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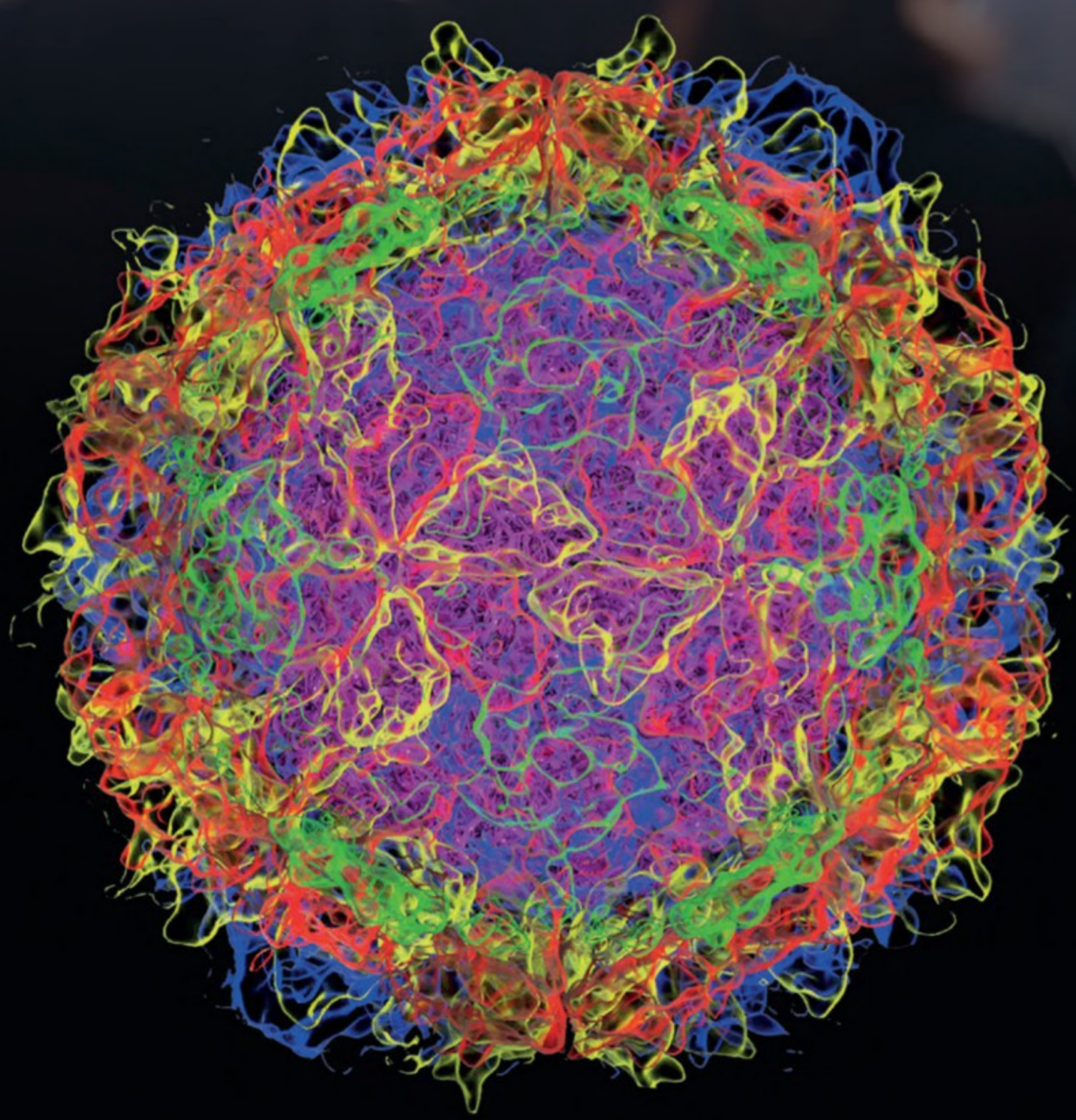

Fenner and White's

MEDICAL VIROLOGY

Christopher J. Burrell | Colin R. Howard | Frederick A. Murphy

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Fenner and White's Medical Virology

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Fifth Edition

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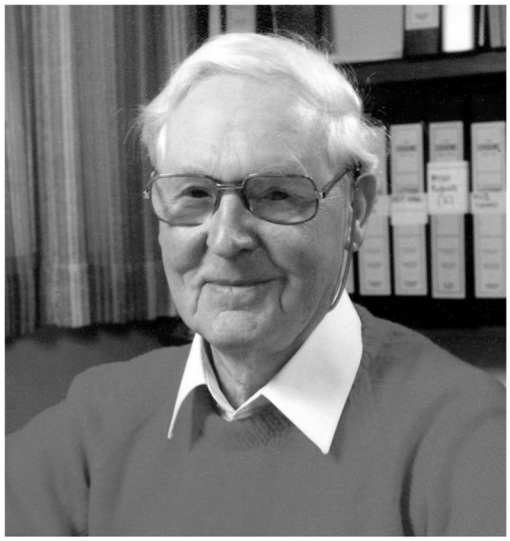
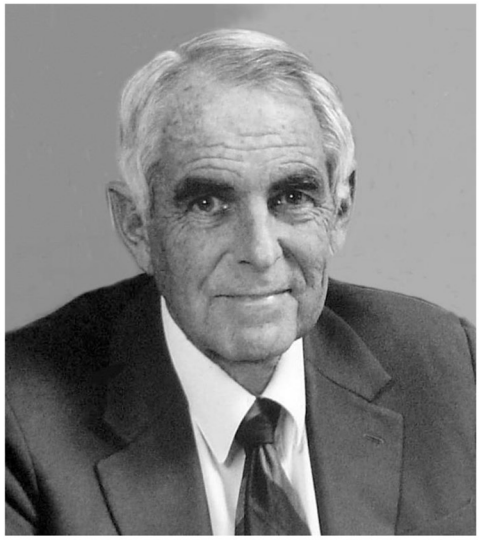


Frank J. Fenner



David O. White

This book is dedicated to our friends Frank J. Fenner (1914–2010) and David O. White (1931–2004), the founders of the series of books now spanning five editions of *MEDICAL VIROLOGY* and five editions of *VETERINARY 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 extending broadly, from its roots in the science of microbiology and the practice of infectious disease, through its key roles in the development of molecular and cell biology, to become an independent scientific and medical discipline and a significant contributor to human, animal, and environmental well-being. We hope our students will come to understand the "big picture" of medical virology as well as Frank and David achieved throughout their outstanding careers.

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

Christopher J. Burrell
Colin R. Howard
Frederick A. Murphy

Foreword

To say that this new version of the classic *Medical Virology* text by Frank Fenner (1914–2010) and David White (1931–2004) is timely is, to say the least, an understatement. It's been 21 years since the fourth edition, with authors Chris Burrell, Colin Howard, and Fred Murphy building on that much respected foundation to produce a greatly refreshed fifth edition. The basic philosophy and structure that made this such a useful volume for many generations of advanced medical students, clinicians, and pathologists remain but, as all who have been even peripherally involved in molecular science over the past two decades will realize, there was an enormous amount of new information that had to be read, sifted, and selected for relevance to the information needs of this target audience.

Basic virology has, of course, been well served by the many revisions of Bernie Fields' (1938–1995) exhaustive text and, following the example set by *Field's Virology*, the fifth edition will appear as *Fenner and White's Medical Virology*. It is a fitting tribute. Taken together, the original authors, and those responsible for this latest version, have variously been active and publishing on one or the other aspect of virus-induced disease and/or pathology since 1948: and the virology lineage goes back even further!

Frank Fenner was the student of MacFarlane Burnet (1899–1985) who, applying technical and conceptual approaches learned from his early studies with bacteriophage, pioneered quantitative mammalian virology, and genetics. David White began his career as a virologist in the university microbiology department headed by Frank Fenner. Both Fred Murphy and I worked for a time and Chris Burrell completed his PhD in that same department, where Rolf Zinkernagel and I did the experiments in viral immunity that led to a Nobel Prize. Reflecting on lineages and the solid foundations that enable the work of subsequent generations, I realize that I've met all the people named above in one or other professional or personal context and have benefitted from either the institutional structures that they established, or the insights they developed. My current office is in the Department of Microbiology and Immunology at the University of Melbourne, which was headed for many years by David White. Perhaps, as Isaac Newton (1642–1726) said "we stand on the shoulders of giants" though, when I've met the "giants" of science, they've pretty much turned out to be hard working, smart, and dedicated people who quietly and systematically built a body of knowledge and a tradition of scholarship. That very much describes the discipline of virology.

Thus, while reflecting on the up-to-date understanding of the authors, and of other researchers who have provided key insights concerning each particular pathogen, *Fenner and White's Medical Virology* reflects a cumulated wisdom that goes back to the early days of 20th century virology. Back then, the thinking and technical range of mammalian virologists was very much focused on pathogenesis and disease process. Now, after decades of illuminating the molecular characteristics of viruses and infected cells, it is gratifying to see these precise investigative tools being applied to the understanding of what actually happens in an infected individual. This is a great time for any medical researcher who is fascinated by the linked issues of disease process, pathology, and treatment.

This fifth edition provides an accessible and informative account of medical virology, based on both contemporary science and what went before. It constitutes an excellent resource for both the physician and the research investigator. New discoveries are constantly being made and, while much remains unclear, the rate of advance in understanding is extraordinary. Hopefully, we will not need to wait another 20 years for the next edition of this seminal text.

Peter C. Doherty
University of Melbourne
December 10, 2015

Preface

Enormous strides have taken place since the fourth edition of this book in regard to our understanding of virus properties and how viral diseases can be controlled, treated, and prevented. Considerable advances have been made across the whole spectrum of virology, particularly in relation to virus structure and replication, "point-of-care" diagnostics and the wide use of antiviral therapies. This progress has done much to contribute to a substantial improvement in public health across the globe.

At the same time, we have seen the emergence of new viral agents, and older agents in new guises, sometimes creating a need for radically new concepts to guide our understanding and clinical and public health actions. Notable examples include HIV/AIDS; SARS and its recently appearing relative MERS; Hendra and Nipah viral diseases; the more recent epidemic manifestations of Ebola; the appearance in humans of novel influenza A viruses; and the ever-increasing range of manifestations of "older" viruses in immunosuppressed individuals. Paradoxically, other aspects of virology, for example, the clinical descriptions of common infections and the principles of management, remain as valid as when they were first made. Our challenge is to transmit hard-learned experiences, within the context of new advances, to an ever-widening audience of newcomers entering the medical, scientific, and related professions.

In keeping with the advice of many colleagues, we have renamed this edition *Fenner and White's Medical Virology*. We have retained the layout of chapters as first set out in 1970 by Frank Fenner and David White, as we believe this structure still provides an excellent framework for the discipline.

Part I deals with the principles of animal virology, and includes chapters on basic virology and viral replication, host immunity, pathogenesis of infection, viral oncogenesis, viral diagnostics, vaccinology, chemotherapy, epidemiology, surveillance, and emergence.

Part II systematically examines in turn each of the virus families containing human pathogens. Chapters in Part II are for the most part set out in a standardized format to allow the reader more rapid access to the information being sought. Of course, virus infections in clinical practice present as syndromes and do not identify themselves by their virus family, so a key final chapter associates common viral syndromes with the etiological agents usually responsible. Much of the approach adopted has been shaped by the authors' experience in teaching virology to science and medical students and graduates—which has itself been shaped by earlier editions of this book.

This edition is designed to meet the needs of advanced medical students, clinicians and pathologists, university teachers, researchers, and public health workers who are seeking a single accessible source of key information about the range of major human viruses and the options for treatment and control. We have included historical perspectives, so that readers gain some insight into the paths and the personalities involved in our reaching this current state of knowledge; we have also highlighted gaps in knowledge, and unmet challenges in the management and control of virus diseases. However, in a book of this size it is not feasible to include all details, and thus readers seeking a more complete discussion of the diagnosis and management of a particular condition should refer to the appropriate source. Similarly, although not intended as a comprehensive summary of current research into a particular viral agent, we hope to prime the reader's enthusiasm to explore further aspects in the scientific literature. We are acutely aware of the need to foster the next generation of virologists, and if reading this book plays a small part in this process, then we will be gratified.

We wish to acknowledge a number of friends and colleagues who kindly agreed to review certain chapters: Peter Balfe (replication), Michael Beard (innate immunity, flaviviruses), Shaun McColl (adaptive immunity), Geoff Higgins (laboratory diagnosis), Yu-Mei Wen (oncogenic viruses, adenoviruses, paramyxoviruses, coronaviruses), Tony Cunningham (herpesviruses), Wendy Howard (influenza), Stephen Locarnini (hepadnaviruses), David Shaw (retroviruses), Sharon Lewin (retroviruses), Barbara Coulson (reoviruses), Lorena Brown (orthomyxoviruses), Peter McMinn (picornaviruses), Bill Rawlinson (caliciviruses, astroviruses), and Mike Catton (viral syndromes). We also thank our graphic artist Richard Tibbitts for his work on the diagrams, and Steven Polyak for the chemical structures of antiviral drugs in the chapter "Antiviral Chemotherapy."

Christopher J. Burrell
Colin R. Howard
Frederick A. Murphy

Chapter 1

History and Impact of Virology

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.

So wrote the great microbiologist Hans Zinsser in his book *Rats, Lice and History*, written in 1935, as he reflected on his life in infectious disease research. Zinsser's thoughts have stimulated generations of students and professionals ever since. Infectious diseases of today present challenges that are different but just as demanding as those facing Zinsser over 80 years ago.

This book presents the subject of medical virology from the perspective of its traditional base as a life science and its application to clinical practice and public health. It is the perspective established by Frank Fenner and David White, who in 1970 conceived the rationale for this book, and maintained it through the previous four editions. It is the perspective that many others have used to teach and learn medical virology.

The foundations of the science of medical virology are intertwined with the other life sciences, particularly microbiology and infectious diseases. Medical virology has a relatively brief history, spanning just over a century, but it is crowded with intriguing discoveries, stories of immense personal courage and numerous practical applications, many of which have had an overwhelmingly positive benefit on humankind. Its origins involved the replacement of centuries-old beliefs and theories with discoveries borne out of rigorous scientific investigation. Targeted prevention and control strategies could only be developed and implemented once the concept of *the specificity of disease causation* had been accepted, namely that infectious diseases are caused not by some common *miasma* (a mysteriously poisonous substance), but rather by specific agents. In a wider sense, the microbial sciences have played a pivotal role in the development of medical thought overall, particularly in applying scientific rigor in understanding pathological processes. Advances in understanding infectious agents have led to improvements in human health and well-being that arguably have exceeded the contribution of any other branch of science. Indeed, no less than 35 workers in this and closely related fields have been awarded the Nobel Prize in Physiology or Medicine in recognition of their achievements.

Infectious disease discoveries have had a profound effect on life expectancy and well-being across the world. For example, epidemics of smallpox, yellow fever, and poliomyelitis, commonplace until well into the 20th century, have been virtually eliminated by the application of various prevention and control strategies. However, hitherto unrecognized diseases have emerged over the past half-century at the rate of at least one per year. Many of the viruses dealt with in this edition were unknown when the first edition was published over 45 years ago. The epidemiology of other viruses has radically changed as humans continue to alter the environment in so many ways. Meeting the challenges posed by emerging diseases requires the medical virologist to acquire ever more increasing expertise and access to ever more complex technologies. Today diseases such as HIV/AIDS, hepatitis C, influenza, and diarrheal diseases represent significant threats to public health. Tomorrow it will be other diseases, the nature and means of control for which are largely unpredictable. One positive note is that all emerging viral diseases of recent years have been found to be caused by members of previously recognized families of viruses. Thus a thorough knowledge of representative members of each family is likely to facilitate and inform the rapid development of knowledge about any new pathogen.

WHY STUDY VIROLOGY?

As many bacterial infections have succumbed to treatment with antibiotics, viral infections now pose proportionally a much greater threat to global public health than was the case, say, a half-century ago. Viral diseases exact a particularly heavy toll among young children and infants in

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the economically less developed nations where healthcare resources are limited. Ironically, there is a resurgence of interest in viruses that target bacteria (bacteriophages) as an alternative strategy for the control of some increasingly drug-resistant bacterial infections (e.g., cholera).

Although this book focuses on viral infections of medical significance, the reader needs to be aware that viruses are a major threat to livestock and plant species, and thereby of great importance in human nutrition and food supply. Human adaptation to diseases of livestock and crops has played a major role in the development of all civilizations.

Virology is much broader than linking a particular disease to a specific pathogen: there are literally hundreds of new viruses being discovered that do not apparently relate to any known pathological condition of either animals or humans. Many of these may in the future be linked to human illnesses and thus the reader needs to be aware of the wider scope of the virological landscape, if not in detail at least to the point of "expecting the unexpected." Conversely, the tantalizing goal remains to clarify what role, if any, viruses may play in well-known diseases of uncertain etiology, for example, multiple sclerosis.

The vast majority of new viral threats emerging annually either originate from an animal host (zoonosis) or are the result of host range extension (that is, "host species jumping"), or other changes in the epidemiology, ecology, and/or pathogenicity of the etiological agent. Since the last edition of this book, virus emergence has become a major focus of virological research.

The discovery of a new human pathogen often stimulates the discovery of related, but hitherto unidentified agents that may, or may not, present threats to human health at some point in the future. A prime example is the emergence of SARS virus, a coronavirus, and the subsequent explosion in our knowledge of coronaviruses of animals. This helped in the later rapid recognition of another human respiratory coronavirus—MERS coronavirus (Middle East Respiratory Syndrome coronavirus).

A BRIEF HISTORY OF VIROLOGY

The history of virology can be divided into a number of eras: these span (1) the discovery of viruses as entities distinct from other disease-causing pathogens, (2) the association of many major human diseases with causative viruses, (3) the development of methods for virus isolation and characterization, (4) the defining of the chemical properties of viruses, and (5) the design and application of vaccines and therapeutics. A summary of the major milestones in the development of virology is given in Table 1.1.

Virology has its foundations in the initial discoveries of bacteria and related diseases. Up to the 19th century the prevailing view was that diseases of humans and animals were the result of miasmas and other environmental influences. This was despite the thesis of Girolamo Fracastoro who suggested as early as 1546 that epidemic diseases were disseminated by minute particles carried over long distances. Anton van Leeuwenhoek first saw bacteria through his microscope in 1676 and Lazarro Spallanzani first grew bacteria in culture in 1775. Remarkably, Edward Jenner developed vaccination against smallpox in 1796 against a backdrop of prevailing opinion that such diseases were caused by environmental factors rather than specific microscopic agents.

The establishment of microbiology as a scientific discipline owes much to the work of Louis Pasteur, who in 1857 discovered the specificity of microbial fermentation, who then went on in 1865 to elaborate the nature of diseases of silkworms. But it was his work on rabies that signaled the start of the virus discovery era. In 1885, Pasteur looked on as his first rabies vaccine was given to a boy, Joseph Meister, bitten severely by a rabid dog, thus opening up the strategy of vaccine development through a process of virus attenuation (Fig. 1.1).

The early pioneering work of the 19th century linking disease to specific bacteria was greatly assisted by the earlier development of the unglazed porcelain ultrafilter by Charles Chamberland who worked in Pasteur's laboratory. These filters originally were used to sterilize water and other fluids by preventing the passage of bacteria. Dimitri Ivanovsky (1892) and Martinus Beijerinck (1898) showed that the agent causing mosaic disease in tobacco plants (now known to be tobacco mosaic virus [TMV]) passed through ultrafilters retaining bacteria. Beijerinck realized he was dealing with something other than a microbe but erroneously thought that the entity that passed through the ultrafilter was an infectious liquid and not a particle—he called it a "contagium vivum fluidum." Friedrich Loeffler and Paul Frosch were the first to correctly conclude that an ultrafilterable infectious agent was indeed a submicroscopic particle. Studying the cause of foot-and-mouth disease of cattle, Loeffler and Frosch found that the causative agent passed through a Chamberland ultrafilter but not the finer Kitasato ultrafilter. Thus these first virologists saw ultrafiltration in a new way—they focused attention on what passed through the ultrafilter rather than what was retained, and thereby established an experimental methodology widely adopted in the early 20th century. In quick succession, further diseases were shown to be caused by ultrafilterable agents: in 1900 the first human virus, yellow fever virus, and its mosquito transmission cycle was discovered by Walter Reed, James Carroll and the US Army Yellow Fever Commission in Havana, Cuba, a discovery that was guided by the earlier work of the Cuban physician Carlos Findlay (Fig. 1.2).

The concept of ultrafilterable infectious agents became more widely acceptable when Karl Landsteiner and Erwin Popper showed conclusively in 1909 that poliomyelitis was caused by an ultrafilterable agent. Importantly, as early as

TABLE 1.1 Some Milestones in the History of Virology

Date	Discoverer(s)	Discovery(ies)
1796	E. Jenner	Application of cowpox virus for vaccination against smallpox
1885	L. Pasteur	Development of rabies vaccine
1892	D. Ivanovsky, M. Beijerinck	Ultrafiltration of tobacco mosaic virus
1898	F. Loeffler, P. Frosch	Ultrafiltration of foot-and-mouth disease virus—clear proof of virus etiology of disease—discovery of the first virus
1898	G. Sanarelli	Discovery of myxoma virus
1900	W. Reed, J. Carroll, A. Agramonte, J. Lazear, C. Finlay	Discovery of yellow fever virus and its transmission by mosquitoes
1903	M. Remlinger, Riffat-Bay, A. di Vestea	Discovery of rabies virus
1907	P. Ashburn, C. Craig	Discovery of dengue viruses
1909	K. Landsteiner, E. Popper	Discovery of polioviruses
1911	P. Rous[a]	Discovery of the first tumor virus: Rous sarcoma virus
1911	J. Goldberger, J. Anderson	Discovery of measles virus
1915	F. Twort, F. d'Herelle	Discovery of bacterial viruses (bacteriophages)
1918		Beginning of global pandemic of influenza
1919	A. Löwenstein	Discovery of herpes simplex virus
1930	K. Meyer, C. Haring, B. Howitt	Discovery of Western equine encephalitis virus
1931	M. Theiler[a]	Attenuation of yellow fever virus—vaccine development
1933	C. Andrews, P. Laidlaw, W. Smith	Isolation of human influenza viruses in ferrets
1933	R. Muckenfuss, C. Armstrong, H. McCordock, L. Webster, G. Fite	Discovery of St. Louis encephalitis virus
1934	C. Johnson, E. Goodpasture	Discovery of mumps virus
1934	M. Hayashi, S. Kasahara, R. Kawamura, T. Taniguchi	Discovery of Japanese encephalitis virus
1935	W. Stanley[a]	Purification/crystallization of tobacco mosaic virus
1936	C. Armstrong, T. Rivers, E. Traub	Discovery of lymphocytic choriomeningitis virus
1937	L. Zilber, M. Chumakov, N. Seitlenok, E. Levkovich	Discovery of tick-borne encephalitis virus (Russian spring summer encephalitis virus)
1938	B. von Borries, H. Ruska, E. Ruska	First electron micrograph of viruses (ectromelia, vaccinia viruses)
1939	E. Ellis, M. Delbrück	Development of one-step growth curve—bacteriophage
1940	K. Smithburn, T. Hughes, A. Burke, J. Paul	Discovery of West Nile virus
1941	G. Hirst	Discovery of agglutination of red blood cells by influenza virus
1945	M. Chumakov, G. Courtois, colleagues	Discovery of Crimean-Congo hemorrhagic fever virus
1948	G. Dalldorf, G. Sickles	Discovery of Coxsackieviruses
1949	J. Enders[a], T. Weller[a], F. Robbins[a]	Development of cell culture methodology for polio, measles, and other vaccines
1950	L. Florio, M. Miller, E. Mugrage	Discovery of Colorado tick fever virus
1952	R. Dulbecco, M. Vogt	Development of plaque assay for animal viruses—polioviruses, Western equine encephalitis virus
1953	W. Rowe	Discovery of human adenoviruses

(*Continued*)

TABLE 1.1 Some Milestones in the History of Virology (Continued)

Date	Discoverer(s)	Discovery(ies)
1954	J. Salk, J. Youngner, T. Francis	Development of inactivated polio vaccine
1958	J. Lederberg[a]	Discovery of genetic recombination and the organization of the genetic material of bacteria
1959	A. Sabin, H. Cox, H. Koprowski	Development of attenuated live-virus polio vaccine
1962	A. Lwoff, R. Horne, P. Tournier	Classification of the viruses based on virion characteristics
1964	M. Epstein, B. Achong, Y. Barr	Discovery of Epstein–Barr virus and its association with Burkitt's lymphoma
1965	D. Tyrrell, M. Bynoe, J. Almeida	Discovery of human coronaviruses (B814 and 229E)
1965	F. Jacob[a], A. Lwoff[a], J. Monod[a]	Discoveries of genetic control of enzymes and virus synthesis: the operon
1967	B. Blumberg[a], H. Alter, A. Prince	Discovery of Australia antigen and its link to hepatitis B
1969	M. Delbrück[a], A. Hershey[a], S. Luria[a]	Discoveries related to the replication mechanism and the genetic structure of viruses
1970	H. Temin[a], D. Baltimore[a], R. Dulbecco[a]	Discoveries related to the interaction between tumor viruses and the genetic material of the cell—reverse transcriptase
1972	A. Kapikian, colleagues	Discovery of Norwalk virus (norovirus)
1973	R. Bishop, G. Davidson, I. Holmes, T. Flewett, A. Kapikian	Discovery of human rotaviruses
1973	S. Feinstone, A. Kapikian, R. Purcell	Discovery of hepatitis A virus
1975	Y. Cossart, A. Field, A. Cant, D. Widdows	Discovery of parvovirus B-19 and its association with aplastic crisis in hemolytic anemia
1975	P. Sharp[a], L. Chow, R. Roberts[a], T. Broker	Discovery of RNA splicing and split genes (adenovirus)
1976	D. C. Gajdusek[a]	Discovery of transmissible spongiform encephalopathies
1976	K. Johnson, P. Webb, J. Lange, F. Murphy, S. Pattyn, W. Jacob, G. Van der Groen, P. Piot, E. Bowen, G Platt, G. Lloyd, A. Baskerville, D. Simpson	Discovery of Ebola virus
1976	J. Bishop[a], H. Varmus[a]	Discovery of the cellular origin of retroviral oncogenes
1977	D. Henderson, F. Fenner, I. Arita, many others	Global eradication of smallpox
1978	D. Nathans[a], W. Arber[a], H. Smith[a]	Discovery of restriction enzymes and their application to problems of molecular genetics
1978	S. Harrison, M. Rossman, N. Olson, R. Kuhn, T. Baker, J. Hogle, M. Chow, R. Rueckert, J. Johnson	Atomic structure of viruses (tomato bushy stunt virus, polioviruses, rhinoviruses)
1980	P. Berg[a]	The development of recombinant-DNA technology
1980	R. Gallo, B. Poiesz, M. Yoshida, I. Miyoshi, Y. Hinuma	Discovery of human T lymphotropic viruses 1 and 2
1981	V. Racaniello, D. Baltimore	Development of an infectious recombinant clone of a virus (poliovirus)
1982	S. Prusiner[a]	Concept of the prion and their etiologic role in spongiform encephalopathies
1982	A. Klug[a]	Crystallographic electron microscopy and structural elucidation of biologically important nucleic acid–protein complexes

(*Continued*)

TABLE 1.1 Some Milestones in the History of Virology (Continued)

Date	Discoverer(s)	Discovery(ies)
1983	F. Barré-Sinoussi[a], L. Montagnier[a], J. Chermann	Discovery of human immunodeficiency virus 1 (HIV1)
1983	M. Balayan	Discovery of hepatitis E virus and its transmission
1985	F. Barin, F. Clavel, M. Essex, P. Kanki, F. Brun-Vézinet	Discovery of human immunodeficiency virus 2 (HIV2)
1988	G. Hitchings[a], G. Elion[a]	Discoveries of important principles for drug treatment—acyclovir
1989	M. Houghton, Q.-L. Choo, G. Kuo, D. Bradley, H. Alter	Discovery of hepatitis C virus
1993	S. Nichol, C. Peters, P. Rollin, T. Ksiazek	Discovery of Sin Nombre virus and its association with hantavirus cardiopulmonary syndrome
1994	Y. Chang, P. Moore	Discovery of human herpesvirus 8—Kaposi sarcoma herpesvirus
1995	K. Murray, P. Hooper, A. Hyatt	Discovery of Hendra virus and its reservoir host fruit bats
1996	P. Doherty[a], R. Zinkernagel[a]	Discovery of the genetic specificity of the cell-mediated immune response
1996	R. Will, J. Ironside, J. Collinge, colleagues	Discovery that bovine spongiform encephalopathy prion is the cause of variant Creutzfeldt–Jakob disease in humans
1999	K. Chua, S. Lam, W. Bellini, T. Ksiazek, B. Eaton, colleagues	Discovery of Nipah virus
1999	D. Asnis, M. Layton, W.I. Lipkin, R. Lanciotti	Extension of West Nile virus range to North America
2001	B. van den Hoogen, A. Osterhaus, colleagues	Discovery of human metapneumovirus
2003	C. Urbani, J. Peiris, S. Lai, L. Poon, G. Drosten, K. Stöhr, A. Osterhaus, T. Ksiazek, D. Erdman, C. Goldsmith, S. Zaki, J. DeRisi, others	Discovery of SARS coronavirus
2003	B. La Scola, D. Raoult, others	Discovery of mimivirus, the largest virus known at the time
2005	J. Taubenberger, P. Palese, T. Tumpey, A. Garcia-Sastre, others	1918 influenza virus genome sequenced and the virus reconstructed
2005		Beginning of global pandemic of chikungunya
2005	E. Leroy, J. Towner, R. Swanepoel, others	Discovery that the reservoir hosts of Ebola/Marburg viruses are bats
2007	T. Allander, D. Wang, Y. Chang, others	Discovery of human polyomaviruses KI, WU, MC
2008	H. zur Hausen[a]	Discovery that human papilloma viruses cause cervical cancer
2008	B. La Scola, D. Raoult, others	Discovery of virophage, Sputnik
2010	W. Plowright and the FAO Global Rinderpest Eradication Programme	Global eradication of rinderpest
2011	B. Hoffmann, M. Beer, T. Mettenleiter, colleagues	Discovery of Schmallenberg virus
2012	A.M. Zaki, R. Fouchier, W.I. Lipkin	Discovery of MERS coronavirus
2014		Beginning of an Ebola hemorrhagic fever epidemic in West Africa, the largest ever
2015		Beginning of a global epidemic of Zika virus disease—discovery of microcephaly as consequence of *in utero* infection

[a]*Scientists who were awarded the Nobel Prize for their work—cited at date of the discovery rather than the date of award.*

FIGURE 1.1 In 1881 and 1882, Louis Pasteur, Charles Chamberland, Émile Roux, and Louis Thuillier began their research toward developing a rabies vaccine. They modified Pierre-Victor Galtier's technique by inoculating nervous tissue from a rabid dog through a long series of dogs via subdural trephination. After many passages, they obtained a virus of maximum virulence and with a fixed incubation period of about 10 days. The degree of attenuation of virus recovered from each passage was measured and virus was then further attenuated in rabbits. This final attenuation procedure consisted of suspending the spinal cord of a rabid rabbit in a flask, in a warm dry atmosphere, to achieve slow desiccation. They succeeded in producing "attenuated viruses of different strengths," the weakest of which could be used to prepare the first dose of a vaccine. Inoculating dogs with a sequence of spinal cords of increasing virulence rendered the recipients resistant to inoculation with fully virulent virus. Within a year, Pasteur and his colleagues reported the results of this treatment in 350 cases of rabies exposure—only one person developed rabies, and this a child who was treated 6 days after exposure. Over the next decades many thousands of people with potential rabies exposures were immunized with ever-improving animal nervous system (brain and spinal cord) vaccines, at the Institut Pasteur in Paris, which was founded in 1888, and in other locations throughout the world. *Louis Pasteur, 1822–95. Painting by Albert Edelfeldt, 1885. From Institut Pasteur, used with permission.*

1911 Peyton Rous also showed similar properties for the etiologic agent of a sarcoma of chickens: Rous sarcoma virus was to play an essential role in determining the basic mechanism by which viruses may trigger the onset of tumors.

The realization that oncogenesis and virus infection went hand in hand was an important milestone in the early days of virology, although it took many decades for its true significance to be appreciated. In 1970 Howard Temin and David Baltimore independently were able to show that oncogenic viruses contain a reverse transcriptase enzyme, thus explaining how an RNA virus could produce DNA copies of its genetic material.

Bacteriophages were independently discovered by Frederick Twort and Felix d'Herelle (1915) who investigated outbreaks of dysentery among troops of the First World War. Presciently, Twort foresaw that the clear plaques in plated *Micrococcus* cultures could be caused by "ultrafilterable viruses."

During the following decades of the 20th century, it was thought by many that viruses represented infectious protein particles. This was a view reinforced by Wendell Stanley's description in 1935 that crystals of pure TMV could be dissolved and transmit infection to healthy plants—he presumed that the crystals were pure protein. This was dispelled, however, when Frederick Bawden and Norman Pirie showed that TMV contained not only protein but also nucleic acid. The importance of this was shown by the classic studies of Oswald Avery, Colin MacLeod, and Maclyn McCarty (1944) and then Alfred Hershey and Martha Chase (1952), who proved DNA was linked to hereditary.

In 1933 the electron microscope was invented by Ernst Ruska and Max Knoll and in 1938 Bodo von Borries, Helmut Ruska, and Ernst Ruska published the first electron micrographs of ectromelia (mousepox) virus and vaccinia virus. It soon became clear that there was great diversity in the size and shape of the various viruses. A major advance was the development of negative-contrast electron microscopy in 1959 by Sydney Brenner and Robert Horne. Using this method, electron-dense stains surround virus particles to produce a negative image of the virus with remarkable resolution; importantly in those early days of medical virology, the method was simple to use. Figs. 1.3 and 1.4 depict the diverse spectrum of morphological shapes represented by animal viruses. By the early 1960s, the fine structure of several viruses was unraveled by Aaron Klug, Donald Caspar, and others using X-ray crystallography—they showed that many viruses are constructed from uniform subunits, following the principles of icosahedral symmetry as first understood for the Platonic solids (regular polyhedra) by the ancient Greeks. Thus through the use of several different approaches the diversity of structural detail among various viruses began to emerge.

Attempts to prevent virus disease using vaccines have paralleled the development of virology, beginning from the early pioneering days of Edward Jenner and Louis Pasteur. Notable developments included the attenuated yellow fever vaccine developed by Max Theiler in 1931, a vaccine that is still in widespread use today and has saved countless thousands of lives. Jonas Salk and Albert Sabin in 1954 and 1959 developed non-replicating (inactivated) and living attenuated virus vaccines against poliovirus, respectively, the use of which has been so extensive that poliovirus infection has all but been eradicated save for a few pockets of infection

FIGURE 1.2 In 1900, Walter Reed and his colleagues discovered yellow fever virus, the first human virus, and its transmission cycle. This is a famous allegorical painting, entitled *Conquerors of Yellow Fever* by Dean Cornwell. It depicts Walter Reed (in white uniform) and Carlos Finlay (with white hair) looking on as Jesse Lazear, who died of yellow fever a month later, applies an infected mosquito to the arm of James Carroll. The painting includes Aristides Agramonte (behind Lazear), Leonard Wood (in brown helmet), Jefferson Kean (in white helmet), and several of the volunteers who subsequently were infected in the same way. Carroll became infected as a result of this experiment—he survived, and went on to have a distinguished career as a microbiologist, but suffered from chronic illness leading to an early death, said to be a consequence of his yellow fever infection. *Purchased copy, used with permission.*

in remote parts of the world (Fig. 1.5). As is described in Chapter 11: Vaccines and Vaccination, vaccine research has often exploited novel concepts, for example, the use of plasma from chronically infected humans as a source of hepatitis B virus (HBV) envelope protein to stimulate immunity against hepatitis B virus (1976), and the use of genetically modified naked DNA preparations to induce the expression of antigens in the tissues of vaccine recipients.

In 1957, Alick Isaacs and Jean Lindemann discovered interferons, molecules that represent the initial mammalian response to infection. Great hope was placed on the use of interferons in the treatment of a wide spectrum of human virus infections: although of proven use in certain conditions, however, the use of interferons has not lived up to the earlier wide promise suggested by laboratory studies.

The sciences of immunology and cell and molecular biology have been intertwined with that of virology: landmark discoveries were made by Peter Doherty and Rolf Zinkernagel, who in 1974 discovered how the cellular immune system recognizes virus-infected cells; and Georges Kohler and Cesar Milstein, who in 1975 developed the first monoclonal antibodies.

THE VIROSPHERE

We live in what many now describe as the *virosphere*, since almost all living multicellular and unicellular organisms are susceptible to virus infection. Take as an example the oceans: every liter of seawater is populated with up to 10 billion viruses. It is estimated that there are around 5×10^{30} bacteria on planet Earth, and that viruses are numerically at least more common; this means there are more viruses in the world than all life forms. The vast majority are most likely viruses of bacteria (bacteriophages) serving to aid the recycling of organic matter, but some have a more sophisticated role in the environment, for example, determining insect behavior as an essential part of an arthropod life cycle. This staggeringly large repertoire of

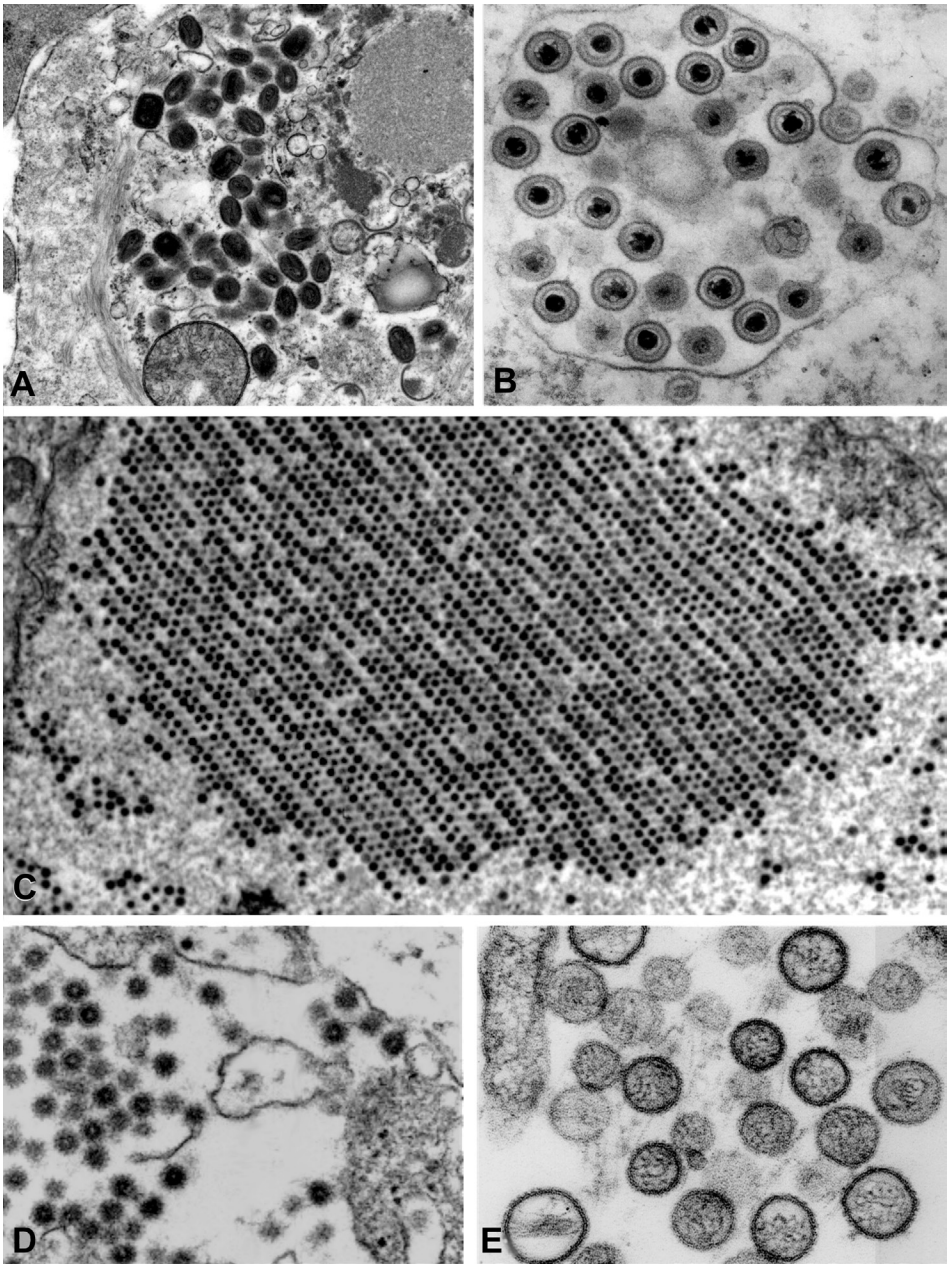

FIGURE 1.3 Thin-section electron microscopy of selected viruses. The remarkable diversity of the viruses is clearly revealed by thin-section electron microscopy of infected cells—and this technique provides important information about morphogenesis and cytopathology. (A) Family *Poxviridae*, genus *Orthopoxvirus*, variola virus. (B) Family *Herpesviridae*, genus *Simplexvirus*, human herpesvirus 1. (C) Family *Adenoviridae*, genus *Mastadenovirus*, human adenovirus 5. (D) Family *Togaviridae*, genus *Alphavirus*, Eastern equine encephalitis virus. (E) Family *Bunyaviridae*, genus *Hantavirus*, Sin Nombre virus. These images represent various magnifications; the details of the morphogenesis of the various viruses are given in the chapters of Part II of this book.

the virosphere is not restricted to inhabiting non-human life forms: we are only recently beginning to study the range of different viruses that humans appear to carry permanently (the human "virome," see Chapter 39: Viral Syndromes), yet appear to cause no harmful effects. One example is the Torque teno (TT) virus, discovered by chance in 1997 during studies of "transfusion-transmitted" infection.

THE NATURE OF VIRUSES

The unicellular *microorganisms* can be arranged in the 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*. Such microorganisms contain DNA as the

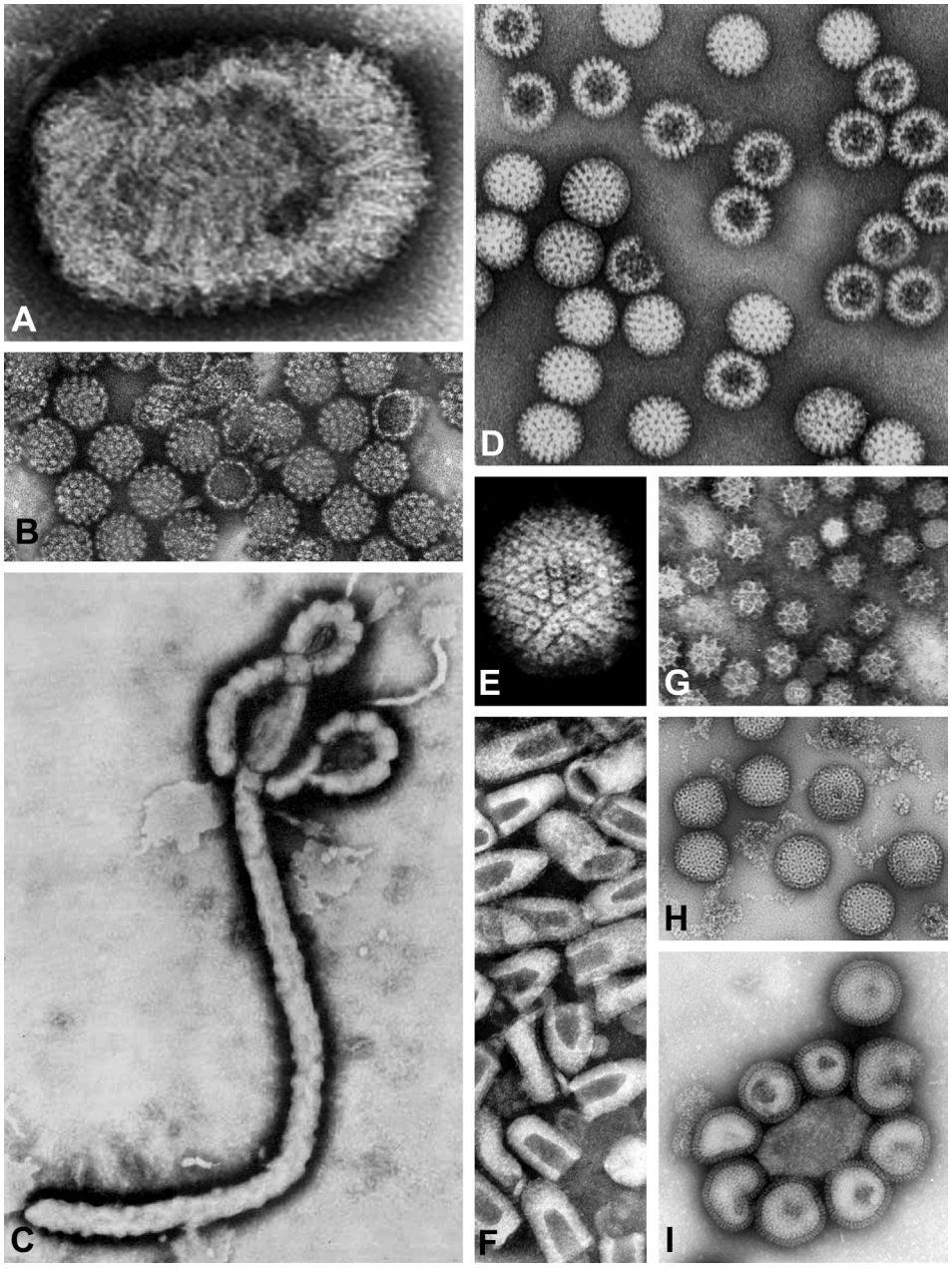

FIGURE 1.4 Negative contrast electron microscopy of selected viruses. The remarkable diversity of the viruses is revealed by all kinds of electron microscopy methods, but none better than by negative staining. (A) Family *Poxviridae*, genus *Orthopoxvirus*, vaccinia virus. (B) Family *Papovaviridae*, genus *Papillomavirus*, human papillomavirus. (C) Family *Filoviridae*, Ebola virus. (D) Family *Reoviridae*, genus *Rotavirus*, human rotavirus. (E) Family *Herpesviridae*, genus *Simplexvirus*, human herpesvirus 1 (capsid only, envelope not shown). (F) Family *Rhabdoviridae*, genus *Lyssavirus*, rabies virus. (G) Family *Caliciviridae*, genus *Norovirus*, human norovirus. (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; the size of the various viruses is given in Chapter 2: Classification of Viruses and Phylogenetic Relationships and in the chapters of Part II of this book.

repository of genetic information, and also contain various species of RNA and most, if not all, of the machinery for producing energy and macromolecules. These microorganisms grow by synthesizing macromolecular constituents (nucleic acids, proteins, carbohydrates, and lipids), and most multiply by binary fission.

Viruses, on the other hand, are neither cellular nor microorganisms. The key differences between viruses and microorganisms are listed in Table 1.2. Viruses do not possess functional organelles (e.g., mitochondria, Golgi, chloroplasts, and endoplasmic reticulum), and thus are totally dependent on the host for the machinery of energy

FIGURE 1.5 The World Health Organization Global Polio Eradication Initiative aims for global eradication of poliomyelitis by about 2018. The Initiative is led by the World Health Organization, UNICEF, and the Rotary and Gates Foundations; it has reduced the number of cases from the many thousands per year to less than 100 (359 cases in 2014; 74 cases in 2015). Polio will be the third disease globally eradicated, after smallpox and rinderpest. The most important step in polio eradication is interruption of endemic transmission by universal infant vaccination using oral vaccine (OPV; often by organizing "national immunization days"), supplementary IPV vaccination campaigns where needed, intensive surveillance of cases of flaccid paralysis, and in some places detection of virus in sewage. Figures (clockwise from top left). An Egyptian stele (slab) thought to depict a polio victim—18th Dynasty (1403–1365 BC); patients with permanent respiratory muscle paralysis after recovery from poliomyelitis would spend the rest of their lives requiring assisted respiration (immersed in an "iron lung"); patients with permanent lower limb weakness following poliomyelitis; those remaining countries reporting cases of poliomyelitis in 2014; oral administration of polio vaccine; in 1921, 39-year-old Franklin D. Roosevelt was diagnosed with poliomyelitis and was left with permanent paralysis from the waist down, but was rarely photographed in a wheelchair. He was elected US president in 1932.

TABLE 1.2 Contrasting Properties of Unicellular Microorganisms and Viruses

Property	Bacteria	Rickettsiae	Mycoplasma	Chlamydiae	Viruses
>300 nm diameter[a]	Yes	Yes	Yes	Yes	No
Growth on non-living media[b]	Yes	No	Yes	No	No
Binary fission	Yes	Yes	Yes	Yes	No
Contain both DNA and RNA	Yes	Yes	Yes	Yes	No[c]
Infectious nucleic acid	No	No	No	No	Many
Functional ribosomes	Yes	Yes	Yes	Yes	No
Sensitivity to antibiotics	Yes	Yes	Yes	Yes	No[d]

[a]*Some mycoplasmas and chlamydiae are less than 300 nm in diameter, and mimiviruses and the other new "giant DNA viruses" are greater than 300 nm in diameter.*
[b]*Chlamydiae and most rickettsiae are obligate intracellular parasites.*
[c]*A few viruses contain both types of nucleic acid, but one of these types acts as the main functional molecule and the other plays a minor role.*
[d]*With very few exceptions.*

production and synthesis of macromolecules. Viruses contain only one type of functional nucleic acid, either DNA or RNA, never both, and differ from microorganisms in having a life cycle divisible into two clearly defined phases. Outside of the host cell, the viruses are metabolically inert and can be considered as complexes of large macromolecules; during this extracellular phase of the viral life cycle, virus transmission is dependent upon movements of air and fluid, and in some cases the life cycle of insect vectors. Once inside the host cell, however, viruses behave with many of the properties of living organisms; viruses are metabolically active in that the viral genome exploits the machinery of the host to produce progeny genome copies, viral messenger RNA, and viral proteins (often along with carbohydrates and lipids), all of which are then assembled to form new virions (*virion*, the complete virus particle). This assembly from pools of precursor molecules is in contrast to the multiplication of cellular organisms by binary fission. In contrast to any microorganism, many viruses can reproduce even if only the viral DNA or RNA genome 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, but mostly not.

Given the unique characteristics of viruses, where might viruses have originated? There are three principal theories that have been argued for many years. First, viruses may have originated as escaped eukaryotic genes, that is nucleic acid sequences, that evolved to encode protective protein coats to allow survival outside of the environment of the cell (transposons and retrotransposons have been suggested as the progenitors of retroviruses). Second, viruses may be degenerate forms of intracellular parasites, having lost most cellular functions (bacteria have been suggested as the progenitors of mitochondria, chloroplasts, and poxviruses); and third, viruses may have originated independently along with other primitive molecules and developed with self-replicating capabilities.

In the absence of fossil remains, insight as to virus evolution relies almost entirely on sequence analyses of virus genomes. For example, the genome of a plant viroid (a subviral agent comprised of infectious naked RNA), potato spindle tuber viroid, seems to be a self-replicating RNA copy of a part of the host potato DNA. Many of the genes of poxviruses are similar to those of eukaryote hosts. In any case, it seems certain from sequence analyses of viral genomes that all presently recognized viruses did not evolve from a single progenitor; rather, different kinds of viruses likely arose independently from different origins, and then continued to diversify and adapt survival and transmission qualities to better fit particular niches by the usual Darwinian process of mutation and natural selection.

It should be stressed that the genetic blueprint of all viruses is under continuing evolutionary pressure, sometimes showing dramatic examples of genetic change and natural selection of those variants that survive the best. Some viruses have continued to evolve in long association with each associated hosts (e.g., herpesviruses, some retroviruses); 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 life forms: for example, some viruses can persist for the lifetime of the host cell by the integration of the DNA genome (or a DNA copy of the RNA genome) into the genome of the host cell, or by the carriage of viral DNA genomes by the host cell in episomal form. Since viruses use the replicative machinery of the host, virus infections present major challenges to antiviral drug development. Drugs that interfere with viral replication nearly always interfere with essential host cell functions. This is in contrast to bacteria, which have unique metabolic pathways different from those of the host, enabling these to be exploited as targets for antibiotics.

The simplest viruses consist of a DNA or RNA genome contained within a protein coat, but there are classes of even simpler infectious agents: (1) *satellites*, which are defective viruses, dependent upon a helper virus to supply essential functions such as nucleic acid replication functions or structural elements such as capsid proteins; (2) *viroids*, which as noted above consist of a naked RNA molecule that is infectious; and (3) *prions*, the agents of the spongiform encephalopathies, consisting of an infectious protein without any associated nucleic acid.

SCOPE OF THIS BOOK

From its beginning medical virology has been intertwined with many related sciences. Even though this book deals with medical virology *per se*—the viruses infecting humans and the diseases so caused—understanding the full scope of the subject requires a continuing appreciation and integration of related sciences, from cell biology to medical epidemiology and extending to human social behavior. The perspective represented by this book, of medical virology as an infectious disease science, is meant to provide a starting point, an anchor, for those who must relate the subject to clinical practice, public health practice, scholarly research, and other endeavors.

Part I of this book thus deals with the properties of viruses, how viruses replicate, and how viruses cause disease. These chapters are then followed by an overview of the principles of diagnosis, epidemiology, and how virus infections can be controlled. This first section is concluded by a discussion on emergence and attempts to predict the next major public health challenges. These form a guide for delving into the specific diseases of interest to the reader as described in Part II.

FURTHER READING

Crawford, D.H., 2011. A Very Short Introduction to Viruses. Oxford University Press, Oxford.

Flint, S.J., Racaniello, V.R., Rall, G.F., Skalka, A.M., Enquist, L., 2015. Principles of Virology, two volumes, fourth ed. ASM Press, Washington, DC, ISBN-10: 1555819516.

Kaslow, R.A., Stanberry, L.R., LeDuc, J.W., 2014. Viral Infections of Humans, Epidemiology and Control, fifth ed. Springer, New York, ISBN 978-1-4899-7447-1.

Knipe, D.M., Howley, P.M., et al., 2013. Field's Virology, sixth ed. Lippincott Williams and Wilkins, Philadelphia, ISBN 978-145-110563-6.

MacLachlan, N.J., Dubovi, E.J., 2011. Fenner's Veterinary Virology, fourth ed. Academic Press, London, ISBN 978-0-12-375158-4.

Waterson, A.P., Wilkinson, L., 1978. An Introduction to the History of Virology. Cambridge University Press, Cambridge, ISBN 978-0-521-21917-5.

Zimmer, C., 2011. A Planet of Viruses. University of Chicago Press, Chicago, ISBN 978-0-226-98335-6.

Zinsser, H., 1935, reprinted 2007. Rats, Lice and History. Transaction Publishers, New Brunswick, USA, ISBN 978-1-4128-0672-5.

Chapter 2

Classification of Viruses and Phylogenetic Relationships

Virus taxonomy brings into sharp focus the debate about the true nature of viruses. A comprehensive classification system should define boundaries within what may at first appear as a continuum of properties. This is often most challenging at the level of genome sequence analysis.

The rules and processes that have been developed are unique to the science of virology, and necessary to accommodate the astonishing variety of viruses. There is now evidence that probably all organisms in the biological world may be infected by at least one virus. Indeed it has been estimated that viruses represent the most abundant biological entities on the planet, existing as pathogens or silent passengers in humans and other animals, plants, invertebrates, protozoa, fungi, and bacteria. To date more than 4000 different viruses and 30,000 different strains and subtypes have been recognized, with particular strains and subtypes often having significant public health importance. Several hundred different viruses are known to cause disease in humans, although this is a small fraction of those viruses encountered in the surrounding environment. Since all viruses, whatever the host, share the properties described in the preceding chapter, virologists have developed a single system of classification and nomenclature that covers all viruses—this is a system overseen by the *International Committee on Taxonomy of Viruses* (ICTV). One challenge of virus classification is to define evolutionary relationships between viruses when minor changes in molecular structures may give rise to pathogens with radically different properties (Fig. 2.1).

VIRAL TAXONOMY

Although it is hierarchical and at most levels reflects evolutionary relationships, the taxonomy of viruses is deliberately non-systematic—that is, there is no intent to relate all viruses to an ancient evolutionary root—in fact, there is good evidence for several separate roots. The earliest efforts to classify viruses were based upon host organism species, common clinical and pathological properties, tropism for particular tissues and organs, and common ecological and transmission characteristics. For example, viruses that cause hepatitis (e.g., hepatitis A virus, family *Picornaviridae*; hepatitis B virus, family *Hepadnaviridae*; hepatitis C virus, family *Flaviviridae*; and Rift Valley fever virus, family *Bunyaviridae*) might have been brought together as "the hepatitis viruses." Such systems have now been superseded.

The initial principles for identifying and distinguishing different viruses involved giving equal weight to the importance of:

1. type of nucleic acid (DNA or RNA);
2. virion size, as determined by ultrafiltration and electron microscopy;
3. virion morphology, as determined by electron microscopy;
4. virion stability, as determined by varying pH and temperature, exposure to lipid solvents and detergents, etc.; and
5. virion antigenicity, as determined by various serological methods.

This approach was practicable in the era before molecular biology, as these characteristics had already been determined for a large number of viruses, and thus these properties could be used to build a taxonomic framework. Subsequently it has been necessary in most cases to determine only a few characteristics in order to place a newly described virus into an established taxon, as a starting point for further work to define its relationship with other members. For example, an isolate from the respiratory tract of a child with croup, identified by negative contrast electron microscopy as an adenovirus, might be submitted immediately for serological identification—it would certainly turn out to be a member of the family *Adenoviridae*, genus *Mastadenovirus* (the adenoviruses of mammals), and would be serologically identified as one of the >50 human adenoviruses—or perhaps, it would turn out to be a new human adenovirus!

Nowadays, the primary criteria for delineation of the main viral taxa are:

1. the type, character, and nucleotide sequence of the viral genome;
2. the strategy of viral replication; and
3. the structure of the virion.

Fenner and White's Medical Virology. DOI: http://dx.doi.org/10.1016/B978-0-12-375156-0.00002-3

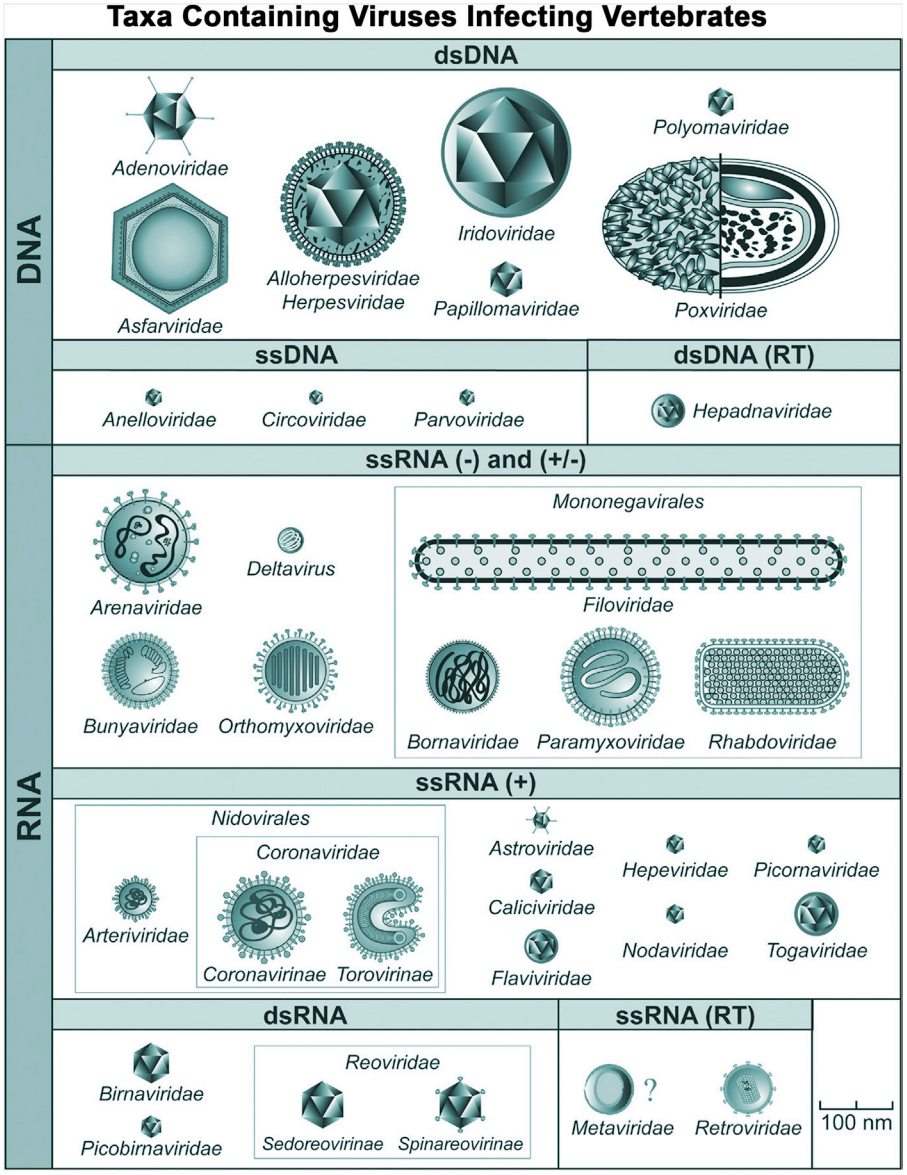

FIGURE 2.1 Diagram illustrating the shapes and sizes of viruses that infect vertebrates. The virions are drawn to scale, but artistic license has been used in representing their structure. In some, the cross-sectional structures of capsid and envelope are shown, with a representation of the genome; with the very small virions, only their size and symmetry are depicted. *Reproduced from King, A.M.Q., Adams, M.J., Carstens, E.B., Lefkowitz, E.J. (Eds.), 2011. Virus taxonomy, classification and nomenclature of viruses. In: Ninth Report of the International Committee for the Taxonomy of Viruses. Academic Press, London, with permission.*

Sequencing, or partial sequencing, of the viral genome provides powerful taxonomic information and now is often done very early in the identification process. Reference genome sequences for all viral taxa are available in public databases (e.g., GenBank, National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, Bethesda, Maryland, United States: <http://www.ncbi.nlm.nih.gov>). Such an approach in most cases allows one to immediately place a virus in a specific taxon.

The universal system of viral taxonomy recognizes five levels, namely *order*, *family*, *subfamily*, *genus*, and *species*. The names of orders end with the suffix *-virales*, families with the suffix *-viridae*, subfamilies with the suffix *-virinae*, and genera with the suffix *-virus*. The names of species also end with the term *virus*, either as a separate word or as a suffix (according to historic precedence). Lower levels, such as *subspecies*, *strains*, and *variants*, are established for practical purposes such as diagnostics, vaccine development, etc., but this is not a matter of formal

classification and there are neither universal definitions nor is there any standard universal nomenclature.

As of 2015 the universal taxonomy system for viruses encompasses seven orders, four of which contain human and animal pathogens (*Picornavirales*, *Herpesvirales*, *Mononegavirales*, and *Nidovirales)*, and 78 families, 27 of which contain human and/or animal pathogens, 348 genera, and 2285 species of viruses (Table 2.1). This situation is constantly changing, and the interested reader should consult the ICTV website for updates (http://www.ictvonline.org). The universal taxonomy system is nearly complete at the level of families and genera; that is, virtually

TABLE 2.1 Major Families of Viruses Infecting Vertebrates—A Subset of the ICTV Universal Virus Taxonomy System, 2015

Family	Subfamily	Genus	Type Species	Viruses Infecting Humans
Double-Stranded DNA Viruses				
Poxviridae	*Chordopoxvirinae*	*Orthopoxvirus*	*Vaccinia virus*	Smallpox (variola)
		Capripoxvirus	*Sheeppox virus*	
		Leporipoxvirus	*Myxoma virus*	
		Suipoxvirus	*Swinepox virus*	
		Molluscipoxvirus	*Molluscum contagiosum virus*	Molluscum contagiosum virus
		Avipoxvirus	*Fowlpox virus*	
		Yatapoxvirus	*Yaba monkey tumor virus*	Yaba monkey tumor virus; Tanapox virus
		Parapoxvirus	*Orf virus*	Orf virus
		Cervidpoxvirus	*Deerpox virus*	
Asfarviridae		*Asfivirus*	*African swine fever virus*	
Iridoviridae		*Ranavirus*	*Frog virus 3*	
		Lymphocystivirus	*Lymphocystis disease virus 1*	
		Megalocytivirus	*Infectious spleen and kidney necrosis virus*	
Alloherpesviridae		*Ictalurivirus*	*Ictalurid herpesvirus 1*	
Herpesviridae	*Alphaherpesvirinae*	*Simplexvirus*	*Human herpesvirus 1*	Herpes simplex viruses 1 and 2
		Varicellovirus	*Human herpesvirus 3*	Varicella-zoster virus
		Mardivirus	*Gallid herpesvirus 2*	
		Iltovirus	*Gallid herpesvirus 1*	
	Betaherpesvirinae	*Cytomegalovirus*	*Human herpesvirus 5*	Human cytomegalovirus
		Muromegalovirus	*Murid herpesvirus 1*	
		Proboscivirus	*Elephantid herpesvirus 1*	
		Roseolovirus	*Human herpesvirus 6*	Human herpesviruses 6 and 7
	Gammaherpesvirinae	*Lymphocryptovirus*	*Human herpesvirus 4*	Epstein-Barr virus
		Macavirus	*Alcelaphine herpesvirus 1*	
		Percavirus	*Equid herpesvirus 2*	
		Rhadinovirus	*Saimiriine herpesvirus 2*	Human herpesvirus 8 (Kaposi sarcoma-associated virus)

(*Continued*)

TABLE 2.1 Major Families of Viruses Infecting Vertebrates—A Subset of the ICTV Universal Virus Taxonomy System, 2015 (Continued)

Family	Subfamily	Genus	Type Species	Viruses Infecting Humans
Malacoherpesviridae		*Ostreavirus*	*Ostreid herpesvirus 1*	
Adenoviridae		*Mastadenovirus*	*Human adenovirus C*	Human adenoviruses A–G
		Aviadenovirus	*Fowl adenovirus A*	
		Atadenovirus	*Ovine adenovirus D*	
		Siadenovirus	*Frog adenovirus*	
		Ichtadenovirus	*Sturgeon adenovirus A*	
Polyomaviridae		*Polyomavirus*	*Simian virus 40*	JC polyomavirus BK polyomavirus; others
Papillomaviridae		*Alphapapillomavirus*	*Human papillomavirus 32*	Human papillomaviruses, many
		Betapapillomavirus	*Human papillomavirus 5*	Human papillomaviruses, many
		Gammapapillomavirus	*Human papillomavirus 4*	Human papillomaviruses, many
		Deltapapillomavirus	*Europeam elk papillomavirus 1*	
		Epsilonpapillopmavirus	*Bovine papillomavirus 5*	
		Zetapapillomavirus	*Equine papillomavirus 1*	
		Etapapillomavirus	*Fringilla coelebs papillomavirus*	
		Thetapapillomavirus	*Psittacus erithacus timneh papillomavirus*	
		Iotapapillomavirus	*Mastomys natalensis papillomavirus*	
		Kappapapillomavirus	*Cottontail rabbit papillomavirus*	
		Lambdapapillomavirus	*Canine oral papillomavirus*	
		Mupapillomavirus	*Human papillomavirus 1*	Human papillomaviruses 1 and 63
		Nupapillomavirus	*Human papillomavirus 41*	Human papillomavirus 41
		Xipapillomavirus	*Bovine papillomavirus 3*	
		Omicronpapillomavirus	*Phocoena spinipinnis papillomavirus*	
		Pipapillomavirus	*Hamster oral papillomavirus*	
Single-Stranded DNA Viruses				
Parvoviridae	*Parvovirinae*	*Parvovirus*	*Minute virus of mice*	
		Erythrovirus	*Human parvovirus B19*	Human parvovirus B19
		Dependovirus	*Adeno-associated virus 2*	AAV 1–5
		Amdovirus	*Aleutian mink disease virus*	
		Bocavirus	*Bovine parvovirus*	Human bocaviruses 1–4
Circoviridae		*Circovirus*	*Porcine circovirus 1*	
		Gyrovirus	*Chicken anaemia virus*	
Anelloviridae		*Alphatorquevirus*	*Torque teno virus*	TTV groups 1–5

(*Continued*)

TABLE 2.1 **Major Families of Viruses Infecting Vertebrates—A Subset of the ICTV Universal Virus Taxonomy System, 2015** (Continued)

Family	Subfamily	Genus	Type Species	Viruses Infecting Humans
Reverse Transcribing Viruses				
Hepadnaviridae (DNA genome)		*Orthohepadnavirus*	*Hepatitis B virus*	Hepatitis B virus genotypes A–H
		Avihepadnavirus	*Duck hepatitis B virus*	
Retroviridae (RNA genome)	*Orthoretrovirinae*	*Alpharetrovirus*	*Avian leukosis virus*	
		Betaretrovirus	*Mouse mammary tumor virus*	
		Gammaretrovirus	*Murine leukaemia virus*	
		Deltaretrovirus	*Bovine leukaemia virus*	Human T-lymphotropic viruses 2 and 3
		Epsilonretrovirus	*Walleye dermal sarcoma virus*	
		Lentivirus	*Human immunodeficiency virus*	HIV-1 and HIV-2
	Spumaretrovirinae	*Spumavirus*	*Simian foamy virus*	
Double-Stranded RNA Viruses				
Reoviridae	*Sedoreovirinae*	*Orbivirus*	*Bluetongue virus*	African horse sickness virus; Kemerovo virus
		Rotavirus	*Rotavirus A*	Rotaviruses A–E; others
		Seadornavirus	*Banna virus*	Banna virus
	Spinoreovirinae	*Coltivirus*	*Colorado tick fever virus*	Colorado tick fever virus
		Orthoreovirus	*Mammalian orthoreovirus*	Mammalian orthoreoviruses 1–4
		Aquareovirus	*Aquareovirus A*	
		Cardoreovirus	*Eriocheir sinensis reovirus*	
Picobirnaviridae		*Picobirnavirus*	*Human picobirnavirus*	Human picobirnavirus
Single-Stranded Negative-Sense RNA Viruses				
Paramyxoviridae	*Paramyxovirinae*	*Respirovirus*	*Sendai virus*	Human parainfluenzaviruses 1 and 3
		Avulavirus	*Newcastle disease virus*	
		Morbillivirus	*Measles virus*	Measles virus
		Rubulavirus	*Mumps virus*	Mumps virus; Human parainfluenzaviruses 2 and 4
		Avulavirus	*Newcastle disease virus*	
		Henipavirus	*Hendra virus*	Hendra virus; Nipah virus
	Pneumovirinae	*Pneumovirus*	*Human respiratory syncytial virus*	Human respiratory syncytial virus
		Metapneumovirus	*Avian pneumovirus*	Human metapneumovirus

(*Continued*)

TABLE 2.1 Major Families of Viruses Infecting Vertebrates—A Subset of the ICTV Universal Virus Taxonomy System, 2015 (Continued)

Family	Subfamily	Genus	Type Species	Viruses Infecting Humans
Rhabdoviridae		*Vesiculovirus*	*Vesicular stomatitis Indiana virus*	
		Lyssavirus	*Rabies virus*	Rabies virus; others
		Ephemerovirus	*Bovine ephemeral fever virus*	
		Novirhabdovirus	*Infectious haematopoietic necrosis virus*	
Filoviridae		*Marburgvirus*	*Lake Victoria marburgvirus*	Lake Victoria marburgvirus
		Ebolavirus	*Zaire ebolavirus*	Zaire; Taï Forest; Reston; Sudan ebolaviruses
Bornaviridae		*Bornavirus*	*Borna disease virus*	Borna disease virus
Orthomyxoviridae		*Influenzavirus A*	*Influenza A virus*	Influenza A virus
		Influenzavirus B	*Influenza B virus*	Influenza B virus
		Influenzavirus C	*Influenza C virus*	Influenza C virus
		Thogotovirus	*Thogoto virus*	Thogoto virus
		Isavirus	*Infectious salmon anaemia virus*	
Bunyaviridae		*Orthobunyavirus*	*Bunyamwera virus*	Bunyamwera virus; California encephalitis virus; Oropouche virus; others
		Hantavirus	*Hantaan virus*	Hantaan virus, Sin Nombre virus; others
		Nairovirus	*Dugbe virus*	Crimean-Congo haemorrhagic fever virus
		Phlebovirus	*Rift Valley fever virus*	Rift Valley fever virus; Sandfly fever Naples virus
Arenaviridae		*Arenavirus*	*Lymphocytic choriomeningitis virus*	Old World (Lassa virus); New World (Junin virus, Machupo virus, others)
Single-Stranded Positive-Sense RNA Viruses				
Coronaviridae	*Coronavirinae*	*Alphacoronavirus*	*Alphacoronavirus 1*	Human coronaviruses 229E and NL43
		Betacoronavirus	*Murine coronavirus*	Human coronavirus HKU1 SARS-related coronaviruses; MERS coronavirus
		Gammacoronavirus	*Avian coronavirus*	
		Torovirus	*Equine torovirus*	Human torovirus
Arteriviridae		*Arterivirus*	*Equine arteritis virus*	
Roniviridae		*Okavirus*	*Gill-associated virus*	
Picornaviridae		*Enterovirus*	*Human enterovirus C*	Human enteroviruses A–D (including polioviruses)
		Rhinovirus	*Human rhinovirus A*	Human rhinoviruses A–C (>100 serotypes)

TABLE 2.1 Major Families of Viruses Infecting Vertebrates—A Subset of the ICTV Universal Virus Taxonomy System, 2015 (Continued)

Family	Subfamily	Genus	Type Species	Viruses Infecting Humans
		Erebovirus	*Equine rhinitis B virus*	
		Hepatovirus	*Hepatitis A virus*	Hepatitis A virus
		Cardiovirus	*Encephalomyocarditis virus*	
		Aphthovirus	*Foot-and-mouth disease virus*	
		Parechovirus	*Human parechovirus*	Human parechoviruses 1–16
		Kobuvirus	*Aichi virus*	Aichi virus
		Teschovirus	*Porcine teschovirus*	
		Sapelovirus	*Porcine sapelovirus*	
		Senecavirus	*Seneca Valley virus*	
		Tremovirus	*Avian encephalomyelitis virus*	
		Avihepatovirus	*Duck hepatitis A virus*	
Caliciviridae		*Vesivirus*	*Vesicular exanthema of swine virus*	
		Lagovirus	*Rabbit haemorrhagic disease virus*	
		Norovirus	*Norwalk virus*	Norwalk viruses
		Sapovirus	*Sapporo virus*	Sapporo viruses
		Nebovirus	*Newbury-1 virus*	
Astroviridae		*Mamastrovirus*	*Human astrovirus*	Human astroviruses 1–8
		Avastrovirus	*Turkey astrovirus*	
Togaviridae		*Alphavirus*	*Sindbis virus*	Equine encephalitis viruses; Ross River and Barmah Forest viruses; Chikungunya virus; others
		Rubivirus	*Rubella virus*	Rubella virus
Flaviviridae		*Flavivirus*	*Yellow fever virus*	Yellow fever virus; Dengue viruses 1–4; Japanese encephalitis virus group; Tick-borne viruses; others
		Pestivirus	*Bovine viral diarrhoea virus type 1*	
		Hepacivirus	*Hepatitis C virus*	Hepatitis C virus, 7 genotypes
Hepeviridae		*Orthohepevirus*	*Orthohepevirus A*	Hepatitis E viruses 1–4
Unassigned				
(single-stranded circular RNA)		*Deltavirus*	*Hepatitis delta virus*	HDV 1–8

all of the viruses mentioned in this book have been placed within a family and assigned to a genus, although there are some "floating genera" where family construction is not yet complete. Subfamilies are used only where needed to deal with very complex interrelationships among the viruses within a particular family.

Virus families are broadly divisible into those with DNA or RNA genomes respectively. Viruses within each family possess broadly similar genome structure, virion morphology, and replication strategy. *Subfamilies* are distinguished in cases where some members of a family can be grouped as possessing distinct and unique properties.

Orders are used to group together those virus families with related but distant phylogenetic properties (e.g., conserved genes, sequences, or domains). Again, since all viruses did not derive from a common ancestor, there is no intent to construct a unified viral evolutionary tree.

Genera are used to bring together viruses with clear, important evolutionary, and biological relationships, which are also usually reflected in antigenic, host range, epidemiological, and/or other relationships.

Species is the most important taxon in the systems used to classify all life forms, but it is also the most difficult to both define and use—this is especially the case with regard to viruses. In recent years the ICTV has determined criteria for defining virus species—different criteria are being used for different families. After some controversy, the ICTV recently redefined the term species:

> A species is a monophyletic ("relating to or descended from one source or taxon") group of viruses, whose properties can be distinguished from those of other species by multiple criteria. The criteria by which different species within a genus are distinguished shall be established by the appropriate Study Group. These criteria may include, but are not limited to, natural and experimental host range, cell and tissue tropism, pathogenicity, vector specificity, antigenicity, and the degree of relatedness of their genomes or genes…

Below the species level, the identification of particular lineages within an individual virus species is often extremely important because of clinical, epidemiological, or evolutionary significance. Such lineages may be designated as serotypes, genotypes, subtypes, variants, escape mutants, vaccine strains, etc. Many different conventions are used for naming at this level, depending on the virus involved—these distinctions lie outside the remit of the ICTV.

Using the above taxonomic system brings a number of practical benefits, including (1) the ability to relate a newly found virus to similar agents that have already been described and thereby to anticipate some of its possible properties, and (2) the ability to infer possible evolutionary relationships between viruses. Even though there has been little disagreement over the use of this system at the order, family, or genus level, there has been considerable confusion at the species level, partly based in misunderstanding over the difference between the man-made taxonomic construction, the species, and the actual entity, the virus. In this book formal ICTV taxonomy and nomenclature will be cited, but virus names will be in the English vernacular.

The discovery of mimiviruses (a virus infecting the protozoan *Acanthamoeba*) in the last decade has challenged the traditional concept of *virus*. The mimivirus genome is able to direct much more than the replication of its own DNA genome, coding as it does for a large number of proteins with functions resembling some eukaryotic proteins and a large number of proteins of unknown function. At the time of writing, no mimivirus-like agent causing human illness has yet been found; still the discovery of mimiviruses has had a profound influence on our understanding of virus evolution and on our sense of what is yet to be discovered. A full discussion regarding the origin of viruses is outside the scope of this book, suffice it to say that some virologists argue that RNA viruses have evolved many aeons before the appearance of DNA viruses.

VIRAL NOMENCLATURE

Formal Usage

In formal usage, the first letters of virus order, family, subfamily, genus, and species names are capitalized and the terms are printed in italics. Further words making up a species name are not further capitalized unless they are derived from a place name (e.g., the species *St. Louis encephalitis virus*). The first letter of the names of specific viruses having the status of tentative species is capitalized, but the names are not italicized. In formal usage, the identification of the taxon precedes the name; for example: "… the family *Paramyxoviridae*" or "… the genus *Morbillivirus*." The following are some illustrative examples of formal taxonomic usage:

> Family *Poxviridae*, subfamily *Chordopoxvirinae*, genus *Orthopoxvirus, Vaccinia virus*, vaccinia virus, strain New York Board of Health Laboratories (Wyeth calf-adapted) [the strain that was used to produce smallpox vaccine in the United States].
>
> Order *Herpesvirales*, family *Herpesviridae*, subfamily *Alphaherpesvirinae*, genus *Simplexvirus*, *Human herpesvirus 1*, herpes simplex virus 1, strain HF [a typical laboratory strain obtainable from the American Type Culture Collection].
>
> Order *Mononegavirales*, family *Rhabdoviridae*, genus *Lyssavirus*, *Rabies virus*, rabies virus, strain CVS 11 [the "challenge virus standard" used in laboratories throughout the world, with passage history back to Pasteur's laboratory].

Informal Usage

In informal vernacular usage, all terms are written in lower case script (except those derived directly from place names); these are not italicized, do not include the formal suffix,

and the name of the taxon follows the name. For example, "…the picornavirus family," "…the enterovirus genus," "poliovirus 1."

One particular problem in vernacular nomenclature lies in the historic use of the same root terms in family and genus names—it is sometimes difficult to determine which level is being cited. For example, the vernacular name "bunyavirus" might refer to the family *Bunyaviridae*, to the genus *Orthobunyavirus*, or perhaps even to one particular species, *Bunyamwera virus*. The solution to this problem is to add an extra word to formally identify which taxon level is being referred to; for example, when referring vernacularly to Bunyamwera virus (capitalized, because the name derives from a place name), a full vernacular description would be "Bunyamwera virus, a member of the genus *Bunyavirus* in the family *Bunyaviridae*…" For each genus there is a type species assigned that creates a link between the genus and the species.

A second problem lies in what seems to be an arbitrary incorporation of the root term, "virus," in some virus names and its separation as a detached word in others. For example, poliovirus *vs.* measles virus. The basis for this lies in history—some, but not all, of the viruses isolated early on assumed the former name style, whereas most viruses discovered more recently have been identified using the latter style. In this book, we have tried to hold to the name style used most commonly for each virus, but since this is mostly a matter of vernacular usage the reader may often find variations.

GROUPINGS OF VIRUSES ON THE BASIS OF EPIDEMIOLOGICAL CRITERIA

There are other informal categories of viruses that are practical and in common usage, distinct from the formal universal taxonomic system and the formal and vernacular nomenclature. These are based upon virus tropism and modes of transmission. Most human pathogens are transmitted by either inhalation, ingestion, injection (including via arthropod bites), close contact (including sexual contact), or congenitally.

Enteric viruses are usually acquired by ingestion (fecal–oral transmission) and replicate primarily in the intestinal tract. The term is usually restricted to viruses that remain localized in the intestinal tract, rather than causing generalized infections. Enteric viruses are included in the families *Picornaviridae* (genus *Enterovirus*), *Caliciviridae, Astroviridae, Coronaviridae, Reoviridae* (genera *Rotavirus* and *Orthoreovirus*), *Parvoviridae*, and *Adenoviridae*.

Respiratory viruses are usually acquired by inhalation (respiratory transmission) or by fomites (inanimate objects carrying virus contagion) and replicate primarily in the respiratory tract. The term is usually restricted to viruses that remain localized in the respiratory tract, rather than causing generalized infections. Respiratory viruses are included in the families *Picornaviridae* (genus *Enterovirus*), *Caliciviridae*, *Coronaviridae*, *Paramyxoviridae* (genera *Respirovirus*, *Rubulavirus*, *Pneumovirus*, and *Metapneumovirus*), *Orthomyxoviridae*, and *Adenoviridae*.

Arboviruses (from "*ar*thropod-*bo*rne viruses") replicate in hematophagous (blood-feeding) arthropod hosts such as mosquitoes and ticks, and are then transmitted by bite to vertebrates, wherein the virus replicates and produces viremia of sufficient magnitude to infect other blood-feeding arthropods. In all cases, viruses replicate in the arthropod vector prior to further transmission: thus, the cycle is perpetuated. The occasional passive transfer of virus on contaminated mouthparts ("the flying pin") does not constitute sufficient grounds for a virus to be identified as an arbovirus. Arboviruses are included in the families *Togaviridae*, *Flaviviridae*, *Rhabdoviridae*, *Bunyaviridae*, and *Reoviridae* (genera *Orbivirus* and *Coltivirus*).

Blood-borne viruses are those that are typically transmitted by transfusion of blood or blood products, by sharing of intravenous injecting equipment, and by other mechanisms of parenteral transfer of blood or body fluids. Some are also transmitted by sexual contact (*sexually transmitted viruses*). This group includes hepatitis B, C, and D, HIV-1 and -2, HTLV-1 and -2, and other viruses can also be transmitted occasionally by this route.

Hepatitis viruses are grouped as such because the main target organ for these viruses is the liver. Hepatitis A, B, C, D, and E viruses each belong to completely unrelated taxonomic families.

Oncogenic viruses usually cause persistent infection and may produce transformation of host cells, which may in turn progress to malignancy. Viruses that have oncogenic potential, in experimental animals or in nature, are included in the families *Herpesviridae*, *Adenoviridae*, *Papillomaviridae*, *Polyomaviridae*, *Hepadnaviridae*, *Retroviridae*, and *Flaviviridae*.

TAXONOMY AND THE CAUSAL RELATIONSHIP BETWEEN VIRUS AND DISEASE

One of the landmarks in the history of infectious diseases was the development of the Henle–Koch postulates that established the evidence required to prove a causal relationship between a particular infectious agent and a particular disease. These simple postulates were originally drawn up for bacteria, but were revised in 1937 by Thomas Rivers and again in 1982 by Alfred Evans in attempts to accommodate the special problem of proving disease causation by viruses. In many cases, virologists have had to rely on indirect causal evidence, with associations based on epidemiology and patterns of antibody prevalence among populations. The framework of virus taxonomy, again, plays

a role, especially in trying to distinguish an etiological, rather than coincidental or opportunistic relationship between a virus and a given disease. Particular difficulty arises where a disease occurs in only a small fraction of infected individuals, where the same apparent disease can be caused by more than one different agent, and in various chronic diseases and certain cancers. These difficulties are confounded in many instances where diseases cannot be reproduced by inoculation of experimental animals, or where the discovered viruses cannot be grown in animals or cell culture: there may even be a "hit and run" relationship where the causative virus may no longer be present in the afflicted individual. Thus scientists have to evaluate the probability of "guilt by association," a difficult procedure that relies heavily on epidemiological observations.

The Henle–Koch postulates were reworked again in 1996 by David Relman and David Fredricks as more and more genomic sequencing criteria came to dominate the subject (Table 2.2). As a test of the value of these criteria, one can consider the level of proof that the human immunodeficiency viruses, HIV-1 and HIV-2, are the etiological agents of human acquired immunodeficiency syndrome (AIDS) (Table 2.3). Early in the investigation of AIDS, before its etiology was established, many kinds of viruses were isolated from patients and many candidate etiological agents and other theories were advanced. Prediction that the etiological agent would turn out to be a member of the family *Retroviridae* was based upon years of research on animal retroviral diseases and many points of similarity with some characteristics of AIDS. Later, after human immunodeficiency virus 1 (HIV-1) was discovered, the morphological similarity of this virus to equine infectious anemia virus, a prototypic member of the genus *Lentivirus*, family *Retroviridae*, highlighted the usefulness of the universal viral taxonomic system and of animal lentiviruses as models for AIDS.

In other examples, the causal relationships of Epstein-Barr (EB) virus to the disease infectious mononucleosis, and of Australia antigen (later known as hepatitis B surface antigen) to clinical hepatitis, were each established by matching serological evidence of acute infection with the timing of onset of clinical disease. Further, the complex role of EB virus in Burkitt's lymphoma was investigated in a large prospective study carried out by the International Agency for Research on Cancer (IARC) on 45,000 children in an area of high incidence of Burkitt's lymphoma in Africa. This showed that:

1. EB virus infection preceded development of the tumors by 7 to 54 months;
2. exceptionally high EB virus antibody titers often preceded the appearance of tumors; and
3. antibody titers to other viruses were not elevated.

In addition, it was demonstrated that the EB virus genome is always present in the cells of Burkitt's lymphomas among African children, and that a malignant lymphoma can be induced in certain primates with EB virus or EB virus-infected lymphocytes (see Chapter 9: Mechanisms of Viral Oncogenesis and Chapter 17: Herpesviruses).

Using a similar approach, Palmer Beasley and coworkers in Taiwan demonstrated unequivocally that persistent

TABLE 2.2 Fredricks and Relman's Molecular Guidelines For Causal Association

1. Strength of the association. Are viral nucleic acid sequences detected in most (all) cases of disease?
2. Specificity of the association. Are viral nucleic acid sequences localized to diseased tissues, and not to healthy tissues? Is the frequency of virus infection reduced significantly in healthy individuals?
3. Response to treatment. Does the copy number of viral nucleic acid sequences fall with resolution of illness or effective treatment, and increase if the disease relapses?
4. Temporality. Does infection with the virus precede and predict disease onset?
5. Plausibility. Do the known biological properties of the virus make sense in terms of the disease?
6. Biological gradient. Is the amount of virus higher in patients with severe disease than it is in persons with mild disease? Is the amount of virus higher in diseased tissues than in healthy tissues?
7. Consistency. Are these findings reproducible by multiple laboratories and by multiple investigators?

TABLE 2.3 Application of Fredricks and Relman's Guidelines to the Cause of Acquired Immunodeficiency Syndrome (AIDS)

1. Strength of the association. Infection with HIV is found in almost all cases that fit a clinical definition of AIDS.
2. Specificity of the association. Human immunodeficiency viruses are found preferentially in target organs (immune cells, lymphoid tissues). HIV infection is not found in healthy individuals, except for those who subsequently develop AIDS or those rare individuals considered long-term non-progressors.
3. Response to treatment. Combination therapy against HIV lowers or completely eradicates circulating virus, resulting in increased CD4 cells, improved immune function, and significant long-lasting clinical improvement.
4. Temporality. HIV infection precedes and predicts disease onset in children born to infected mothers, in medical personnel infected via needle-stick accidents, and in recipients of blood transfusions from infected persons.
5. Plausibility. HIV infects and kills CD4+ T cells and macrophages. SIV causes AIDS in experimentally inoculated macaques.
6. Biological gradient. HIV-1 RNA load is highest in lymphoid tissues and brain (diseased tissues). HIV-1 RNA load predicts the rate of disease progression.
7. Consistency. These findings are consistently reproducible, worldwide.

hepatitis B infection increased the subsequent risk of primary liver cancer, but not other cancers, by approximately 100-fold.

These studies are examples of important concepts now widely understood in situations where a virus has been shown to cause a specific disease, namely that not all cases of the infection may necessarily develop the clinical disease, and not all cases of the clinical disease may be caused by the particular virus in question. Thus, for many associations between a virus and a clinical disease, the concept of infection representing a "risk factor" is more appropriate than it being an absolute "cause." It also now happens frequently using modern diagnostic methods, that viruses are recovered from individuals with some ongoing disease; however, careful work is essential in such cases to distinguish a true causative role from an unrelated infection of no clinical significance occurring at the same time.

GENOME SEQUENCING AND VIRUS EVOLUTION

The breath-taking advances in genome sequencing now enable the complete genomes of many hundreds of virus isolates to be sequenced in a matter of days, if not hours. Multiple sequence alignment and the construction of phylogenetic trees are now commonplace when virologists are confronted with either a potentially new virus or an isolate with new or unexpected properties. These data are rapidly challenging previous ideas about the origin and evolution of many viruses of medical importance. Detailed phylogenetic analysis of RNA viruses in particular sometimes provides unexpected answers that in turn create more questions; for example, hepadnaviruses share a similar reverse transcriptase-based replication strategy that is common to the caulimoviruses of plants—does this reflect a common ancestor or convergent evolution?

Deep evolutionary relationships among the higher virus taxa have led to the construction of several Orders—the *Herpesvirales*, *Mononegavirales*, *Nidovirales*, and *Picornavirales*. The common conserved sequences employed here are at the lower limit of significance, but similarities in some functional and structural protein domains still appear among otherwise unrelated viruses in various taxa. Sequence analyses also suggest that it is unlikely that many more associations of diverse taxa will be found that warrant construction of further Orders.

At the other extreme, namely clarifying the phylogenetic relationships among viruses in the same taxa (i.e., families or genera), great progress is being made continually. For example, the origin of the 2009 influenza (H1N1) pandemic has been found to be complex indeed: the virus is a reassortant with genes from four different ancestral viruses—North American swine influenza, North American avian influenza, human influenza, and swine influenza virus typically found in Asia and Europe. Similarly, some member viruses of the family *Bunyaviridae* have been found to be natural reassortants with genes from known and unknown ancestors.

Thus, the development of a robust, yet flexible and continually evolving taxonomic system for viruses underpins, and gives structure to, all facets of research, management, and control of virus diseases.

FURTHER READING

Adams, M.J., Lefkowitz, E.J., King, A.M.Q., Carstens, E.B., 2013. Recently agreed changes to the International Code of Virus Classication and Nomenclature. Arch. Virol. Available from: http://dx.doi.org/10.1007/s00705-013-1749-9.

Anthony, S.J., Epstein, J.H., Murray, K.A., et al., 2013. A strategy to estimate unknown viral diversity in mammals. mBio., 4. Available from: http://dx.doi.org/10.1128/mBio.00598-13.

Fredricks, D.N., Relman, D.A., 1996. Sequence-based identification of microbial pathogens: a reconsideration of Koch's postulates. Clin. Microbiol. Rev. 9, 18–33.

Holmes, E.C., 2009. The Evolution and Emergence of RNA Viruses. Oxford University Press, Oxford, ISBN 978-0-19-921112.

Virus taxonomy, classification and nomenclature of viruses. King, A.M.Q., Adams, M.J., Carstens, E.B., Lefkowitz, E.J. (Eds.), 2011. Ninth Report of the International Committee for the Taxonomy of Viruses, Academic Press, London, ISBN 978-0-12-384684-6.

Krupovic, M., Bamford, D.H., 2010. Order to the viral universe. J. Virol. 84, 12476–12479. Available from: http://dx.doi.org/10.1128/JVI.01489-10.

Chapter 3

Virion Structure and Composition

The virus particle or *virion* represents a virus in its *extracellular* phase, in contrast to the different *intracellular* structures involved in virus replication. To ensure survival of a virus, the virion must fulfill two roles: (1) protecting the genome from environmental damage, for example, from heat, desiccation, chemicals; and (2) facilitating the passage of the virus to the next host, that is, from the point of release from the original host, passage through the environment to the point of encountering a new host, followed by entry into the cells of the new host. There are many different ways that different viruses achieve these two roles, and viral genomes and virion structures show enormous variety in both size and composition—yet there are many features or principles of assembly that are shared by most viruses. Notably, many key structures within the virion are assemblies and subassemblies of a large number (usually hundreds) of identical protein subunits that lock together sterically to form a stable shell (capsid or envelope); the employment of large numbers of one or a few different primary units (structural units, capsomeres) allows the genetic coding of relatively large macromolecules by a very small number of different viral genes.

PHYSICAL METHODS FOR STUDYING VIRUS STRUCTURE

Electron Microscopy

The development of electron microscopy was pivotal in the establishment of virology as a scientific discipline. Viruses are smaller than the limit of resolution of the light microscope, which is about 0.3 µm, or 300 nm. Poxviruses were for many years considered amongst the largest of viruses, being just about visible in the light microscope using dark-field optics or certain staining techniques. In recent years, much larger viruses have been discovered but so far none of these have been shown to be human pathogens. Nevertheless, these newly discovered viruses are driving a re-examination of the limits of the concept of *virus*. For example, the virions of megavirus and mimivirus, both infectious for *Acanthamoeba*, are about 0.5 µm in diameter, have a DNA genome up to 1.26 Mb in size and code up to 1120 proteins. The virions of pandoraviruses are 1–1.2 µm in size, have a linear DNA genome up to 2.8 Mb, and code up to 2500 proteins. An even larger virus, Pithovirus (recently isolated from Siberian permafrost), is 1.5 µm in size, the size of a small bacterium, with >2500 putative protein-coding sequences, of which only 6% have recognizable relationships with genes from known viruses, microorganisms, or eukaryotes. At the same time, viruses that are smaller than those in previously known taxa have been found, so virologists must now be prepared to work with viruses as large as bacteria and as small as large protein molecules (Table 3.1).

The first electron microscopy of viruses by Bodo von Borries, Ernst Ruska, and Helmut Ruska in 1938 employed a simple preparative method that did not show much more than the outline of virions. Later, metal shadow-casting of purified virus preparations improved the visualization of virions, but still not enough. Beginning in the 1950s, ultra-thin sectioning of virus-infected cells became widespread, providing more virion detail and also the beginning of the science of ultrastructural cytopathology—virion morphogenesis, intracellular localization of virus structures and cellular organelles and coincident damage to host cell structures. In 1959, visualization of viral ultrastructure was taken to a still higher level of resolution when Sydney Brenner and Robert Horne developed negative staining. In this method, a solution of potassium phosphotungstate (or other electron-dense salts) is added to a virus suspension on a coated specimen grid; the metal ions surround and fill the interstices in the surface of virions giving a negative image in the electron beam, thus revealing structural details not previously seen. A remarkable diversity of virus structures can be seen in negatively stained preparations. Electron micrographs of virions and infected cells from different families of viruses are shown in the various chapters of this book (see Figs. 1.3 and 1.4 for comparison of ultra-thin sectioning versus negative staining).

In the past few years, the above methods have been complemented by several new microscopy technologies, particularly scanning electron microscopy and cryo-electron microscopy, the latter using computer-based image construction of images of snap frozen, unstained virion

Fenner and White's Medical Virology. DOI: http://dx.doi.org/10.1016/B978-0-12-375156-0.00003-5

TABLE 3.1 Relative Sizes of Common Objects in the Biological World

Size	Equivalent Practical Units	Observations
1 m	3 ft 3 in.	Humans, adult males, are about 1.8 m tall
10 cm	4 in.	Human adult hand is about 10 cm wide
1 cm	1 cm	*Aedes aegypti*, adult, mosquito is about 1 cm long
1 mm	1 mm	*Ixodes scapularis* tick, nymphal stage, is about 1 mm long
0.1 mm	100 µm	Smallest things visible to the naked eye
0.01 mm	10 µm	Lymphocytes are about 20 µm in diameter
		Bacillus anthracis, among the largest of pathogenic bacteria, is 1 µm wide and 5 to 10 µm long
0.001 mm	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
0.1 µm	100 nm	Influenza viruses and retroviruses, typical medium-sized viruses, are about 100 nm in diameter
	100 nm	Flaviviruses, such as yellow fever virus, typical smaller-sized viruses, are about 50 nm in diameter
0.01 µm	10 nm	Picornaviruses, such as polioviruses, typical small viruses, are about 30 nm in diameter
		Circoviruses, the smallest of the viruses of vertebrates, are 17 to 22 nm in diameter
0.001 µm	1 nm, 10 Å	Smallest things visible in transmission electron microscope; DNA double helix diameter is 2 nm
0.1 nm	1 Å	Diameter of atoms is about 2 to 3 Å

preparations. This technique has the advantage of showing viruses in a hydrated state rather than the desiccated conditions of negative staining in electron microscopy. The resolution of these techniques is rapidly approaching that obtained by X-ray diffraction of crystallized virions and viral substructures (Fig. 3.1).

X-Ray Crystallography of Viruses

X-ray crystallography of viruses provides another technique for visualizing virion structural organization showing structural details to near atomic resolution, the location of antigenic sites on the surface of virions, and aspects of virion attachment and penetration into cells. For example, applying this technique to several picornaviruses revealed that the polypeptides of each of the three larger structural proteins are packaged to form wedge-shaped eight-stranded antiparallel β-barrel subassemblies (Fig. 3.2). The overall contour of picornavirus virions reflects the packing of these subassemblies. Relatively unstructured amino acid chains form loops that project from the main wedge-shaped domains. Some loops form flexible arms that interlock with the arms of adjacent wedge-shaped subassemblies, 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, and to study the structure of these viruses it is usually necessary to separate well-defined substructures and examine crystals of these structures by X-ray diffraction. Rotaviruses are an excellent example of this approach, being composed of a core and two capsid layers, each component exhibiting unique structural details, fitting together in a precise fashion to form the complete virion.

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. Today, determination of new variations in the amino acid sequence and hence the microstructure of the influenza hemagglutinin is used in the development of updated vaccines. Many individual viral proteins have been analyzed to the level of 2–3 Å resolution, revealing

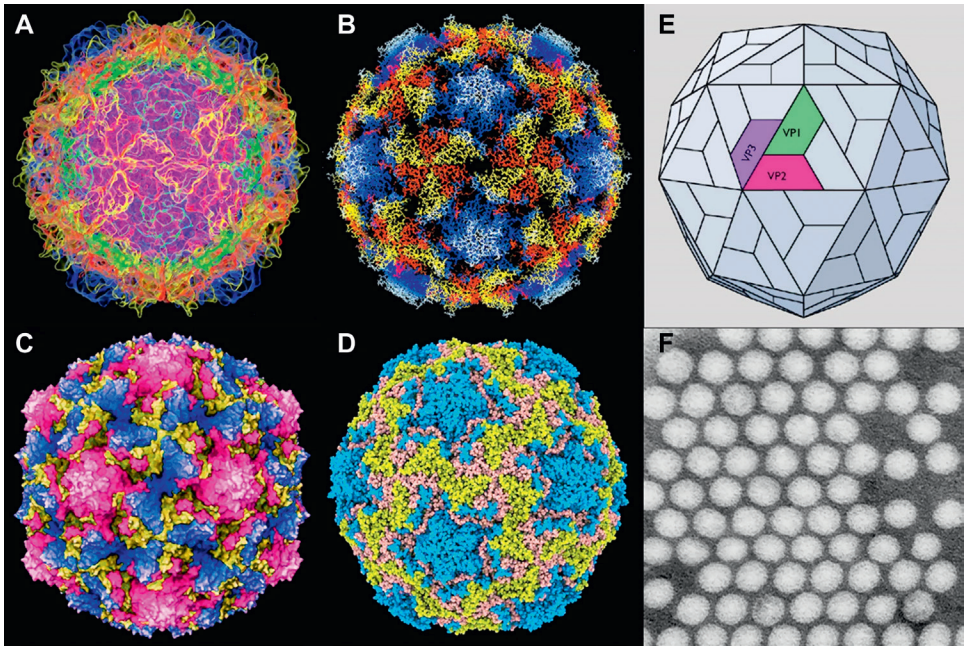

FIGURE 3.1 Picornavirus structural studies. (A–D) Computer-based virion reconstructions from cryo-electron microscopy and X-ray diffraction images. (A) Poliovirus 1, protein chain model. *From Jason Roberts and colleagues, with permission.* (B) Poliovirus 1, string model. (C) Coxsackie B3 virus, space-filling model. (D) Human rhinovirus B14, Qutemol rendering model. *(B–D) from Jean-Yves Sgro, with permission.* (E) Model of poliovirus icosahedral capsid showing location of the three proteins making up the capsid surface, VP1, VP2 and VP3 (VP4 is buried on the inner face of the capsid). (F) Poliovirus, negative contrast electron microscopy from Joseph Esposito (deceased), showing little or no virion surface detail—such was the resolution available before X-ray diffraction and cryo-electron microscopy technologies, both of which require massive computer compilation to reconstruct image data.

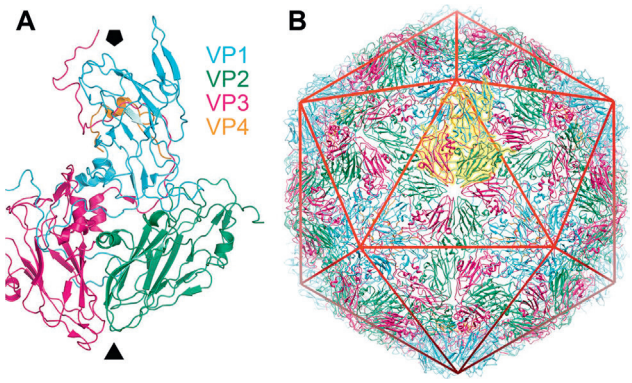

FIGURE 3.2 Structure of a typical picornavirus. (A) X-ray crystallographic structure of a native virus particle. A single substructural unit (protomer) is shown in a string-ribbon representation. The viral proteins are colored blue (VP1), green (VP2), magenta (VP3), and orange (VP4), and the approximate positions of the viral 5- and 3-fold axes are indicated by the solid black pentagon and triangle, respectively. (B) The intact, T = 3 capsid structure, again in a string-ribbon representation. The unit shown in panel (A) is highlighted in yellow, and the layout of the virion icosahedron is included as a red line overlay. *Adapted from Bakker, S.E., Groppelli, E., Pearson, A.R., Stockley, P.G., Rowlands, D.J., Ranson, N.A., 2014. Limits of structural plasticity in a picornavirus capsid revealed by a massively expanded equine rhinitis A virus particle. J. Virol. 88: 6093–6099.*

potential targets for new antiviral compounds. Notable is the development of antiviral drugs for the treatment of influenza through analysis of the detailed structure of the viral neuraminidase (Fig. 3.3).

CHEMICAL COMPOSITION OF VIRIONS

Viruses are distinguished from other macromolecular forms by a possessing rather simple, repetitive chemical composition. The *virion*, that is the complete infectious virus particle, includes a genome comprising one or a few molecules of either DNA or RNA, surrounded by a morphologically defined protein coat, the *capsid*; the capsid and the enclosed nucleic acid together constitute the *nucleocapsid.* A small number of additional proteins may be present within the virion as enzymes. The nucleocapsid of some viruses is surrounded by a lipoprotein bilayer *envelope* into which are inserted viral proteins (*peplomers*): these may or may not be glycosylated. Sometimes a matrix protein is also associated with the inner aspect of the viral envelope. The simplest virus (e.g., tobacco necrosis virus satellite, a defective virus that needs a helper virus to

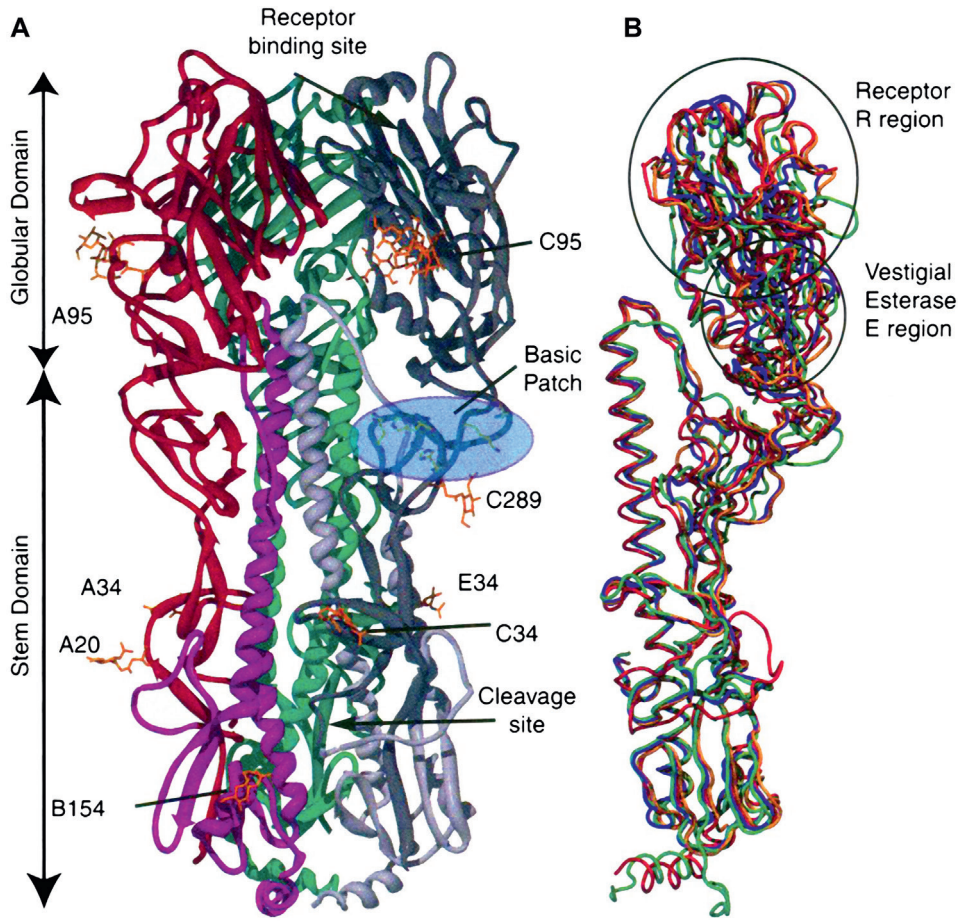

FIGURE 3.3 Crystal structure of the hemagglutinin (HA) protein of the influenza 1918 virus, and comparison with other human, avian, and swine HAs. (A) Overview of the HA0 trimer, represented as a ribbon diagram. Each of the three monomers is colored differently. Carbohydrates are colored orange and labeled with the asparagine to which each is attached. The basic patch is indicated in the light blue ellipse and consists of HAl residues. The locations of the three receptor binding sites, and cleavage sites, are shown for only one of the three monomers. (B) Structural comparison of different HA0 monomers, showing influenza 1918 HA0 (red), human H3 (green), avian H5 (orange), and swine H9 (blue). *Reproduced from MacLachlan, N.J., Dubovi, E.J., 2011. Veterinary Virology, fourth ed. Academic Press, London (Fig. 1.4), with permission.*

provide some of its functions) directs the synthesis of only one protein; many important viruses direct the synthesis of five to ten proteins; large viruses, such as the poxviruses and herpesviruses, direct the synthesis of up to 200 proteins and the recently discovered megaviruses up to 2500 proteins: still this is very few relative to the number of proteins involved in the life processes of bacteria (>5000 proteins) and eukaryotic cells (between 250,000 and 1,000,000 proteins). There are many variations on these constructions and diverse additional components are found among larger and more complex viruses (Fig. 3.4).

Viral Nucleic Acids

Viral genes are encoded in either DNA or RNA molecules; both DNA and RNA genomes can be either *double-stranded* or *single-stranded*, as well as *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 viruses of the families *Reoviridae*, *Birnaviridae*, and *Picobirnaviridae* have a double-stranded RNA genome and these genomes are segmented (*Reoviridae*: 10, 11, or 12 segments, depending on the genus; *Birnaviridae* and *Picobirnaviridae*: two segments). All viral genomes are haploid, that is, they contain only one copy of each gene, except for retrovirus genomes, which are diploid. When carefully extracted from the virion, the nucleic acid of viruses of certain families of both DNA and RNA viruses is directly infectious; that is, when transfected into a cell there is sufficient genetic information to initiate a complete cycle of viral replication and produce a normal yield of progeny virions.

The sequence in which the various virus families are described in Part II of this book reflects the essential characters and diversity of viral genomes. The remarkable variety of

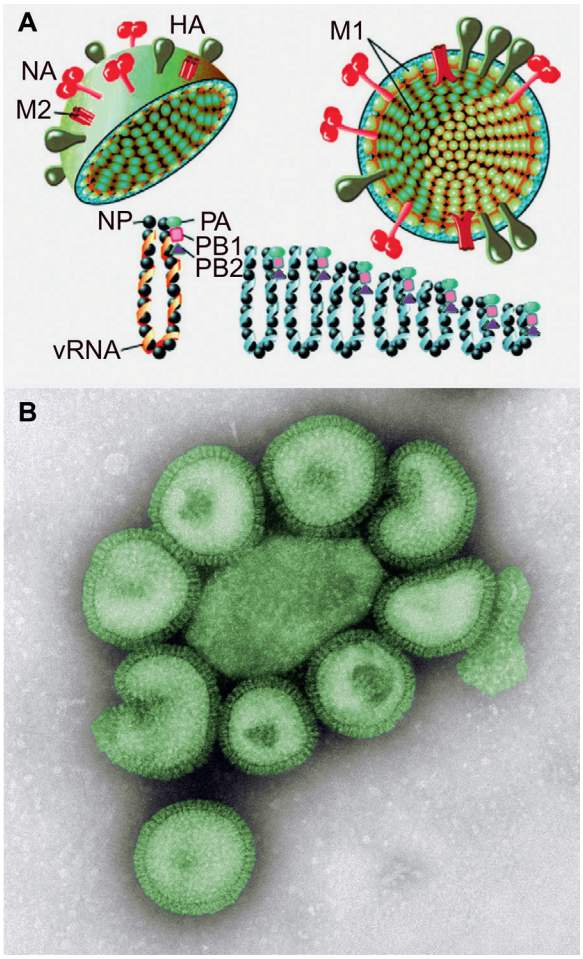

FIGURE 3.4 Structure of influenza A virions. (A) There are two major glycoproteins embedded in the lipid envelope, viz. the trimeric hemagglutinin (HA) which predominates, and the tetrameric neuraminidase (NA). The envelope also contains a small number of M2 membrane ion channel proteins. Inside the envelope lies the matrix protein (M1); the viral ribonucleoprotein which consists of the RNA genome in segments, each segment associated with nucleocapsid protein molecules; and the PA, PB1, and PB2 polymerase proteins. (B) Negative contrast EM of influenza A particles. *(A) Reproduced from MacLachlan, N.J., Dubovi, E.J., 2011. Veterinary Virology, fourth ed. Academic Press, London, Fig. 21.3, with permission.*

viruses is reflected in diverse ways in which the information encoded in the viral genome is transcribed into mRNA, then translated into proteins, and the ways in which the viral nucleic acid is replicated (see Chapter 4: Virus Replication).

Viral DNA Genomes

The genomes of all DNA viruses of vertebrates are monopartite, consisting of a single molecule that is, double-stranded except in the case of the parvoviruses, anelloviruses, and circoviruses. DNA genomes may be linear or circular, depending on the virus family. The DNA of papillomaviruses, polyomaviruses, hepadnaviruses, anelloviruses, and circoviruses is circular. Additionally, the circular DNA of the papillomaviruses and polyomaviruses is supercoiled. The DNA genome of hepadnaviruses is partially double-stranded, although the single-stranded gap is closed during replication to form a covalently closed circular DNA (cccDNA: see Chapter 22: Hepadnaviruses and Hepatitis Delta).

Most linear viral DNAs have characteristics that can facilitate a circular configuration, a requirement for replication by a rolling circle mechanism (see Chapter 4: Virus Replication). The two strands of poxvirus DNA are covalently cross-linked at each terminus (forming *hairpin ends*), so that upon 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 contain repeat sequences at the ends of the molecule that permit circularization. Adenovirus DNA contains inverted terminal repeats; these are also a feature of the single-stranded DNA of parvoviruses. Another type of terminal structure occurs in adenoviruses, hepadnaviruses, and parvoviruses (and some single-stranded RNA viruses such as the picornaviruses and caliciviruses); all of these viruses contain a protein covalently linked to the 5′-terminus (the 5′ cap) with an essential function in priming replication of the genome.

The size of viral DNA genomes ranges from 1.7 kilobases (kb) for some circoviruses to over 200 kilobase pairs (kbp) for the double-stranded DNA of herpesviruses and poxviruses—and up to 2.8 mbp for the double-stranded DNA of the giant pandoraviruses and pithovirus of amoeba. As 1 kb, or for double-stranded DNA 1 kbp, contains enough genetic information to code for about one average-sized protein, it might be surmised that viral DNAs contain anywhere between two and 200 genes, coding for some two to 200 proteins in poxviruses and more than 2500 proteins in the giant viruses of amoeba. However, the relationship between any particular nucleotide sequence and its protein product(s) is not straightforward. On the one hand, the DNA of most of the larger viruses, similar to that of mammalian cells, contains what appears to be redundant information in the form of repeat sequences, thus the coding capacity of large viral genomes may be overestimated. On the other hand, coding capacity might be underestimated: first, a given DNA or mRNA sequence may be read in up to three alternate reading frames, producing up to three proteins with different amino acid sequences; second, both strands of a double-stranded viral DNA molecule may be transcribed, each transcript yielding a different protein; 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 a number of distinct mRNAs, each of which may be translated into a different protein, or a single polyprotein translation product may be subsequently cleaved by proteolysis to yield multiple discrete proteins.

Viral DNAs contain several kinds of non-coding sequences essential for genome expression and replication, some of which have been conserved throughout evolutionary