Antiviral Agents and Viral Diseases of Man

Second Edition

Editors

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Preface to the Second Edition

Since the first edition of this book, much progress has been made with antiviral agents and in antiviral therapy; witness the fact that acyclovir did not appear in the first edition index. Yet antiviral agents remain novel in clinical medicine. Their proper application is complex due to the interweaving of virus replication with host processes. Other relevant progress, in addition to the several new agents currently under study, include the impact of the new biotechnology that has made a considerable contribution to viral diagnosis and new insights in immunology that have led to a better understanding of pathogenesis of disease. It is our hope that the second edition of this book will acquaint the reader with the contemporary agents and current concepts of the diseases they manage, indications for therapy, parameters responsive to therapy, and in general, the state of the art of antiviral agent development and application. It is also our expectation that this book will serve as a guidepost for future directions of development, identifying both fertile fields and dead-end areas.

The prediction in the preface of the First Edition has proven correct: we do have new antiviral agents available to the clinician. Progress will continue to be steady. Time will divulge what the ultimate limitations are for the control of viral disease. The standards and effective strategies are identified so that antiviral therapy can continue to advance on a sound, rational basis.

We expect that there will be greater efforts in the development of targeted antiviral agents and that many of the planned clinical studies will result in clinically effective drugs. As in all clinical matters, drug usage will ultimately depend on the discretion of the individual physician and the patient's responses. New, unanticipated applications and side effects of drugs will continue to appear as biologically active therapeutic agents are made available and are accepted by those who care for patients. Hence, this book is presented as a contemporary picture of a continually changing perspective.

This Second Edition will be useful to the student, laboratory scientist, and clinician. The student will glean an overview of virology, the diseases caused by viruses, and the role of antivirals. The laboratory scientist is presented the necessary background for further effective and efficient development of new agents. A reasonable strategy for the development of new agents is presented. Finally, the physician providing primary care should benefit by having access to this contemporary knowledge of viral diseases and their management as seen today.

Preface to the First Edition

There is no question that antivirals are important for modifying infections in man. Viral infections are among the greatest causes of human morbidity and resulting economic loss; this, together with the rapid advances being made in other areas of clinical management, accentuates the increasing need for measures to control viral infections. In many instances, the immunologic manipulation of patients that is required for optimal treatment of certain diseases renders the patients extremely susceptible to infections that are not often seen in otherwise healthy populations.

In the past, antivirals usually have been discovered by fortuitous means. Screening programs, in many instances seeking other products such as anticancer agents, have yielded compounds with some potential; these have then been developed by means of tissue culture and animal model systems to determine the feasibility of applying them clinically as antiviral drugs. The development of antivirals has been slow because their effectiveness is closely related to cellular metabolism. Simply put, viral replication is intracellular, and it involves the use of cellular functions for viral synthesis. Until we fully understand viral replication and can clearly uncouple it from normal cellular metabolic processes, tailored antivirals cannot be developed.

Of equal importance to the field is a thorough understanding of the pathogenesis of disease. Recent developments in diagnostic techniques not only have permitted more accurate diagnosis but also have vastly improved our understanding of the natural history of viral diseases. It is only through such understanding that the feasibility of antivirals can be determined.

A third consideration in antiviral development is the basic issue of whether or not they will work. Some experience in this area has been gained with idoxuridine. This compound, in ointment form, has been licensed for a number of years for topical treatment of herpetic keratitis; however, systemic administration has been both ineffective and toxic. Very little experience has been accumulated with other compounds. However, the past 2 years have seen considerable advances in chemoprophylaxis and chemotherapy. Although amantadine has been licensed for prophylaxis against Asian influenza (H2N2) since the late sixties, it was not until 1976 that it became licensed for use against all influenza A strains. Vidarabine ointment has recently been licensed for topical treatment of herpetic keratitis, including cases refractory to idoxuridine. This compound has also been licensed for systemic use against herpes encephalitis.

Interferon was shown in late 1976 to hold some promise in treatment of chronic active hepatitis as well as herpes zoster. Suddenly progress is accelerating, and good news is being heard after a long wait. Several other studies are under way to evaluate the clinical roles of vidarabine and vidarabine monophosphate, interferon and ribavirin, and other antivirals against a spectrum of viral diseases. On the immunologic front, smallpox has fallen by the wayside and become a disease of historical interest only.

It is through advances in all these areas that control of viral disease can be extended beyond the level achieved with vaccines. This book was developed with these problems in mind. Significant progress has been made in all these areas, and the time seems appropriate for a text reviewing the progress and the potential of antiviral research.

Another consideration important in the planning of this text was identification of the audience to whom it is directed. It should be of value to the widest audience of scientists interested in

antivirals; it is not intended solely for clinicians or laboratory scientists. It is not a compendium of diseases and information about how they should be treated, nor is it a list of antivirals and their modes of action. Rather, it attempts a synthesis of these areas, discussing the clinical, diagnostic, epidemiologic, pharmacologic, and molecular biologic aspects of the interrelationships of viruses, antivirals, and disease, with particular emphasis on antivirals that are currently available and disorders in which antivirals may be of value if they can be developed. The book is intended for those who will most need it in the coming years: the medical student/resident who is interested in infectious diseases, the clinician who will inform him of the current state of the art, the microbiologist who will apprise him of new developments in the field, and the research scientist who, it is hoped, will be encouraged to undertake further work in the field.

In order to reach such a wide and diverse audience, all aspects of antiviral work must be covered. In order to understand how an antiviral is to be of value in the clinic, one must understand viral replication. Therefore, we begin with the basics of virology, the biology of viral infections, and the pharmacology of antiviral action. If antivirals are to be of value, it is important that rapid and accurate diagnosis be made; we must understand the diagnostic tools available so that we can do more than say that the patient has a fever and the flu. We then proceed to the pathogenesis of various diseases and the roles of antivirals in their control. This is done by means of arbitrary divisions, on the basis of organ systems whenever possible. There are many instances of overlap, but this is not considered to be undesirable. In many instances it is done for completeness and emphasis, as well as for strength when different disciplines must contribute.

The practical aspects of using antivirals must be addressed if the goal is clinical application. Some exciting new developments are also described, even though their practical applications are still being developed. This is particularly pertinent in the case of exogenous interferon. It is our assumption that if an antiviral is found to be efficacious and of clinical importance, the mechanisms for making it economically feasible will be found.

The reader will notice that the chapters in this book vary somewhat in terms of length, organization, and style. These variations arise from the differing natures of the topics being discussed. We have elected to maintain these differences, since the state of the art varies widely from one area to another.

We anticipate that we are entering a new era in the development and application of antivirals. It is hoped that this book will be useful in coordinating many factors and much new knowledge at this pivotal point. We believe that the next several years will see great progress in antiviral developments and applications. If this book enlightens some and, more important, stimulates a few to pursue studies in this field, its intended goal will be accomplished.

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Chapter 1

Fundamentals of Virus Structure and Replication

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STRUCTURAL CHARACTERISTICS OF MAJOR GROUPS OF ANIMAL VIRUSES

Architecture of Virions

In the context of a book on antivirals, a brief review of the essential structural features of animal viruses is important for at least two reasons. First, virus architecture provides a basis for classification, without which no comprehensive text on viruses and viral infections could be conceived. Second, the structure of a virus determines the nature of many essential steps in the process of replication, which in turn influences the susceptibility of the virus to the actions of antivirals. For instance, the processes of attachment and penetration that occur in the initial stages of the infectious cycle, as well as the processes of maturation and release, can differ profoundly depending on whether a virus does or does not have an envelope. Thus information about the presence of an envelope in the virion can be essential in making the choice of the appropriate antiviral agent.

Students of viral architecture have developed a relatively simple vocabulary that permits precise description of the essential morphologic features of viruses (Fig. 1). On the basis of the structural characteristics of their *nucleocapsids*, most (but not all) viruses can be divided into two groups: viruses with helical symmetry and viruses with icosahedral symmetry of the nucleocapsid.

A plant virus, tobacco mosaic virus (TMV), is the most thoroughly studied example of a virion with *helical symmetry*. The TMV particle *(virion)* has a rigid rodlike structure formed

by a perfectly helical ribonucleoprotein tube. The RNA helix is tightly bound to the protein *structural units*, which are also arranged in helical form and extend to the outside of the virion (Fig. 2a). Each structural unit is composed of a single polypeptide. In comparison with the nucleocapsid of TMV, the nucleocapsids of animal viruses with helical symmetry are much less rigid. Rather than forming perfect rods, the nucleocapsids in animal viruses may be folded into several parallel strands, as in orthomyxoviruses (Fig. 2b), or they may be arranged in irregular patterns, as in paramyxoviruses (Fig. 2c). Helical nucleocapsids of animal viruses are always enclosed in envelopes.

Icosahedral symmetry is characterized by a highly structured rigid shell *(capsid)* enclosing the viral nucleic acid in a condensed form. The icosahedron has 20 triangular facets and 12 corners (apices). The structure is formed by self-assembly of the protein building blocks, *capsomeres*. Unlike the structural units of helical virions, capsomeres may be made up of more than one polypeptide molecule. The laws of crystal structure determine the possible numbers of capsomeres, forming an icosahedron. Thus the adenovirus virion contains 252 capsomeres, with 240 hexons and 12 pentons (Fig. 2d). The 12 apices are formed by single capsomeres with five neighboring capsomeres (pentons). Each of the remaining capsomeres has six adjacent capsomeres (hexons). Capsids of herpesviruses have 150 hexons and 12 pentons. (Unlike many other icosahedral viruses, capsids of herpesviruses are enclosed in envelopes.) The much smaller virion of Papovaviridae is made up of a total of 72 capsomeres (60 hexons and 12 pentons).

Not all virus families can be neatly classified as having nucleocapsids with either helical or icosahedral symmetry. Members of the family Poxviridae (the largest and structurally most complex of all animal viruses) are morphologically unrelated to any of the other viruses (Fig. 2e).

Analysis of Major Components of Virions

Most information about the morphology of viruses is provided by various techniques of electron microscopy. Additional important information is derived from physiochemical analysis of purified virus particles.

Nucleic Acid

Unlike more complex microorganisms, viruses contain only one type of nucleic acid—RNA or DNA; each can be present in either *single-stranded form* or *double-stranded form*. Most DNA-containing viruses have double-stranded DNA, while of the RNA viruses most have single-stranded RNA, but there are some notable exceptions (Table 1).

There are other characteristics of the virion nucleic acid that aid in their classification and help us to understand the life cycles of viruses. One such characteristic is the size of the nucleic acid, which increases with the complexity of the virion. The smallest genomes among animal viruses are found in Parvoviridae (2×10^6 d, single-stranded DNA) and Picornaviridae ($2\text{-}3 \times 10^6$ d, single-stranded RNA). Assuming that the entire genomes of these viruses can code for protein, there will be enough genetic information to code for proteins with total molecular weights of approximately 220,000 and 270,000, respectively. This conclusion stems from the *coding ratio*: the molecular weight of single-stranded nucleic acid divided by the molecular weight of coded protein is 9. The largest viruses, Poxviridae, have double-stranded DNA genomes with molecular weights up to 2×10^8; assuming that only one of the DNA strands is transcribed into mRNA, this amount of nucleic acid can code for protein with a total molecular weight of more than 1×10^7 (or about 500 different average-size proteins). In reality, the number and size of proteins synthesized by a virus may exceed the apparent coding capacity of the viral genome. This may be due to either transcriptional regulation, or posttranscriptional and posttranslational processing. For in-

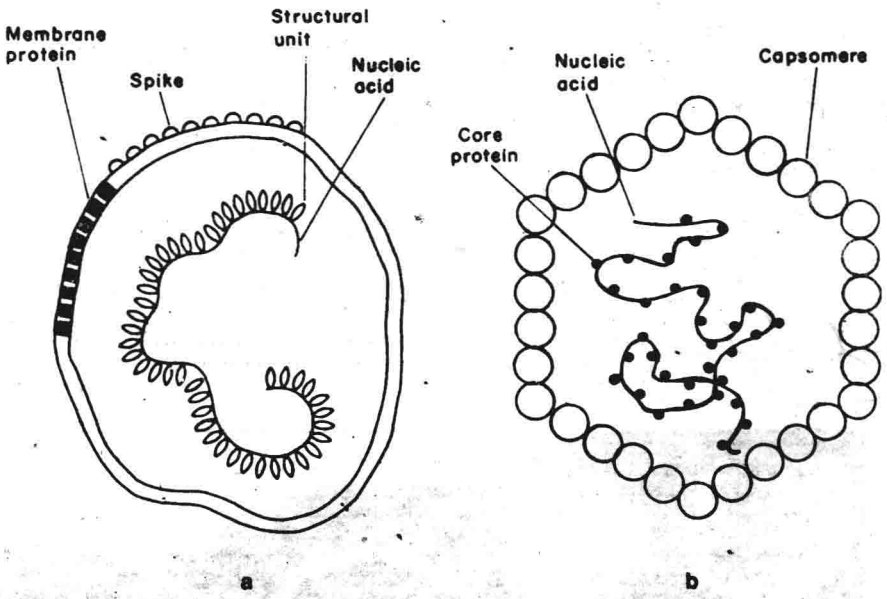
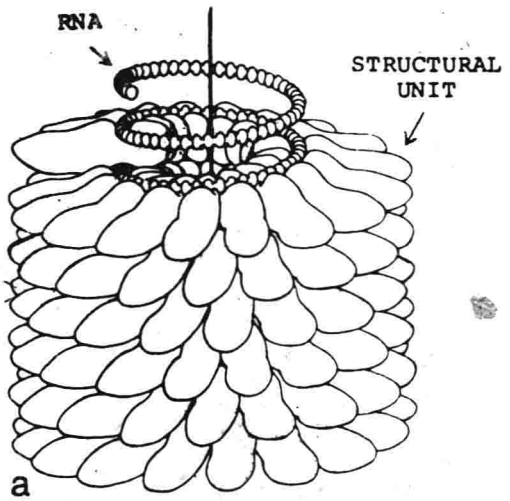
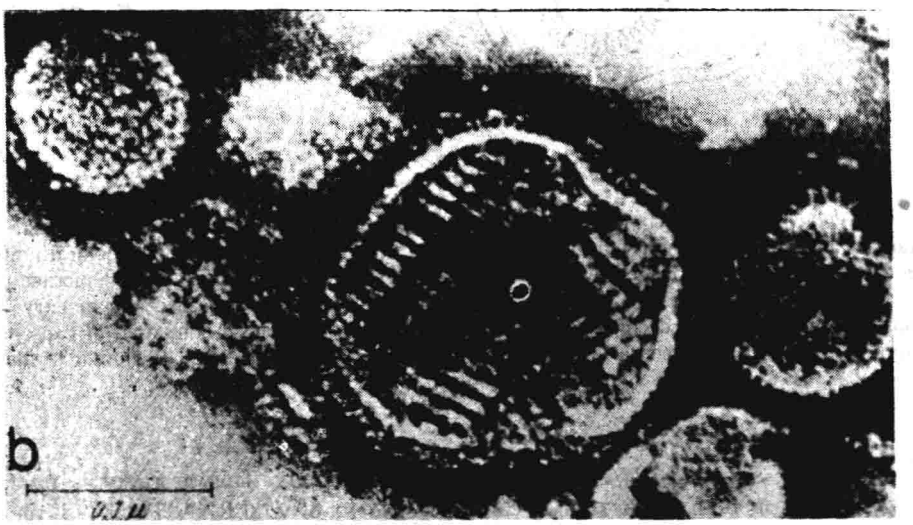


FIG. 1. Typical structural components of viruses with helical or icosahedral symmetry. **a:** Schematic diagram of an enveloped virion with helical nucleocapsid. Viral nucleic acid and structural units form the nucleocapsid, which is surrounded by an envelope composed of a lipid bilayer with membrane protein and protruding glycoprotein spikes. **b:** Schematic diagram of a virion with icosahedral symmetry. The core is composed of nucleic acid and core protein enclosed in the protein capsid composed of capsomeres. Note that some viruses with icosahedral symmetry may, in addition, have an envelope similar to that shown in panel A.

stance, in double-stranded DNA viruses, overlapping regions on both DNA strands can be transcribed into mRNAs coding for distinct proteins. Sometimes the same stretch of DNA can code for more than one protein sequence as a result of a shift in the reading frame. Specific examples of posttranscriptional (splicing) and posttranslational processing (proteolytic cleavage) will be mentioned in sections of this chapter describing the replication cycle of viruses.

Viruses with genomes of single-stranded RNA can be divided into two groups: one group of viruses contains RNA that can be translated directly into protein [referred to as messenger or positive (+) strand]; another group of viruses contains RNA that acts as the template for the viral mRNA [antimessenger or negative (−) strand]. This property is referred to as *polarity* of viral RNA. Polarity is an important characteristic because it determines the basic strategy of the virus during replication in the cell. In order to survive in nature, *negative-stranded RNA virus* must carry as part of its virion a *transcriptase enzyme* that on penetration of the virion into the cell will catalyze the first virus-specific biosynthetic event—i.e., transcription of viral +RNA which in turn can be translated into viral proteins. *Positive-stranded RNA viruses* do not have virion transcriptase; following penetration in the cell, the +RNA can start directing the synthesis of virus-specific proteins, including enzymes needed for subsequent transcription and replication of the viral genome. (Details of the replicative processes of positive- and negative-stranded RNA





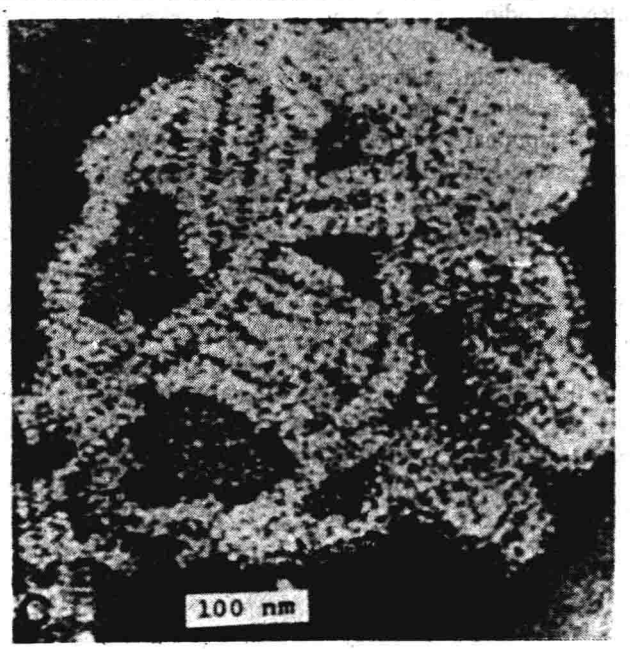
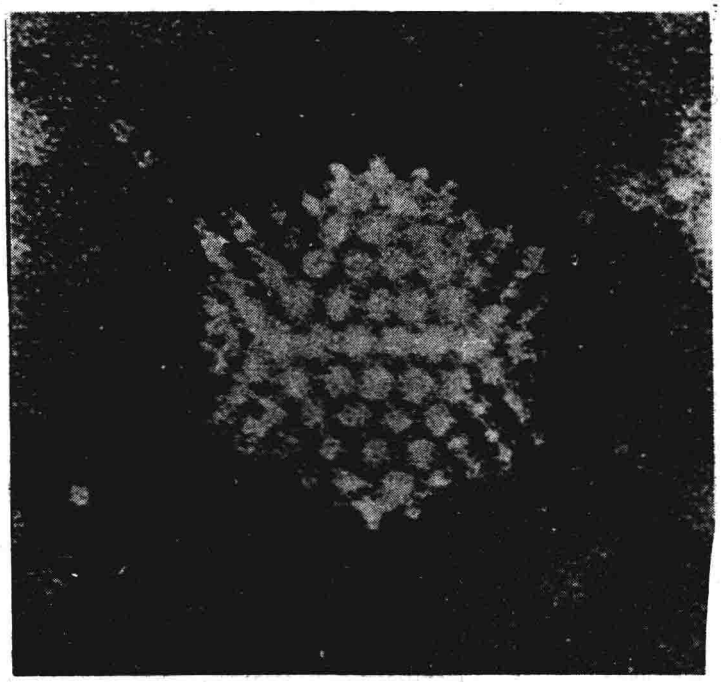
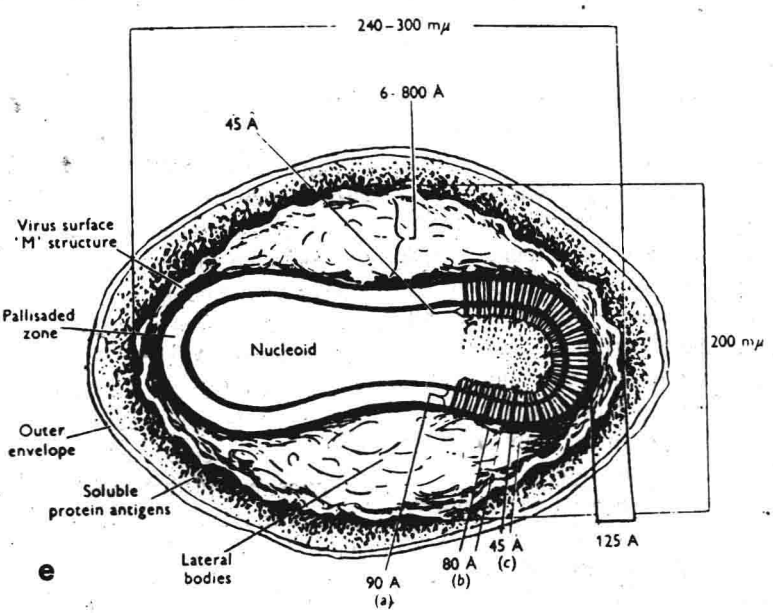


FIG. 2. **a:** Segment of helical nucleocapsid of TMV. (From Klug and Caspar, ref. 42, with permission.) **b:** Electron micrograph of influenza A2 virus after negative staining. Three or four separate internal coiled components can be seen inside large particle enclosed within envelope. (From Hoyle, ref. 33, with permission; photograph by J. Almeida and A. P. Waterson.) **c:** Electron micrograph of helical nucleocapsid of Newcastle disease virus (member of Paramyxoviridae family). (From Horne, ref. 32, with permission.) **d** *(facing page):* Electron micrograph of adenovirus particle revealing icosahedral array of capsomeres. (From Valentine and Pereira, ref. 81, with permission.) **e** *(facing page):* Diagram of poxvirion. The structure labeled "nucleoid" is the virion core containing DNA. (From Westwood et al., ref. 84, with permission.)





viruses will be reviewed in subsequent sections of this chapter.) Similar to cellular mRNAs, positive-stranded viral RNAs usually have a *poly(A) segment* attached at the 3′ end and a 7-methylguanosine *cap* at the 5′ end. The guanosine residue blocks the 5′-terminal penultimate base of the RNA through a unique 5′-5′ triphosphate linkage (5). However, in certain vi-

TABLE 1. *Most important structural characteristics of major groups of animal viruses*

Family name[a]	Representative species	Genome[b]	Approximate diameter of virion (nm)	Symmetry of nucleocapsid[c]	Other structural features
Parvoviridae	Adeno-associated viruses	DNA(SS)	20	I	
Papovaviridae	Wart viruses	DNA(DS)	50	I	
Adenoviridae	Adenoviruses	DNA(DS)	80	I	Penton fibers
Herpesviridae	Herpes simplex viruses	DNA(DS)	180	I	Envelope
Poxviridae	Vaccinia virus	DNA(DS)	100 × 250 × 300		Brick-shaped, complex structure
Picornaviridae	Polioviruses	RNA(SS)	25	I	
Togaviridae	Yellow fever virus	RNA(SS)	40–70	I	Envelope
Coronaviridae	Infectious bronchitis virus	RNA(SS)	75–160	H	Envelope
Bunyaviridae	California encephalitis virus	RNA(SS)	100	H	Envelope
Orthomyxoviridae	Influenza viruses	RNA(SS)	80–120	H	Envelope
Paramyxoviridae	Parainfluenza viruses	RNA(SS)	150	H	Envelope
Rhabdoviridae	Rabies virus	RNA(SS)	75 × 180	H	Bullet-shaped, envelope
Arenaviridae	Lymphocytic choriomeningitis virus	RNA(SS)	80–120	H	Envelope
Retroviridae	Avian leukosis viruses	RNA(SS)	100	I/H(?)[d]	Envelope
Reoviridae	Rotaviruses	RNA(DS)	70	I	

[a]According to Matthews (51).
[b]SS = single stranded; DS = double stranded.
[c]I = icosahedral; H = helical.
[d]Probably icosahedral capsid with helical ribonucleoprotein core.

ruses—e.g., Picornaviridae—the genome RNA is linked covalently to a protein with a molecular weight varying from 2,400 to 12,000 (86). Similar genome proteins are also found in some DNA viruses: e.g., the genome of adenoviruses and hepatitis B virus have a protein covalently linked to or near the 5′ termini.

Recognition of the distinct characteristics of positive- and negative-stranded RNA viruses provides a rational basis for the empirically determined differences in the *infectivities of isolated virion RNAs*. Provided that the nucleic acid is introduced into the cell in intact form, RNA isolated from positive-stranded viruses is infectious: i.e., it will initiate a replicative cycle of the virus similar to the cycle occurring after penetration of the intact virus. On the other hand, RNA isolated from negative-stranded viruses is not infectious in the absence of virion transcriptase.

Most viruses contain a single molecule of nucleic acid. However, in some RNA viruses the *genome is segmented*, and the number and size of individual pieces of RNA present in the virion are characteristic for each virus (Table 2). In some instances each segment of RNA serves as the template for the mRNA specific for a single virus protein. For instance, influenza viruses A and B contain eight distinct segments of single-stranded RNA with antimessenger polarity (negative strands). Individual segments represent the genes for single virus proteins, except that segments 7 and 8 of type A influenza virus may contain templates for more than one mRNA (44).

The nucleic acid in most RNA viruses has a linear open-ended structure. However, in one group of animal viruses (Bunyaviridae), the viral RNA exists in three segments and each of the segments can form a circle by a noncovalent link between inverted complementary sequences at the 5′ and 3′ ends (7). (Since Bunyaviridae are negative-stranded RNA viruses,

TABLE 2. *Characteristics of viral nucleic acids and related properties*

Family name	Genome[a]	Approximate molecular weight (× 10^6 d)	Number of segments	Polarity	Infectivity of isolated nucleic acid	Transcriptase present
Parvoviridae	DNA(SS)	2	1	− or +	+	None
Papovaviridae	DNA(DS)	3–5	1	±	+	None
Adenoviridae	DNA(DS)	20–25	1	±	+	None
Herpesviridae	DNA(DS)	80–150	1	±	+	None
Poxviridae	DNA(DS)	85–240	1	±	−	DNA→RNA
Picornaviridae	RNA(SS)	2–3	1	+	+	None
Togaviridae	RNA(SS)	4	1	+	+	None
Coronaviridae	RNA(SS)	6	1	+	+	None
Bunyaviridae	RNA(SS)	5	3	−	−	RNA→RNA
Orthomyxoviridae	RNA(SS)	5	8	−	−	RNA→RNA
Paramyxoviridae	RNA(SS)	5–7	1	−	−	RNA→RNA
Rhabdoviridae	RNA(SS)	4	1	−	−	RNA→RNA
Arenaviridae	RNA(SS)	4	2	−	−	RNA→RNA
Retroviridae	RNA(SS)	5[c]	1[c]	+	−[b]	RNA→DNA
Reoviridae	RNA(DS)	12–20	10	±	−	RNA→RNA

[a]SS = single stranded; DS = double stranded.
[b]Although isolated virion RNA is not infectious, DNA isolated from transformed cells has been shown to cause "transfection," i.e., transfer of the virus genome from one cell to another.
[c]Contains two identical copies of virus genome. Molecular weight value is for one monomeric copy.

the viral RNA has neither a terminal poly(A) segment nor a cap.) The RNA of Retroviridae (RNA tumor viruses) is unique because it contains two identical segments held together near their 5′ ends (9,78).

Complexity of the nucleic acid structure is also evident among DNA viruses. The viral genome may consist of a linear single-stranded DNA, as found in parvovirus (83), or a closed supercoiled circular double-stranded DNA, as in Papovaviridae (77). Circularization of the DNA in papovaviruses aids in integration of the viral nucleic acid into the chromosomal DNA of host cells—usually a prerequisite for malignant transformation by these viruses. Hepatitis B virions contain a partially double-stranded circular DNA with a single-stranded portion of variable size (62). In most viruses with linear double-stranded DNA, the nucleic acid contains inverted terminal repetitions of nucleotide sequences (terminal redundancy). A striking feature of poxvirus DNA is the presence of a short polynucleotide chain that covalently links the strands of the linear double-stranded molecule at or near each terminus (29). The DNA of the Herpesviridae is among the most complex in size and content. The virion DNA consists of a linear double-stranded molecule, which contains two covalently linked regions L and S (73). The L and S components represent 82% and 18%, respectively, of the viral genome and contain unique fragments U_L and U_S. These unique fragments in each component are bracketed by specific sequences and their inverted repeats. Components L and S are inverted relative to each other and, as a result, DNA extracted from virions consists of four equimolar populations differing in the relative orientation of the L and S components. Naturally, replication of such a genome involves mechanisms much more complex than those seen with other simpler genomes.

The *base composition* of viral nucleic acids is of taxonomic importance, and it also may have some functional significance during the process of integration of oncogenic viruses. The G + C content of DNA viruses ranges from the low of about 35% (Poxviridae) to about 75% (some Herpesviridae).

Proteins

Together with the nucleic acid, proteins are indispensable components of all virions. Although nucleic acids isolated from some virions may be infectious (Table 2), their infectivity can be demonstrated only under artificial laboratory conditions (e.g., in the presence of hypertonic medium, polycations, $CaCl_2$, etc.). As far as is known, a protein shell is always required for virions to be infectious under natural conditions.

An apparent exception from this rule is a group of agents causing infections in plants, termed *viroids* (21). These agents exist in the form of "naked" infectious RNA, lacking demonstrable protein. Viroid RNA is characteristically circular and contains regions of unusual secondary structure. Until recently it was thought that agents similar to plant viriods may be the causative agents of transmissible slow neurological disorders in animals and man, such as scrapie, kuru, and Creutzfeldt-Jakob disease. However, more recent evidence suggests that these diseases may be caused by a class of proteinaceous infectious particles *(prions)* whose infectivity apparently does not require nucleic acid (57).

A protein structure that is present in all conventional virions is the *capsid*. In a virion lacking an envelope, the capsid provides the only protection for the nucleic acid. Since it is the structure that first comes into contact with the surface of the host cell, in viruses lacking an envelope the capsid determines the host range. In many viruses the virion nucleic acid is tightly bound to a basic *core protein*, which may resemble histones. *Envelope proteins*, many of them glycosylated, form the integral part of the viral envelope (see below).

The discovery of a variety of *virion enzymes* has aided greatly in providing an understanding of the life cycles of viruses. Only members of certain families of viruses do not have virion-associated enzymes (e.g., Picornaviridae and Togaviridae); others have up to a half-dozen different enzymes present in the virion (e.g., Poxviridae). Some of the enzymes present may be of host cell origin, serving no apparent useful function for the virus. However, the presence of some virus-coded enzymes is essential for virus infectivity (17,37).

Most important among the virus specific enzymes are the *transcriptases* (also called polymerases) present in some virions. There are four different types of virion transcriptases:

1. A *DNA-dependent RNA transcriptase* is present in the core of Poxviridae. These viruses with double-stranded DNA genomes multiply in the cell cytoplasm. With all other DNA viruses, transcription takes place in the cell nucleus; these latter viruses utilize polymerases of their host cells, and none are found in the virion.
2. All negative-stranded RNA viruses have *RNA-dependent RNA transcriptase*. No host-cell enzyme capable of transcribing RNA on the RNA template is known to be present in animal cells, although there is evidence for the presence of such an enzyme in uninfected plant cells (35).
3. For a similar reason, there is *RNA transcriptase in viruses with double-stranded RNA genomes*.
4. Viruses belonging to the group of RNA tumor viruses (Retroviridae) are unique in that they possess a *reverse transcriptase* (76), an enzyme that in defiance of the central dogma of molecular biology catalyzes DNA synthesis on the template of single-stranded RNA. As will be explained later in this chapter, this enzyme is responsible for the exclusive life cycle of these viruses.

Viral Envelopes

Envelopes of viruses are derived from host-cell membranes: the Golgi apparatus; endoplasmic reticulum; and nuclear, vacuolar, or plasma membrane. It is not surprising that viral envelopes structurally resemble cellular membranes, featuring a lipid bilayer with protein molecules embedded in it and, frequently, *glycoprotein spikes* protruding on the outer surface. For instance, Orthomyxoviridae feature two types of spikes, representing the virion hemagglutinin and neuraminidase, respectively.