contents

PREFACE

The aim of "Veterinary Virology" is to present the fundamental principles of virology to students of veterinary medicine and related medical, biomedical, ecological, environmental, and comparative medical sciences. It will also serve as a useful resource for clinicians, teachers, and those involved in research in many related fields of comparative medicine. The pace of change since the previous edition has been so great that the book has been completely rewritten and greatly expanded. Coverage of zoonotic viruses and the diseases they cause has been expanded as has coverage of the viruses and viral diseases of laboratory animals, poultry, fish, and wildlife. We have tried to weave the concept of new, emerging, and reemerging viral diseases into the fabric of the book, reflecting the new perspective this concept has brought to veterinary and zoonotic virology and related fields.

The arrangement of the previous editions has been retained, but our account of the molecular biology of viral infections is much more detailed and more thoroughly integrated into the overall subject. Similarly, our account of viral genetics, phylogeny, and evolution has been expanded and has become a more integral part of the book. This, of course, is built on accurate and up-to-date viral taxonomic usage. The classification and nomenclature decisions of the International Committee on Taxonomy of Viruses, to May 1998, have been incorporated in this new edition.

Part I presents an overview of the principles of animal virology, starting with the viruses themselves and progressing to the infections they cause at the level of the cell, the individual animal host, and the host population. The emphasis is on pathogenesis, that is the events in the war between virus and host that we see as acute, chronic-persistent, and more subtle forms of disease. Our focus on pathogenesis naturally leads to emphasis on subjects pertaining to the host response to infection and to the means of intervening in the course of infection: immunology, diagnostics, vaccinology, epidemiology, prevention, and control.

Part II is arranged by virus family, with major subsections in each chapter providing more specific information about the viruses, their classification, their molecular properties and their replication, and on the important veterinary and zoonotic diseases caused by specific viruses. The diseases are covered from the perspective of their clinical features, their pathogenesis, pathology and immunity, their laboratory diagnosis, and their epidemiology, prevention, and control.

In order to focus on major virologic concepts and mechanisms that form the bases for our understanding of specific clinical diseases, minutiae have been omitted and much of the factual information is consolidated into tables and figures. Likewise, statements are not individually supported by citation of research papers; however, selective lists of authoritative books, reviews, and some key recent papers are provided at the end of each chapter to simplify the reader's entry into the virologic and infectious disease literature.

The pattern of producing "Veterinary Virology" and its companion volume "Medical Virology" involves alternating research, writing and editing between the two, with the authors' sense of the advances in the science pertaining to veterinary medicine being incorporated into one volume and at the same time into the files from which the next volume pertaining to human medicine is initiated; and, then, vice versa. Of course, this system pertains mostly to Part I and to the first part of each chapter in Part II of each book. We believe that this cross-fertilization between the veterinary and human medical sciences is a valuable and unique aspect of these volumes and a useful foundation for the concept of comparative medicine. Comparative medicine, in our view, is a powerful impetus in advancing both human and animal health. Thus, this book has as its base the fourth edition of "Medical Virology" published in 1994. In turn, this volume will serve as a foundation for the fifth edition of "Medical Virology."

We would like to acknowledge the help of the many colleagues who in so many ways have helped us in preparing this book: this has ranged from discussions on the qualities and shortcomings of the earlier editions and expectations for this edition, to the provision of new information from ongoing research work, to insights into the future of the science and its integration into clinical practice. We also acknowledge the help of many colleagues with the illustrations used throughout this book. Without all this help we would not have had the incentive to revise the book to the extent that we hope is evident. In this regard, we would also like to acknowledge the help of our teachers and our students who in their own ways have provided life-long incentives. Veterinary students, graduate students, and postdoctoral

fellows were all in mind as we pondered questions of "too little" or "too much?"

Finally, we would like to acknowledge the devotion of Ms. Shirley Light of Academic Press in the preparation and production of this edition. Ms. Light has served in this role from the first edition and has played a central role in the continuing improvements in the style and layout that are evident as one looks at all the editions in the series.

Frederick A. Murphy
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PART I

Principles of Virology

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CHAPTER 1

The Nature of Viruses as Etiologic Agents of Veterinary and Zoonotic Diseases

Veterinary and Zoonotic Virology as Infectious Disease Sciences

> Infectious disease is one of the few genuine adventures left in the world. The dragons are all dead and the lance grows rusty in the chimney corner. . . . About the only sporting proposition that remains unimpaired by the relentless domestication of a once free-living human species is the war against those ferocious little fellow creatures, which lurk in the dark corners and stalk us in the bodies of rats, mice, and all kinds of domestic animals; which fly and crawl with the insects, and waylay us in our food and drink and even in our love.

This quote is taken from the book "Rats, Lice and History," written in 1935 by the great microbiologist Hans Zinsser as he reflected on his life in infectious disease science. Zinsser's thought has challenged generations of students and professionals ever since, and now it challenges those who use this book, those who, by their own clinical and scholarly experiences, understand that the infectious diseases of today are as demanding as those that faced Zinsser.

This book presents the subject of veterinary and zoonotic virology from the perspective of its traditional base as an infectious disease science. It is the perspective established by Frank Fenner and David White, who in 1970 conceived the venue for a book, "Medical Virology," now in its fourth edition, and in 1987 its complement, "Veterinary Virology," this being its third edition. It is the perspective that many others have used to teach medical and veterinary and zoonotic virology in many countries.

It seems fitting to start with a sense of the roots of the subject, with a sense of how the roots of veterinary and zoonotic virology are intertwined with those of all the other infectious disease sciences. The history of veterinary and zoonotic virology is brief, spanning only about a century, but it is crowded with wonderful discoveries and practical applications. It centers on the replacement of centuries-old beliefs, conceptions, and theories with scientific proofs. Scientific proofs established the concept of *specificity of disease causation;* i.e., particular viral diseases are caused not by some common miasma (a mysteriously poisonous substance), but rather by specific viruses. This concept led to the introduction of specific prevention and control strategies, specific diagnostic tests, and specific therapeutic approaches.

This concept and the reformation of thought that it caused involved bitter struggle against entrenched opposition, but in the end the scientific method, the evidence-based method, won out. In a larger sense, the infectious disease sciences have played a paramount role in the reformation of all veterinary and medical thought: the concept of specificity of disease causation and the requirement for verifiable scientific proofs have been extended universally throughout all medical and veterinary sciences. At the same time, the practical application of the infectious disease sciences has led to improvements in animal and human health and well-being that have exceeded the contribution of any other branch of science.

Proof of this practical value of the infectious disease sciences is seen in the momentous effects of scientific discoveries on animal productivity, life expectancy, and well-being, worldwide. For example, the great epidemics of foot-and-mouth disease, rinderpest, hog cholera, and fowl plague, to name a few, that were so common in the 19th century have been virtually eliminated from developed countries by the application of various prevention and control strategies. At the same time, many of the zoonotic and food-borne diseases that were the causes of many human deaths have largely been controlled in developed countries.

However, even as the great epidemic infectious diseases have been conquered, new diseases have emerged, in every case requiring increasing expertise and more complex technological solutions than ever imagined when diseases like foot-and-mouth disease and hog cholera were the primary targets of control efforts. Just a few years ago it was canine parvovirus disease that took center stage and today it is bovine spongiform encephalopathy that represents the need for advanced veterinary virologic expertise, technologies, and control strategies. Tomorrow it will be other diseases, their cause, nature, and means of control totally unpredictable. In any case, our virologic knowledge base, the stuff of this book, is the starting point for managing the viral diseases that affect domestic and wild animals and often humans in direct and indirect contact with animals. This is what the authors mean in presenting the subject of veterinary and zoonotic virology as an infectious disease science.

Discoverers and Discoveries

The foundation of the virologic/infectious disease sciences predates the concept of the specificity of disease causation and is heavily dependent on initial discoveries about bacteria. There are great names and discoveries to be remembered: Hippocrates, the Greek physician and father of medicine, who in the 4th century, made important epidemiologic observations on many infectious diseases, including rabies; Fracastoro, who theorized in 1546 that epidemic diseases were disseminated by minute particles carried over long distances; van Leewenhoek, who in 1676 first saw bacteria with his microscope; Spallanzani, who in 1775 first grew bacteria in culture; Jenner, who in 1796 introduced vaccination against the viral disease smallpox; Semmelweis and Holmes, who in the 1840s developed practical methods of cleanliness and antimicrobial disinfection; Davaine, who in 1850 first associated an infectious organism, the anthrax bacillus, with disease; and Darwin, Wallace, and Mendel, who from 1859 onward revolutionized thinking in genetics and evolution.

Pasteur's Influence

On this foundation, Pasteur (Figure 1.1) established the microbiologic/virologic/infectious disease sciences, first in 1857 by discovering the specificity of microbial fermentations (wine, beer, cheese), then in 1865 by extending the concept to infectious diseases of silkworms, and finally between 1877 and 1895 by extending the concept to animal and human infectious diseases. His early infectious disease work centered on septic war wounds; he then turned to anthrax and other bacterial diseases and finally to rabies. In each instance, he moved quickly from studies aimed at discovering the causative agent to the development of specific intervention. In 1885, Pasteur gave the first rabies vaccine to a boy, Joseph Meister, bitten severely by a rabid dog—that day marks the opening of the modern era of infectious disease science aimed at disease prevention and control. Clearly, Pasteur deserves his title of father of the microbiologic/virologic/infectious disease sciences.

Pasteur was joined by Koch, who discovered the causative agents of tuberculosis and cholera and contributed much to the development of laboratory methods in bacteriology. As a result, the identification of the causative agents of many important human bacterial diseases proceeded at breakneck pace around the turn of the 20th century. At the same time, the seminal work of Salmon, Smith, Kilborne, Curtice, and others identified the specific cause of many important animal pathogens, including the causative agent of Texas fever (*Babesia bigemina*, transmitted by the tick *Boophilus annulatus*—the first proof that arthropods can transmit infectious agents). These successes in turn nurtured the founding of the science of virology.

Founders of the Science of Virology

In the field of virology, there are great names and discoveries to be remembered: Beijerinck and Ivanovski, who in the 1890s discovered the first virus, tobacco mosaic virus, and Loeffler and Frosch, who in 1898, working

FIGURE 1.2.

F. A. J. Loeffler (left) and P. Frosch, in 1898, working with Robert Koch (right), discovered the first virus of vertebrates, foot-and mouth disease virus.

with Koch (Figure 1.2), discovered the first virus of vertebrates, foot-and-mouth disease virus. They described the filterability of the virus, noting that "the filtered material contained a dissolved poison of extraordinary power or an as yet undiscovered agent that is so small that it is able to pass through the pores of a filter definitely capable of retaining the smallest known bacteria." On the basis of its virulence after successive dilutions in experimental animals, they concluded that the causal agent of foot-and-mouth disease was not soluble but "corpuscular." Loeffler and Frosch gave filtration a new emphasis by focusing attention on what passed through the filter rather than what was retained and establishing an experimental methodology that was adopted widely in the early 20th century in research on viral diseases.

There are other great names to be remembered: Sanarelli, who in 1898 discovered myxoma virus; Reed and Carroll, who discovered the first virus of humans, yellow fever virus, and who, influenced by the work of Salmon, Curtice, and their colleagues, also discovered its mosquito transmission cycle; M'Fadyean, who in 1900 discovered African horse sickness virus; Centanni, Lode, and Gruber, who in 1901 discovered fowl plague virus; Remlinger and Riffat-Bay, who in 1903 discovered rabies virus; DeSchweinitz and Dorset, who in 1903 discovered hog cholera virus; Arnold Theiler, who in the early 1900s made breakthrough discoveries concerning rinderpest, African horse sickness, and other animal diseases; Ellermann and Bang, who in 1908 discovered avian leukemia virus, the first cancer-causing virus; Landsteiner and Popper, who in 1909 discovered poliovirus; Rous, who in 1911 discovered the first solid tumor virus now known as Rous sarcoma virus; Laidlaw and Dunkin, who in 1926 discovered canine distemper virus; Shope, who in 1931 discovered swine influenza virus; Andrewes, Laidlaw, Smith, and Burnet, who in 1933 first isolated influenza virus, just 15 years after the great influenza pandemic of 1918–1919 in which 25 to 40 million people died; Max Theiler (Arnold Theiler's son), who in 1935 developed the yellow fever vaccine that is still in use today; Olafson, Pritchard, Gillespie, Baker, and colleagues, who in the 1940s–1950s determined the cause of bovine viral diarrhea; Sigurdsson, who in the 1950s in studies of scrapie and maedi/visna in sheep proposed the concept of slow infectious diseases; Salk and Sabin, who in 1954 and 1957 developed inactivated virus and attenuated virus polio vaccines; Montagnier and colleagues, who in 1984 discovered human immunodeficiency virus (HIV); Carmichael, Parrish, and colleagues, who in 1978 discovered canine parvovirus; Pedersen and colleagues, who in 1987 discovered feline immunodeficiency virus; the many British veterinary virologists, who in 1986 discovered the agent of bovine spongiform encephalopathy, and Prusiner, who discovered the nature of prions, the etiologic agents of bovine spongiform encephalopathy, scrapie, and similar diseases and who in 1997 was awarded the Nobel Prize in Medicine for his work.

The field of viral disease pathogenesis also celebrates great names and discoveries: the field was pioneered by Fenner, who in the 1940s in classical studies of ectromelia (mousepox) introduced viral quantitation methods for determining the sequential events in the course of infection, from portal of entry, viremic spread, seeding of target organs, to transmission. Fenner was followed by Mims, who starting in the 1960s used immunofluorescence to visualize the development of viral antigens in serially collected experimental animal tissues. In turn, Appel in the 1960s applied these methods to the study of canine distemper virus infection in dogs.

The sciences of immunology and cell and molecular biology have been intertwined with virology and the infectious disease sciences from their beginnings: these sciences also call to mind great names and discoveries that have influenced modern veterinary and zoonotic virology greatly. In immunology, we acknowledge Metchnikoff, Bordet, and Ehrlich, who by discoveries made between 1883 and 1909 outlined the nature of the immune system; Loeffler, Rous, Yersin, and Behring, who in 1888 discovered bacterial toxins and antitoxins; Avery and Lancefield, who between 1928 and 1933 developed the basic concepts of infectious disease diagnostics; Porter, Edelman, and Nisonoff, who in 1959 described the structure and molecular function of antibodies; Jerne and Burnet, who in 1974 conceived clonal selection as the basis of the immune response; Doherty and Zinkernagel, who in 1974 discovered how the cellular immune system recognizes virus-infected cells; and Kohler and Milstein, who in 1975 developed the first monoclonal antibodies. In cell biology we recognize Carrel, Steinhardt, Eagle, Puck, Dulbecco, Enders, and others, who from the 1910s to the 1960s invented cell culture methods, and Palade, Claude, Porter, and de Duve, who in the 1960s and 1970s described the fine structure of cells and organelles and their biochemical functions. In molecular biology we acclaim the work of Avery, Hershey, and Chase, who between 1944 and 1952 showed that DNA carries all hereditary specificity; Watson and Crick, who in 1953 discovered the structure of DNA and thereby the molecular basis for heredity; Nierenberg, Ochoa, Matthaei, and Khorana, who between 1961 and 1966 deciphered the genetic code; and Cohen and Boyer, who in 1973 developed recombinant-DNA technology.

From this brief, far from complete, history, it is clear that from its beginning veterinary and zoonotic virology has been intertwined with medical virology and the other infectious disease sciences. In fact, in the early 1900s, there did not seem to be any separation whatsoever, and today, in some areas of veterinary virology, notably those dealing with the zoonotic and arthropod-borne viruses, the seamless cloth is still intact. Even though this book now proceeds to deal with veterinary and zoonotic virology per se—the viruses infecting animals and the diseases they cause—understanding the full scope of the infectious diseases requires continuing integration of this subject with all the other infectious disease sciences—this is the perspective of "comparative medicine," "comparative virology," and the perspective of "lifelong learning." It is the perspective that all students and professionals who use this book will want to practice.

Viruses, "At the Edge of Life"

The unicellular *microorganisms* can be arranged in order of decreasing size and complexity: protozoa, fungi, and bacteria (the latter including mycoplasmas, rickettsiae, and chlamydiae). These microorganisms, however small and simple, are *cells*. They contain DNA as the repository of their genetic information; they also contain RNAs and they have most or all of their own machinery for producing energy and macromolecules. These microorganisms grow by synthesizing their own macromolecular constituents (nucleic acids, proteins, carbohydrates, and lipids), and most multiply by binary fission.

Viruses, however, are not cells, are not microorganisms. They possess no functional organelles and are completely dependent on their host for the machinery of energy production and synthesis of macromolecules. They contain only one type of functional nucleic acid, either DNA or RNA, never both, and they differ from microorganisms in having two clearly defined phases in their life cycle. Outside their host cell, the viruses are metabolically inert; this is the phase of their life cycle involved in transmission. Inside their host cell, the viruses are metabolically active; this is their replicative phase in which the viral genome exploits the machinery of the host cell to produce progeny genome copies, viral messenger RNA, and viral proteins (often along with carbohydrates and lipids) that assemble to form new virions (*virion*, the complete virus particle). Unlike any microorganism, many viruses can reproduce even if only their genomic DNA or RNA is introduced into the host cell. These qualities have been used to argue the question, "Are viruses alive?" One answer is to envision "*viruses, at the edge of life,*" in some ways fulfilling the criteria we use to define life, in some ways not. The key differences between viruses and microorganisms are listed in Table 1.1.

Given their unique characteristics, where might viruses have originated? There are two theories that have been argued for many years: viruses are either degenerate bacteria that have lost most of their cellular functions or they are escaped eukaryotic genes, i.e., nucleic acids that have learned to survive outside the environment of the cell. Viral genomic sequence analyses may at last resolve this question: for example, the genome of a plant viroid (a subviral agent composed of naked RNA), potato spindle tuber viroid, seems to be a self-replicating RNA copy of a bit of host DNA. Many of the genes of poxviruses are similar to those of eukaryotes. In any case, it seems certain from viral genomic sequence analyses that all the viruses did not evolve from a single progenitor; rather the different kinds of viruses likely arose independently and then continued to diversify and adapt their survival and transmission qualities to better fit particular niches by the usual Darwinian mutation/selection mechanisms. Some viruses have continued to evolve in long association with their evolving hosts (e.g., the herpesviruses); others have evolved by "host species jumping" (e.g., influenza viruses) and yet others by developing zoonotic transmission schemes (e.g., rabies virus).

Several important practical consequences follow from understanding that viruses are different from microorganisms and all other life forms: for example, (1) some viruses can persist for the lifetime of their host by the integration of their genomic DNA (or a DNA copy of their genomic RNA) into the genome of their host cell or by the carriage of their genomic DNA episomally in their host cell, and (2) most viruses have defied all antiviral drug development. Because viruses use the replicative machinery of their host cell, drugs that interfere

Table 1.1
Properties of Unicellular Microorganisms and Viruses

Property	Bacteria	Rickettsiae	Mycoplasmas	Chlamydiae	Viruses
>300 nm diameter[a]	+	+	+	+	−
Growth on nonliving media[b]	+	−	+	−	−
Binary fission	+	+	+	+	−
DNA and RNA	+	+	+	+	−
Infectious nucleic acid	−	−	−	−	−[c]
Functional ribosomes	+	+	+	+	−
Metabolism	+	+	+	+	−
Sensitivity to antibiotics	+	+	+	+	−[d]

[a]Some mycoplasmas and chlamydiae are less than 300 nm in diameter.
[b]Chlamydiae and most rickettsiae are obligate intracellular parasites.
[c]Some, among both DNA and RNA viruses.
[d]With very few exceptions.

with viral replication nearly always interfere with essential cell functions (bacteria have unique metabolic pathways, different from those of their host, and antibiotics are directed at these pathways).

The simplest viruses consist of a DNA or RNA genome contained within a protein coat, but there are classes of even simpler infectious agents: viroids, which as noted earlier consist of a naked RNA molecule that is infectious, and prions, the agents of the spongiform encephalopathies, which consist of an infectious protein, with no associated nucleic acid.

Viral Morphology

We know that viruses are smaller than microorganisms, but we forget how unbelievable this realization was at first and we forget just how small the viruses are—in fact, it is very difficult to understand from the perspective of direct human experience sizes of entities much smaller than we can see with the naked eye (Table 1.2). The first unequivocal discovery of an animal virus occurred in 1898, when Loeffler and Frosch demonstrated that the etiologic agent of foot-and-mouth disease, then an important disease of cattle throughout Europe and many other regions of the world, could be transferred by material that had been passed through a filter with pores too small to allow the passage of bacteria. The new infectious agents became known as *filterable viruses,* quickly shortened to *viruses.* Once the concept of filterability of viruses took hold, this experimental procedure was applied to many candidate infectious agents. The early discoveries of many important pathogenic viruses of animals and humans, as cited at the beginning of this chapter, were all made using ultrafiltration (together with animal inoculation of the filtrates).

Electron Microscopy of Viruses

For a time the viruses were also called "ultramicroscopic," as they are smaller than the limit of resolution of the light microscope (which is about 0.3 μm, i.e., 300 nm; poxviruses, the largest viruses, are just about this size but are visible in the light microscope using only dark-field optics or certain staining techniques). Only with the advent of the electron microscope was it possible to visualize the viruses. It then became apparent that the viruses range in size from about the size of the smallest microorganisms down to the size of large protein molecules.

Early electron microscopic studies of viruses by Ruska in 1939–1941, which employed metal shadowing of purified virus preparations, were expanded during the 1950s to include ultrathin sectioning of virus-infected cells. In 1959, visualization of viral ultrastructure was transformed when negative staining was developed. For negative staining, a solution of potassium phosphotungstate, which is electron dense, is added to a virus suspension on a coated specimen grid; it surrounds and fills the interstices in the surface of virions, giving a negative image of the virion, showing details not previously seen (Figure 1.3). The remarkable diversity of the viruses is evident when one compares the morphology of various viruses as they appear when negatively stained. This diversity is revealed in a different way when one com-

Table 1.2
Perspective on the Size of Viruses

10^{0}	1 m	1 m	Humans, adult males, are about 2 meters tall
10^{-1}	0.1 m		Human, adult, hand is about 10 cm wide
10^{-2}	0.01 m	1 cm	*Aedes aegypti,* adult, mosquito is about 1 cm long
10^{-3}	0.001 m	1 mm	*Ixodes scapularis* tick, nymphal stage, is about 1 mm long
10^{-4}	0.0001 m	100 μm	Smallest things visible to the naked eye
10^{-5}	0.00001 m	10 μm	Lymphocytes are about 20 μm in diameter
			Bacillus anthracis, among the largest of pathogenic bacteria, is 1 μm wide and 5–10 μm long
10^{-6}	0.000001 m	1 μm	Smallest things visible in light microscope are about 0.3 μm in size
			Poxviruses, the largest of the viruses of vertebrates, are 300 nm (or 0.3 μm) in their longest dimension
10^{-7}	0.0000001 m	100 nm	Influenza viruses and retroviruses, typical medium-sized viruses, are about 100 nm in diameter
			Pestiviruses, such as bovine viral diarrhea virus, typical smaller-sized viruses, are about 50 nm in diameter
10^{-8}	0.00000001 m	10 nm	Picornaviruses, such as foot-and-mouth disease viruses, typical small viruses, are about 30 nm in diameter
			Circoviruses, the smallest of the viruses of vertebrates, are 17–22 nm in diameter
10^{-9}	0.000000001 m	1 nm	Smallest things visible in transmission electron microscope; DNA double helix diameter is 2 nm
10^{-10}	0.0000000001 m	1 Å	Diameter of atoms is about 2–3 Å

pares the morphology of various viruses as they appear in ultrathin sections of infected cells (Figure 1.4).

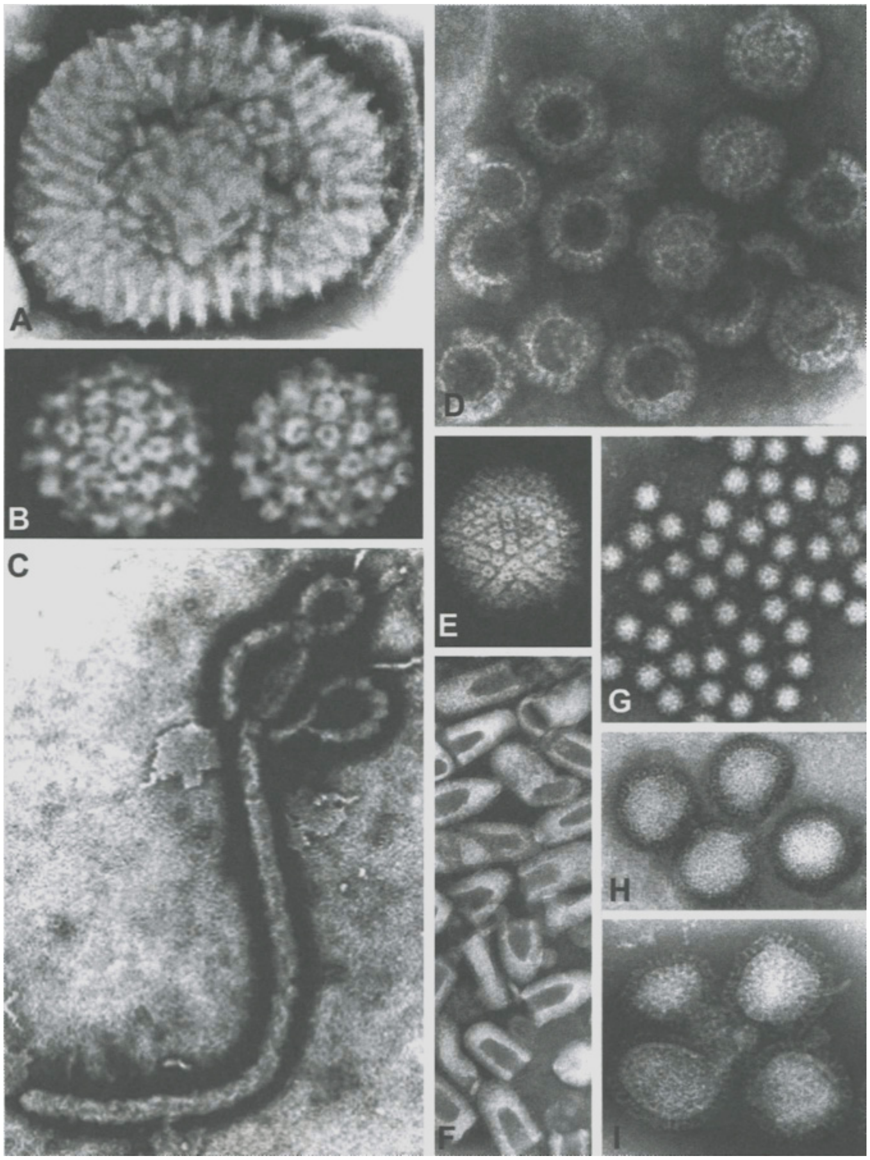
Electron micrographs of virions and infected cells representing the families of viruses that cause animal and zoonotic diseases are shown in the chapters of Part II of this book. In the past few years, ultrathin sectioning of virus-infected cells and tissues and negative staining of purified virions have been complemented by several new microscopy technologies, particularly scanning electron microscopy and computer-based image constructions of frozen, unstained virions. It seems that each new technology reveals the viruses as more and more beautiful—but this is a terrible beauty (Figures 1.5A and 1.5B).

X-Ray Crystallography of Viruses

Electron microscopic approaches have added much to our understanding of the nature and structure of viruses, but in the past few years there has not been much improvement in their resolving power—for this, X-ray crystallography and computer analysis of resulting diffraction patterns have been employed to advance our knowledge of virion structural details at near atomic resolution (Figure 1.6A). X-ray crystallographic analyses of many important viruses have provided remarkable insight into virion organization and assembly, the location of antigenic sites on the surface of virions, and aspects of virion attachment and penetration into cells. For example, in several picornaviruses, the amino acids of each of the three larger structural proteins have been found to be packaged so as to form wedge-shaped, eight-stranded antiparallel β-barrel domains (Figures 1.6B and 1.6C). The overall contour of picornavirus virions reflects the packing of these domains. In the same way, other amino acid chains have been found to form loops that project from the main wedge-shaped domains. Some loops form flexible arms that interlock with the arms of adjacent wedge-shaped units, thereby providing physical stability to the virion. Other loops, those involved in virion attachment to the host cell, harbor the *antigenic sites (epitopes)* that are the targets of the host's neutralizing antibody response against the virus.

Larger viruses are much more complex in structure (Figures 1.3 and 1.4). To study the fine structure of the

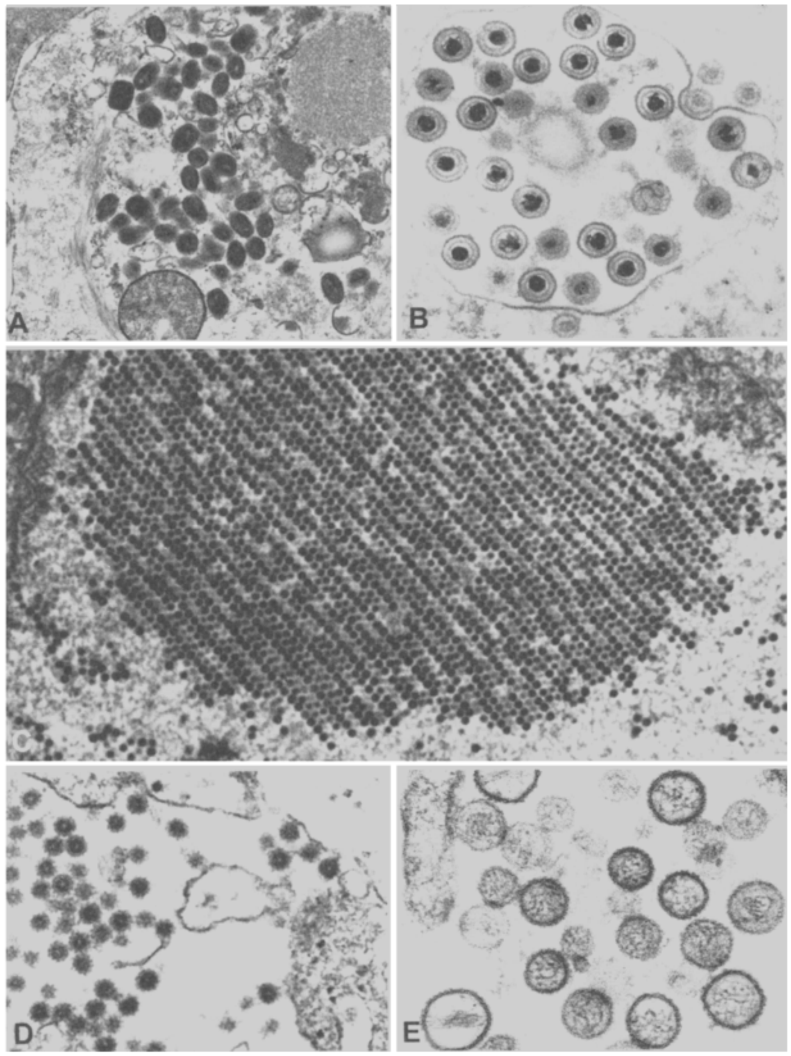
FIGURE 1.3.



Negative contrast electron microscopy of selected viruses. The remarkable diversity of the viruses is revealed by all kinds of electron microscopic methods, but none better than by negative staining. (A) Family *Poxviridae*, genus *Orthopoxvirus*, vaccinia virus. (B) Family *Papovaviridae*, genus *Papillomavirus*, bovine papillomavirus 1. (C) Family *Filoviridae*, unnamed genus, Ebola virus. (D) Family *Reoviridae*, genus *Orthoreovirus*, a new reovirus isolated from colonized baboons with central nervous system disease. (E) Family *Herpesviridae*, genus *Simplexvirus*, human herpesvirus 1 (capsid only, envelope not shown). (F) Family *Rhabdoviridae*, genus *Lyssavirus*, rabies virus. (G) Family *Caliciviridae*, genus *Vesivirus*, feline calicivirus. (H) Family *Bunyaviridae*, genus *Phlebovirus*, Rift Valley fever virus. (I) Family *Orthomyxoviridae*, genus *Influenzavirus A*, influenza virus A/Hong Kong/1/68 (H3N2). These images represent various magnifications; sizes of the various viruses are given in Chapter 2 and in the chapters in Part II of this book.

more complex viruses, it is necessary to separate well-defined substructures, crystallize them, and subject the crystals to X-ray diffraction analysis. For example, bluetongue viruses are composed of a subcore and two capsid layers. The subcore structure, as determined by X-ray crystallography, is composed of 120 copies of VP3 that self-assemble and then seem to direct the assembly of the surrounding capsid layers (Figure 1.7). The structure of the entire bluetongue virus virion was determined by X-ray crystallography in 1997—intact virions are

FIGURE 1.4.



Thin-section electron microscopy of selected viruses. The remarkable diversity of the viruses is also revealed by thin-section electron microscopy of infected cells. (A) Family *Poxviridae*, genus *Orthopoxvirus*, variola virus. (B) Family *Herpesviridae*, genus *Simplexvirus*, human herpesvirus 1. (C) Family *Adenoviridae*, genus *Mastadenovirus*, adenovirus 5. (D) Family *Togaviridae*, genus *Alphavirus*, eastern equine encephalitis virus. (E) Family *Bunyaviridae*, genus *Hantavirus*, Sin Nombre virus. These images represent various magnifications; details of the morphogenesis of the various viruses are given in the chapters in Part II of this book.

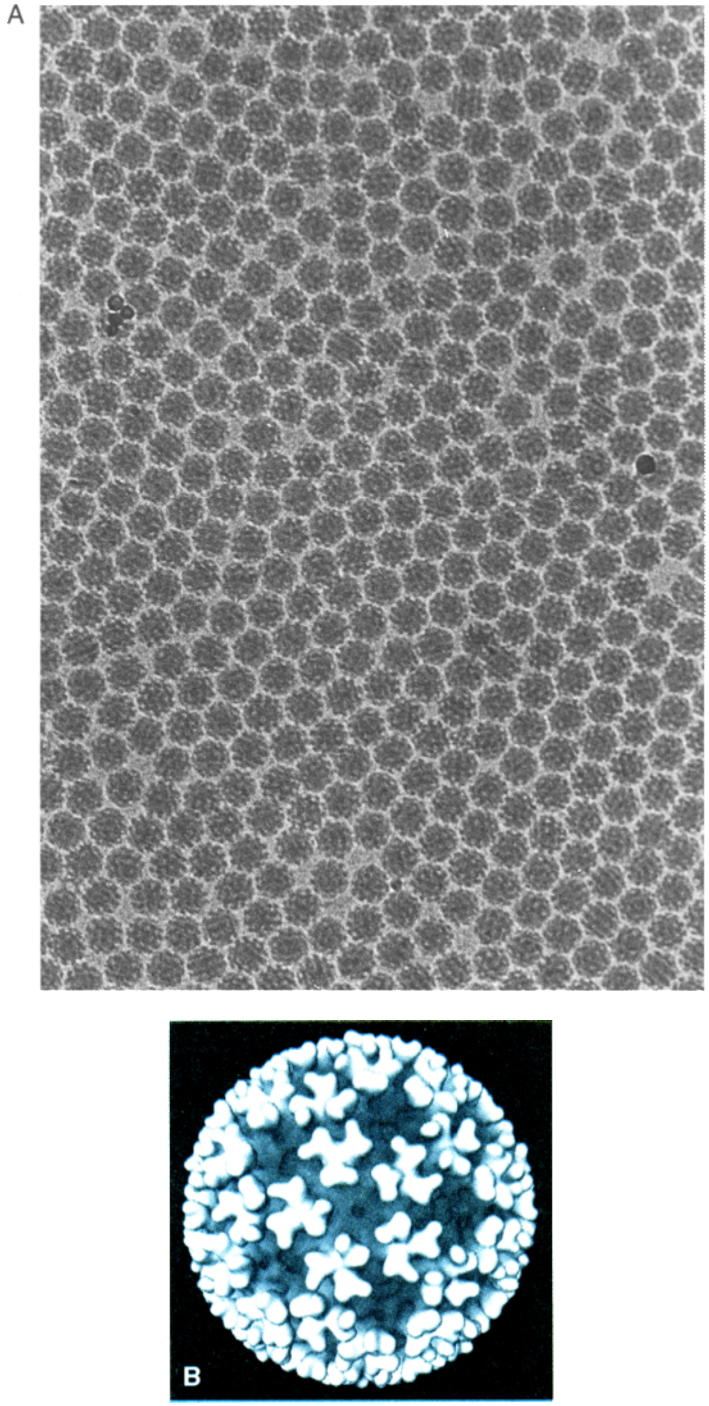
composed of more than 1000 separate protein molecules (see Chapter 24).

One of the pioneering studies of viral structure was the determination by X-ray crystallography of the structure of the hemagglutinin molecule of influenza viruses and the placement and variation of neutralizing epitopes on this molecule. This has been described in exquisite detail (Figure 1.8). Today, the determination of new variations in the amino acid sequence and the structure of the influenza hemagglutinin are used in the development of updated vaccines and antiviral drugs. Of course the largest viruses, such as the poxviruses, asfarviruses, and herpesviruses, are even more complex and their study requires even more dissection of viral proteins and substructures (see Chapters 16, 17, and 18).

Capsid Structure

The *virion*, i.e., the complete virus particle, of the simplest viruses consists of a single molecule of nucleic acid (DNA or RNA) surrounded by a morphologically de-

FIGURE 1.5.



(A) Semliki Forest virus, a member of the family *Togaviridae,* genus *Alphavirus.* Purified virus was placed in a monolayer on a coated grid and frozen instantaneously in liquid helium; the unstained virus preparation, embedded in a microfilm of ice, was examined in an electron microscope equipped for cryoelectron microscopy. Details of virion structure are revealed that were never seen by other methods; such virion images are now digitized routinely and large numbers of such images are subjected to computer analysis. (B) The result of computer analysis of many cryoelectron microscopic images of the virus showing the arrangement of the structural units that make up the icosahedral capsid. [A, courtesy of C.-H. von Bonsdorff; B, from R. G. Webster and A. Granoff, eds., "Encyclopedia of Virology," 2nd ed. (CD-ROM). Academic Press, London, 1998.]

FIGURE 1.8.

Classical schematic diagram of the three-dimensional structure of the influenza virus hemaglutinin trimer as determined from X-ray crystallography. Regions of β strands (flat, twisted arrows) and regions of α-helix (helices) are represented. (Courtesy of F. Hughson and D. Wiley.)

fined protein coat, the *capsid;* the capsid and enclosed nucleic acid constitute the *nucleocapsid.* The nucleocapsid of some viruses is surrounded by a lipoprotein *envelope* (Figure 1.3). There are many variations on these constructions and diverse additional components are found in the more complex viruses (Figure 1.9).

New information on the structure and organization of the components of the viral capsid obtained from X-ray crystallographic analyses requires a new synthesis of the terminology used to describe virions. Some features pertain to morphologically defined structures, others to molecular components themselves. *Capsomers* or *morphologic units* are discernible features (protrusions, depressions, etc.) seen on the surface of virions by electron microscopy (Figure 1.3). They may or may not correspond to individual molecular components. Folded polypeptide chains, specified by the viral genome, comprise *protein subunits;* assemblages of these protein subunits comprise *structural units* and in turn sets of these structural units comprise *assembly units,* which are the major intermediates in the formation of viral capsids. Only the simplest virions are assembled from the primary products of biosynthesis, i.e., protein subunits; in most cases, virions are constructed from distinct *subassemblies* by processes involving sequential synthesis and modification or cleavage of precursors. One crucial need in virion assembly is the incorporation of the viral nucleic acid into the nascent virion—several different mechanisms driving this process have been recognized, including the presence of *packaging signals* in the genomic nucleic acid sequence.

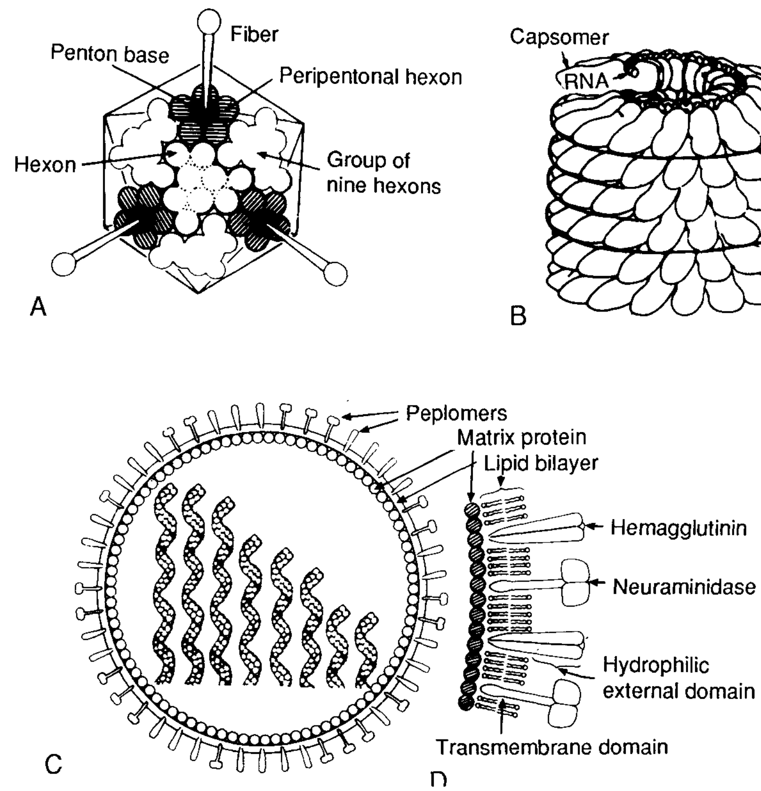
Virion Symmetry

For reasons of evolutionary progression and genetic economy, virions are assembled from multiple copies of one or a few kinds of protein subunits—the repeated occurrence of similar protein–protein interfaces leads to assembly of the subunits into symmetrical capsids. This efficiency of design also depends on principles of *self-assembly,* wherein structural units are brought into position through random thermal movement and are bonded in place through weak chemical bonds. Viruses come in a variety of shapes and sizes, depending on the shape, size, and number of their protein subunits and the nature of the interfaces between these subunits; however, only two kinds of symmetry have been recognized, *icosahedral* and *helical* (Figure 1.9).

Icosahedral Symmetry

The symmetry found in isometric viruses is invariably that of an icosahedron, one of the five classical *platonic solids* of geometry; virions with *icosahedral symmetry* have 12 vertices (corners), 30 edges, and 20 faces, each face an equilateral triangle. Icosahedra have axes of two-, three-, and fivefold rotational symmetry, which pass through their edges, faces, and vertices, respectively (Figure 1.10). The icosahedron is the optimum solution to the problem of constructing, from repeating subunits, a strong structure enclosing a maximum volume. The same principles were exploited by the architect Buckminster Fuller in his design of icosahedral buildings (geodesic domes). Only certain arrangements of structural units can form the faces, edges, and vertices of viral icosahedra. The structural units or capsomers on the faces and edges of adenovirus virions, for example, bond to six neighboring capsomers and are called *hexons;* those at the vertices bond to five neighbors and are called *pentons* (Figure 1.8). In the virions of some viruses, both *hexons* and *pentons* are composed of the same polypeptide(s), whereas in those of other viruses they are formed from different polypeptides. The arrangements of structural units on the surface of virions of three small icosahedral model viruses are shown in Figure 1.10. Because of variations in the arrangement of the structural units on different viruses, some appear rather hexagonal in outline and some appear nearly spherical. Even within rather smooth overall surface configurations, functional protrusions, bulges, and projections (often housing cellular attachment ligands and neutralizing epitopes) and depressions, clefts, and canyons

FIGURE 1.9.



Features of virion structure, exemplified by adenovirus (A), tobacco mosaic virus (B), and influenza A virus (C, D). (A) Icosahedral structure of an adenovirus virion. All hexon capsomers are trimers of the same polypeptide, distinguished as peripentonal or group of nine by their location in the capsid. The penton base is a pentamer of another polypeptide; the fiber a trimer of a third polypeptide. (B) The structure of helical nucleocapsids was first 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 forms a capsomer and 2130 capsomers assemble in a helix. The 6-kb RNA genome fits in a groove on the inner part of each capsomer and is wound to form a helix that extends the length of the virion. (C) Structure of virion of influenza A virus. All animal viruses with a helical nucleocapsid and some of those with an icosahedral capsid are enveloped. The nucleocapsids with helical symmetry are long and thin and in influenza A virus occur as eight segments, which may be connected loosely (not shown). The viral RNA is wound helically within the helically arranged capsomers of each segment, as shown for tobacco mosaic virus. (D) The envelope of influenza virus consists of a lipid bilayer in which several hundred glycoprotein peplomers or spikes are inserted; beneath the lipid bilayer there is a virus-specified matrix protein. The glycoprotein peplomers of influenza virus comprise two different proteins, hemagglutinin (a rod-shaped trimer) and neuraminidase (a mushroom-shaped tetramer), each of which consists of a hydrophobic internal domain, a transmembrane domain, and a hydrophilic external domain. Some 50 molecules of a small membrane-associated protein, M2 (not shown), form a small number of pores in the lipid bilayer. [A, from J. Mack and R. M. Burnett, *in* "Biological Macromolecules and Assemblies: Virus Structures" (F. Jurnak and A. McPherson, eds.), Vol. 1, p. 337. Wiley, New York; 1984; B, from C. F. T. Mattern, "Molecular Biology of Animal Viruses" (D. P. Nayak, ed.), p. 5. Dekker, New York, 1977.]

(also housing attachment ligands but usually not neutralizing epitopes) may be seen at higher resolution (Figures 1.11A and 1.11B).

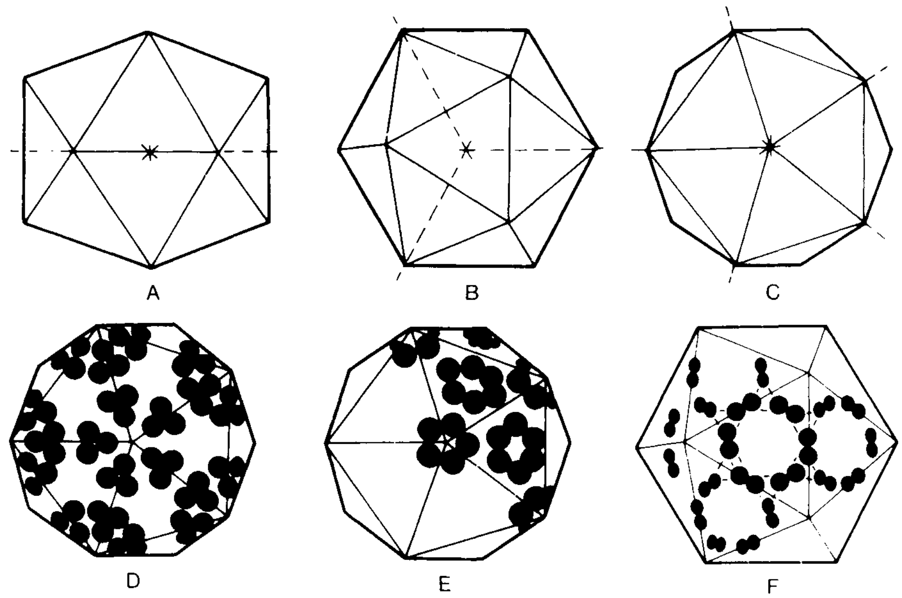
Helical Symmetry

The nucleocapsid of several RNA viruses self-assembles as a cylindrical structure in which the protein structural units are arranged as a helix, hence the term *helical symmetry*. It is the shape and repeated occurrence of identical protein–protein interfaces on the structural units that lead to the symmetrical assembly of the helix. In helically symmetrical nucleocapsids the genomic RNA forms a spiral within the nucleocapsid (Figure 1.9). Many of the plant viruses with helical nucleocapsids are rod shaped, flexible or rigid, and nonenveloped. However, in all such animal viruses the helical nucleocapsid is wound into a secondary coil and enclosed within a lipoprotein envelope.

Viral Envelopes

The virions of the member viruses of many different virus families are enveloped, and in most cases the integrity of

Figure 1.10.



(Upper row) An icosahedron viewed along (A) twofold, (B) threefold, and (C) fivefold axes of symmetry. (Lower row) Differences in the clustering of capsid polypeptides are responsible for the characteristic appearances of particular viruses as seen by negative contrast electron microscopy. (D) When capsid polypeptides are arranged as 60 trimers, structural units themselves are difficult to see; this is the case with foot-and-mouth disease virus. (E) When capsid polypeptides are grouped as 12 pentamers and 20 hexamers they form bulky capsomers, as is the case with parvoviruses. (F) When capsid polypeptides form dimers on the faces, they produce ring-like features on the virion surface, as is the case with caliciviruses.

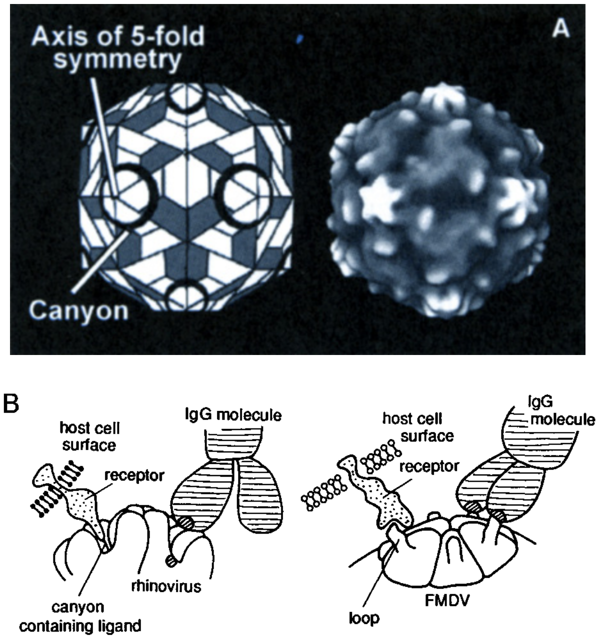
the envelope is necessary for viral infectivity. Enveloped virions acquire their outer layer when their nucleocapsid is extruded through one of the cellular membranes—this process is known as *budding*. The lipids of the viral envelope are derived directly from the cellular membrane, but the proteins associated with the envelope are virus coded. There are several kinds of envelope-associated proteins associated with at least four crucial activities: receptor binding, membrane fusion, uncoating, and receptor destruction. *Glycoproteins*, usually in the form of dimers or trimers, assemble into the virion peplomers (peplos = envelope) or spikes (Figure 1.9) seen in electron micrographs on the surface of orthomyxoviruses, paramyxoviruses, rhabdoviruses, filoviruses, coronaviruses, bunyaviruses, arenaviruses, and retroviruses. *Fusion proteins* are glycosylated and are also associated with peplomers; they are involved in key steps in viral entry and viral release. *Matrix proteins* are nonglycosylated and are found as a layer on the inside of the envelope of orthomyxoviruses, paramyxoviruses, rhabdoviruses, filoviruses, and retroviruses, but not coronaviruses, bunyaviruses, and arenaviruses. Matrix protein provides added rigidity to the virion; for example, the helical nucleocapsid of rhabdoviruses is closely apposed to a rather rigid layer of matrix protein, which in turn is tightly bound to the viral envelope and the internal domain of the surface glycoprotein peplomers.

Virion Structure/Function Relationships

Viral capsids and envelopes are not just packages—they must be stable enough in their expected environment to protect their contained nucleic acid, yet at the same time they must be in a primed status to facilitate uptake and infection of target cells. This primed status usually involves conformational rearrangements in virion surface structures in response to various triggers. For example, upon entry of the host, the hemagglutinin of influenza viruses is cleaved by extracellular enzymes, generating a primed modified structure. Upon entry of the host cell via endocytosis, the primed hemagglutinin is activated when exposed to the low pH within the endosome. This activated hemagglutinin mediates endosome membrane damage, thereby allowing viral RNA entry into the cytoplasm.

From this example, it is clear that knowledge of the fine structure of virions can shed light on practical matters: (1) the steps in virion attachment, penetration,

FIGURE 1.11.



(A) The location of the canyon containing the cellular receptors surrounding the axes of fivefold symmetry on the rhinovirus virion. (B) Model of the interactions between receptors on host cells and ligands on virions, using rhinovirus and foot-and-mouth disease virus (FMDV) as examples. In the interaction of rhinovirus with its host cell, the ligands are situated within surface depressions ("canyons") near axes of fivefold symmetry. This location of the ligands serves to prevent antibody binding at those crucial sites. Antibodies specific for other antigenic sites on the surface of rhinoviruses do not necessarily block virion–cell receptor interaction. In the interaction of foot-and-mouth disease virus with its host cell, the ligands are situated on flexible, sequence-variable loops extending from the surface of the virion. These loops are not protected from antibody binding, which does block virion–cell interaction. (Courtesy of M. G. Rossmann and R. R. Rueckert.)

and uncoating as targets for antiviral drug design; (2) the steps in virion assembly, again as targets for antiviral drug design and use; (3) the bases for virion integrity as targets for disinfectant design; and (4) the mechanisms of viral shedding and the patterns of viral transmission as targets for vaccine development. In these ways, knowledge of virion structure contributes to the development of disease prevention and control strategies.

Perhaps most important of virion structure/function relationships and virus/host relationships are those that pertain to virion attachment and entry into the host cell. In this context the terms *receptor* and *ligand* have often been used in imprecise ways. In this book, the term receptor is used to designate specific molecule(s) or structure(s) on the surface of host cells that are involved in virus attachment. The term ligand is used for the receptor-binding molecule(s) on the surface of the virus. For example, the hemagglutinin of the influenza virus is the ligand that binds to the receptor on the host cell surface, in this case a glycoconjugate terminating in *N*-acetylneuraminic acid.

Chemical Composition of Virions

Viruses are distinguished from all other forms of life by their simple chemical composition, which includes a genome comprising one or a few molecules of either DNA or RNA, proteins that form the virion (i.e., structural proteins, including capsid proteins, in some cases tegument proteins, envelope proteins such as glycoproteins, fusion proteins, and matrix proteins), proteins that are needed for virion assembly (nonstructural proteins), proteins that facilitate the viral takeover of host cell machinery (enzymes involved in viral replication, including replicases, polymerases, transcriptases, etc.), some of which are incorporated in the virion, and in some viruses carbohydrates (mostly as side chains on glycoproteins) and lipids. The simplest virus (tobacco necrosis virus satellite, a defective virus that needs a helper virus to provide some of its functions) directs the synthesis of only one protein; many important viruses direct the synthesis of 5–10 proteins; and the largest viruses, such as poxviruses and herpesviruses, direct the synthesis of up to 200 proteins—still this is very few relative to the number of proteins involved in the life processes of bacteria (more than 5000 proteins) and eukaryotic cells (more than 100,000 proteins).

Viruses exhibit a remarkable variety of strategies for the expression of their genes and for the replication of their genomes. Knowledge of these schemes has much practical significance, especially in understanding infection and disease pathogenesis and the application of rational means of disease prevention and control. Many of the viruses discovered most recently have very limited host ranges and tissue tropisms (e.g., feline immunodeficiency virus). As more and more such viruses are discovered, we can expect to find additional novel genome replication and expression strategies, each of which will require new research approaches.

Viral Nucleic Acids

Viral genes are encoded in either DNA or RNA genomes; genomic DNA and RNA can be *double stranded* or *single stranded* and can be *monopartite* (all viral genes contained in a single molecule of nucleic acid) or *multipartite* (segmented: viral genes distributed in multiple molecules or segments of nucleic acid). For example, among the RNA viruses, only the member viruses of the families *Reoviridae* and *Birnaviridae* have a double-stranded RNA genome, and these genomes are segmented (*Reoviridae:* 10, 11, or 12 segments, depending on the genus; *Birnaviridae:* 2 segments). All viral genomes are haploid, i.e., they contain only one copy of each gene, except for retrovirus genomes, which are diploid. These characters, and others, have been used to order the presentation of the families of viruses containing pathogens of animals (and humans) in this book (Table 1.3).

When extracted carefully from the virion, the nucleic acid of viruses of certain families of both DNA and RNA viruses is itself infectious, i.e., when experimentally introduced into a cell it can initiate a complete cycle of viral replication, with the production of a normal yield of progeny virus. The essential features of the genomes of viruses of vertebrates are summarized in Table 1.4. 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 3).

Viral Genomic DNA

The genome of all DNA viruses of vertebrates is monopartite, consisting of a single molecule which is double stranded, except in the case of the parvoviruses and the

TABLE 1.3
Families of Viruses Containing Pathogens of Animals and Humans

- DNA viruses
  - Double-stranded DNA
    - *Poxviridae*
    - *Asfarviridae*
    - *Iridoviridae*
    - *Herpesviridae*
    - *Adenoviridae*
    - *Papovaviridae*
  - Single-stranded DNA
    - *Parvoviridae*
    - *Circoviridae*
- DNA and RNA reverse-transcribing viruses
  - Double-stranded/single-stranded DNA
    - *Hepadnaviridae*
  - Single-stranded RNA
    - *Retroviridae*
- RNA viruses
  - Double-stranded RNA
    - *Reoviridae*
    - *Birnaviridae*
  - Single-stranded RNA
    - Negative sense,
      - Nonsegmented Order: *Mononegavirales*
        - *Paramyxoviridae*
        - *Rhabdoviridae*
        - *Filoviridae*
        - *Bornaviridae*
    - Negative sense, Segmented
      - *Orthomyxoviridae*
      - *Bunyaviridae*[a]
      - *Arenaviridae*[a]
    - Positive sense, Nonsegmented nested set transcription
      - Order: *Nidovirales*
      - *Coronaviridae*
      - *Arteriviridae*
    - Direct transcription
      - *Picornaviridae*
      - *Caliciviridae*
      - *Astroviridae*
      - *Togaviridae*
      - *Flaviviridae*
- Subviral agents: Satellites and prions
  - Single-stranded RNA (Negative sense, defective)
    - Floating genus: *Deltavirus*
  - No known nucleic acid, self-replicating protein
    - Prions

[a]Some member viruses of the family *Bunyaviridae* and all member viruses of the family *Arenaviridae* have ambisense genomes.

Table 1.4
Structure of Viral Genomes

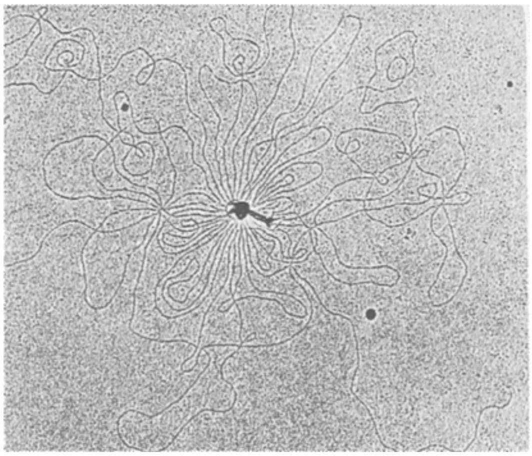
Family	Structure of genome
Poxviridae *Asfarviridae* *Iridoviridae*	A single molecule of linear double-stranded DNA, both ends covalently closed, with inverted terminal repeats
Herpesviridae	A single molecule of linear double-stranded DNA, containing terminal and internal reiterated sequences, usually forming two covalently linked components (L and S) that result in the formation of two or four isomeric forms oriented differently in the various herpesviruses
Adenoviridae	A single molecule of linear double-stranded DNA with inverted terminal repeats and a covalently bound terminal protein
Papovaviridae	A single molecule of circular supercoiled double-stranded DNA
Parvoviridae	A single molecule of linear single-stranded DNA, negative or positive sense,[a] with palindromic sequences at ends that allow circularization of the DNA during replication
Circoviridae	A single molecule of circular single-stranded DNA, ambisense or positive sense
Hepadnaviridae	A single molecule of circular double-stranded DNA with a region of single-stranded DNA
Retroviridae	Diploid linear single-stranded RNA, positive sense, each molecule hydrogen bonded at 5′ end with 3′-termini polyadenylated and 5′ ends capped
Reoviridae	Ten, 11, or 12 molecules of linear double-stranded RNA (segmented genome)
Birnaviridae	Two molecules of linear double-stranded RNA (segmented genome)
Order: *Mononegavirales* *Paramyxoviridae* *Rhabdoviridae* *Filoviridae* *Bornaviridae*	A single molecule of linear single-stranded RNA, negative sense
Orthomyxoviridae	Eight, 7, or 6 molecules of linear single-stranded RNA, negative sense (segmented genome)
Bunyaviridae	Three molecules of linear single-stranded RNA, negative sense or ambisense, with "sticky ends" that allow circularization (segmented genome)
Arenaviridae	Two molecules of linear single-stranded RNA, negative sense or ambisense, with "sticky ends" that allow circularization (segmented genome)
Order: *Nidovirales* *Coronaviridae* *Arteriviridae*	A single molecule of linear single-stranded RNA, positive sense, with nested set transcription
Picornaviridae *Caliciviridae* *Astroviridae* *Togaviridae* *Flaviviridae*	A single molecule of linear single-stranded RNA, positive sense, 3′ end polyadenylated (except most member viruses of the family *Flaviviridae*), 5′ end capped, or protein covalently bound (*Picornaviridae, Caliciviridae, Astroviridae*)
Deltavirus	A single molecule of circular single-stranded RNA, negative sense
Prions	No nucleic acid, self-replicating infectious prion protein (PrP)

[a]Varying among the various parvoviruses.

circoviruses (Figure 1.12). DNA genomes may be linear or circular, depending on the virus family. The DNA of papovaviruses, hepadnaviruses, and circoviruses is circular. The circular DNA of hepadnaviruses is partially double stranded, partially single stranded. The circular DNA of the papovaviruses is also supercoiled.

Most of the linear viral DNAs have characteristics that enable them to adopt a circular configuration, which is a requirement for replication by what is called a rolling circle mechanism. The two strands of poxvirus DNA are covalently cross-linked at their termini (forming *hairpin ends*), so that on denaturation, the molecule becomes a large single-stranded circle. The linear double-stranded DNA of several DNA viruses (and the linear single-stranded RNA of retroviruses) contains repeat sequences at the ends of the molecule that permit circularization. In adenovirus DNA there are inverted terminal repeats; these are also a feature of the single-stranded DNA of

FIGURE 1.12.



For this classical image, the DNA was released osmotically from a single T4 bacteriophage virion and floated on a liquid surface. From there, the spread-out DNA and the now empty virion head were transferred to a coated electron microscope grid and shadowed with atoms of a heavy metal. An amazing amount of DNA, up to 200 kbp, is coiled into the heads of the large DNA bacteriophages.

parvoviruses. Another type of terminal structure occurs in adenoviruses, hepadnaviruses, parvoviruses, and some single-stranded RNA viruses such as the picornaviruses and caliciviruses. In these viruses, a protein, which has an essential function in replication of the genome, is linked covalently to the 5′ terminus.

The size of viral DNA genomes ranges from 1.7 kb for some circoviruses, to over 200 kbp for the largest of the double-stranded DNA herpesviruses and poxviruses. As 1 kb or 1 kbp for double-stranded DNA contains enough genetic information to code for about one average-sized protein, it might be surmised that viral DNAs contain roughly between 2 and 200 genes, coding for some 2 to 200 proteins. However, the relationship between any particular nucleotide sequence and its protein products is not as straightforward as this. On the one hand, the DNA of most of the larger viruses, like that of mammalian cells, contains what appears to be redundant information in the form of repeat sequences, so the coding capacity of large viral genomes might be overestimated. On the other hand, the coding capacity might be underestimated: first, a given DNA or mRNA sequence may be read in up to three different reading frames, giving rise to two or three proteins with different amino acid sequences; second, either or both strands of double-stranded viral DNA may be transcribed, and in either direction, each yielding different proteins; third, genes may overlap, yielding various transcripts and protein products; and finally, a single primary RNA transcript may be spliced or cleaved in several different ways to yield several distinct mRNAs, each of which may be translated into a different protein.

Viral DNAs contain several kinds of noncoding sequences, some of which have been conserved throughout evolutionary time because they encode vital functions; these include DNA replication initiation sites, RNA polymerase recognition sites, translation initiation and termination sites, RNA splice sites, promoters, enhancers, and so on.

Viral Genomic RNA

With the exception of reoviruses and birnaviruses, the genomes of all vertebrate RNA viruses are single stranded. They can be monopartite or multipartite: for example, retroviruses, paramyxoviruses, rhabdoviruses, filoviruses, coronaviruses, arteriviruses, picornaviruses, togaviruses, and flaviviruses have monopartite genomes, whereas orthomyxoviruses, bunyaviruses, and arenaviruses have multipartite genomes. The genomes of arenaviruses consist of 2 segments, bunyaviruses 3, orthomyxoviruses 6, 7, or 8 (depending on the genus), birnaviruses 2, and reoviruses 10, 11, or 12 (depending on the genus). Each RNA molecule in these viruses is unique (often encoding a single protein). Except for the very small circular single-stranded RNA of delta hepatitis virus (the structure of which resembles that of viroids of plants), no animal virus RNA genome is a covalently linked circle. However, the single-stranded RNA of bunyaviruses and arenaviruses appears to be "circular" because of "sticky" hydrogen-bonded ends. The genomes of single-stranded RNA viruses have a considerable secondary structure, regions of base pairing causing the formation of loops, hairpins, and so on, which probably serve as signals controlling nucleic acid replication, transcription, translation, and/or packaging into the capsid.

Single-stranded genomic RNA can be defined according to its sense (also known as polarity). If it is of the same sense as mRNA, i.e., it can direct the synthesis of protein, it is said to be of *positive sense.* This is the case with the picornaviruses, caliciviruses, togaviruses, flaviviruses, coronaviruses, and retroviruses. If, however, the genomic nucleotide sequence is complementary to that of mRNA, it is said to be *negative sense.* Such is the case with paramyxoviruses, rhabdoviruses, filoviruses, orthomyxoviruses, arenaviruses, and bunyaviruses, all of which have an RNA-dependent RNA polymerase (transcriptase) in the virion, which in the infected cell transcribes positive-sense RNA using the viral genome as the template. With arenaviruses and at least one genus of bunyaviruses, one of the RNA segments is ambisense, i.e., part positive sense, part negative sense. Where the viral RNA is positive 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).

The size of single-stranded RNA viral genomes varies from 1.7 to 21 kb (M_r approximately 1–10 million) and that of double-stranded RNA viruses from 18 to 27 kbp, a much smaller range than found among double-stranded DNA viruses. Accordingly, these viruses encode fewer proteins than many DNA viruses, generally less than a dozen. Most of the segments of the genomes of orthomyxoviruses and reoviruses are individual genes, each coding for one unique protein.

Anomalous Features of Viral Genomes

Viral preparations often contain some particles with an atypical content of nucleic acid. Several copies of the complete viral genome may be enclosed within a single virion, or virions may be formed that contain no nucleic acid (empty particles) or that have an incomplete genome (defective interfering particles). Moreover, host cell DNA may sometimes be incorporated into virions (e.g., papovavirus), whereas fragments of ribosomal RNA have been found in orthomyxovirus and arenavirus virions.

Viral Proteins

The virions of all viruses of vertebrates contain several different proteins, the number ranging from 1 in the simplest virus to >100 in the most complex. Some virus-coded proteins are *structural,* i.e., they are used to construct the capsid and other components of the virion. Other proteins are *nonstructural;* some of these that are not part of the mature virion are involved in virion assembly whereas others are involved in the various aspects of viral replication processes. The latter are enzymes, most of which are involved in nucleic acid replication, transcription, and translation and in the shutdown of host cell functions and the subversion of its machinery to viral synthetic activities. These include various types of (1) replicases (e.g., DNA-dependent DNA replicases) and other enzymes involved in viral DNA replication, (2) transcriptases that transcribe mRNA from viral genomic double-stranded DNA or double-stranded RNA or negative-sense single-stranded RNA, and (3) various proteases, helicases, and ligases. Reverse transcriptase, which transcribes DNA from RNA, is found uniquely in retroviruses and hepadnaviruses. Other unique enzymes found in retroviruses are involved in the integration of the DNA product of reverse transcription into the cellular chromosomal DNA. Poxviruses, which replicate in the cytoplasm and therefore have less access to cellular machinery, carry a number of unique enzymes for processing RNA transcripts and replicating their DNA.

Viral Glycoproteins

Most viral glycoproteins occur as membrane-anchored peplomers (spikes) extending outward from the envelope of enveloped viruses, but the virions of some of the more complex viruses also contain glycosylated internal or outer capsid proteins. Oligosaccharide side chains (glycans) are attached by N-glycosidic or, more rarely, O-glycosidic linkages. Because they are synthesized by cellular glycosyl transferases, the sugar composition of these glycans corresponds to that of host cell membrane glycoproteins.

Viral Envelope Lipids

Most lipids found in enveloped viruses are present as a typical lipid bilayer in which the virus-coded glycoprotein peplomers (spikes) and, in some cases, other viral proteins are embedded. As a consequence, the composition of the lipids of particular viruses differs according to the composition of the membrane lipids of the host cells from which they came. The composition of the membrane lipids of viruses also varies with the particular membrane system employed for virion budding. For example, the lipids of paramyxoviruses that bud from the plasma membrane of host cells differ from those of bunyaviruses and coronaviruses, which bud from the membranes of intracytoplasmic organelles. Lipids constitute about 20–35% of the dry weight of most enveloped viruses; some 50–60% of viral envelope lipid is phospholipid and most of the remainder is cholesterol.

Stability of Viral Infectivity

In general, viruses are more sensitive than bacteria or fungi to inactivation by physical and chemical agents, but there are important exceptions. A knowledge of specific viral 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.

Temperature

The principal environmental condition that may adversely affect the infectivity of viruses is temperature. Surface proteins are denatured within a few minutes at temperatures of 55–60°C, with the result that the virion is no longer capable of normal cellular attachment, penetration, and/or uncoating. At ambient temperature the rate of decay of infectivity is slower but significant, especially in the summer or in the tropics. To preserve infectivity, viral preparations must therefore be stored at low temperature; 4°C (wet ice or a refrigerator) is usually satisfactory for a day or so, but longer-term preservation requires much lower temperatures. Two convenient temperatures are −70°C, the temperature of solid CO_2 (dry ice) and of some mechanical 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. Enveloped viruses are more heat labile than nonenveloped viruses. Enveloped virions, e.g., those of the genus *Pneumovirus* in the family *Paramyxoviridae,* are also susceptible to repeated freezing and 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 cold gel packs.

In the laboratory, it is often necessary to preserve virus stocks 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° or −196°C, or (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 used universally in the manufacture of attenuated virus vaccines. The most prominent exception to this is the prions, which are amazingly stable under virtually all environmental conditions, surviving boiling, freezing, many physical and chemical insults, and even large doses of γ-irradiation (see Chapter 40).

Ionic Environment and pH

On the whole, viruses are best preserved in an isotonic environment at physiologic pH, but some tolerate a wide ionic and pH range. For example, whereas most enveloped viruses are inactivated at pH 5–6, rotaviruses and many picornaviruses survive the acidic pH of the stomach.

Lipid Solvents and Detergents

Because the infectivity of enveloped viruses is destroyed readily by lipid solvents such as ether or chloroform or detergents such as sodium deoxycholate, these agents must be avoided in laboratory procedures concerned with maintaining the viability of viruses. However, detergents are used commonly by virologists to solubilize viral envelopes and to liberate proteins for use as vaccines or for chemical analysis.

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