ANTIVIRAL AGENTS AND VIRAL DISEASES OF MAN

EDITED BY
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Preface

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 which are not often seen in otherwise healthy populations.

In the past, antivirals have usually 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. Adenine arabinoside 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 adenine arabinoside and adenine arabinoside

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 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 also are 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, these nucleocapsids 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 12 apices are always formed by single capsomeres with five neighboring capsomeres (pentons). Each of the remaining capsomeres has six adjacent capsomeres (hexons). 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). Capsids of herpesviruses have 150 hexons and 12 pentons. (Unlike the virions of most other icosahe-

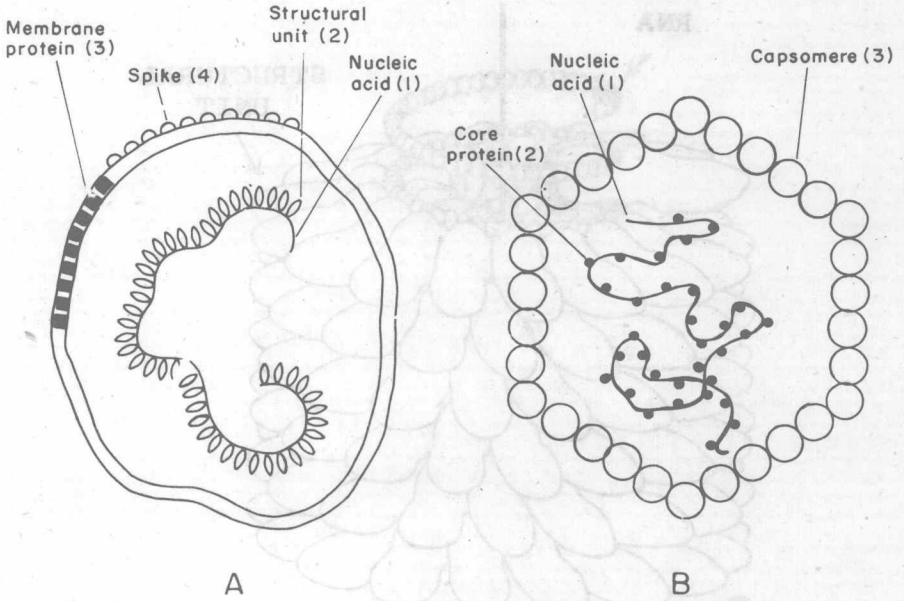


FIG. 1. Typical structural components of viruses with helical or icosahedral symmetry. **A:** Schematic diagram of an enveloped virion with helical nucleocapsid. Viral nucleic acid (1) and structural units (2) form the nucleocapsid, which is surrounded by an envelope composed of a lipid bilayer with membrane protein (3) and protruding glycoprotein spikes (4). **B:** Schematic diagram of a virion with icosahedral symmetry. The core is composed of nuclei acid (1) and core protein (2) enclosed in the protein capsid composed of capsomeres (3). Note that some viruses with icosahedral symmetry may, in addition, have an envelope similar to that shown in panel A.

dral viruses, each herpesvirus virion also has an envelope.) 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 at least one family, the 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 physicochemical analysis of purified virus particles.

Nucleic Acid

The genome of a virus can be either RNA or DNA; the *type* of nucleic acid contained in the virion is the most important characteristic of any virus.

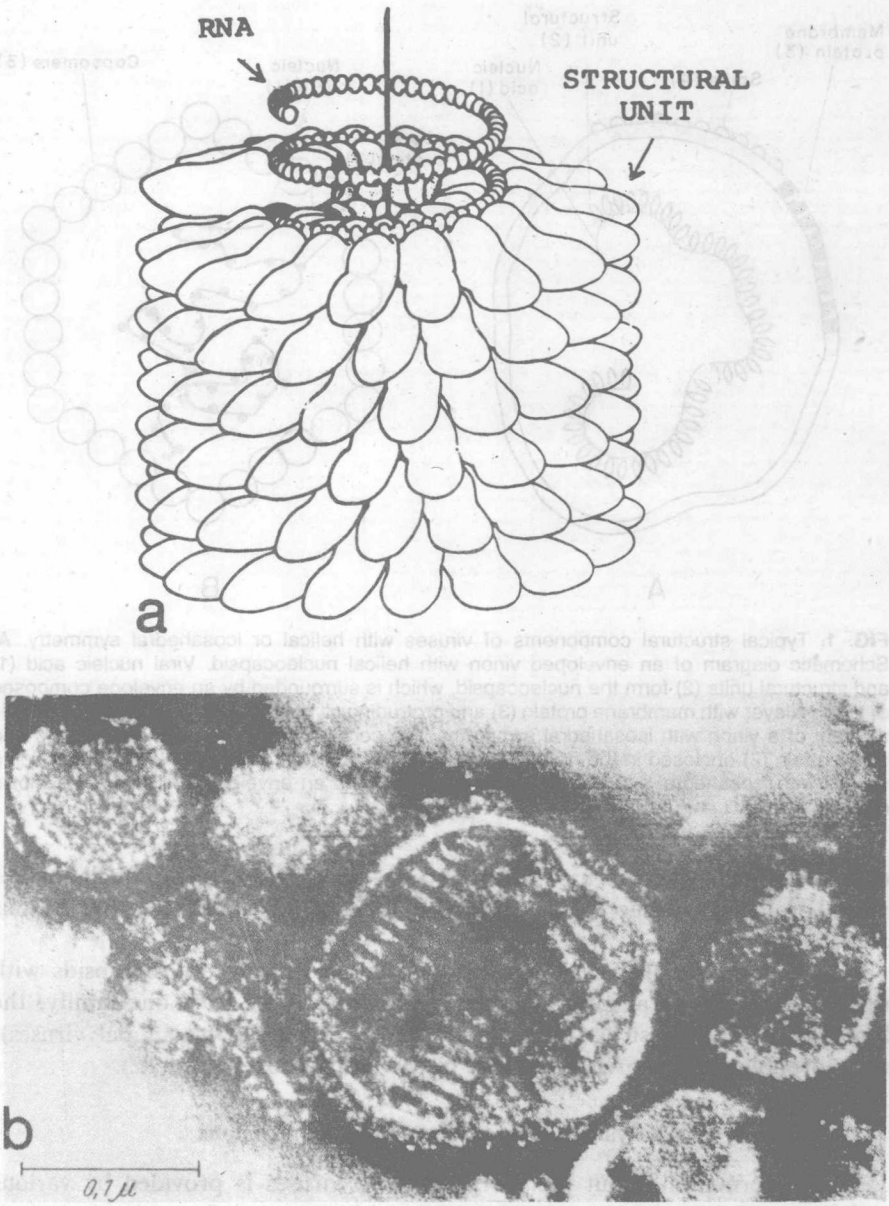
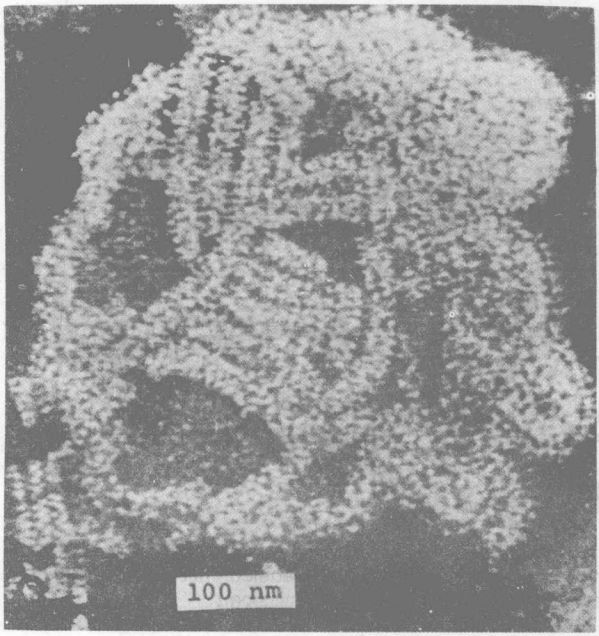


FIG. 2. A: Segment of helical nucleocapsid of TMV. (From Klug and Caspar, ref. 34, with permission.) **B:** Electron micrograph of influenza A2 virus after negative staining. Three or four separate internal coiled components can be seen inside large particle enclosed within envelope. (From Hoyle, ref. 23, 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. 22, with permission.) **D:** Electron micrograph of adenovirus particle revealing icosahedral array of capsomeres. (From Valentine and Pereira, ref. 68, with permission.)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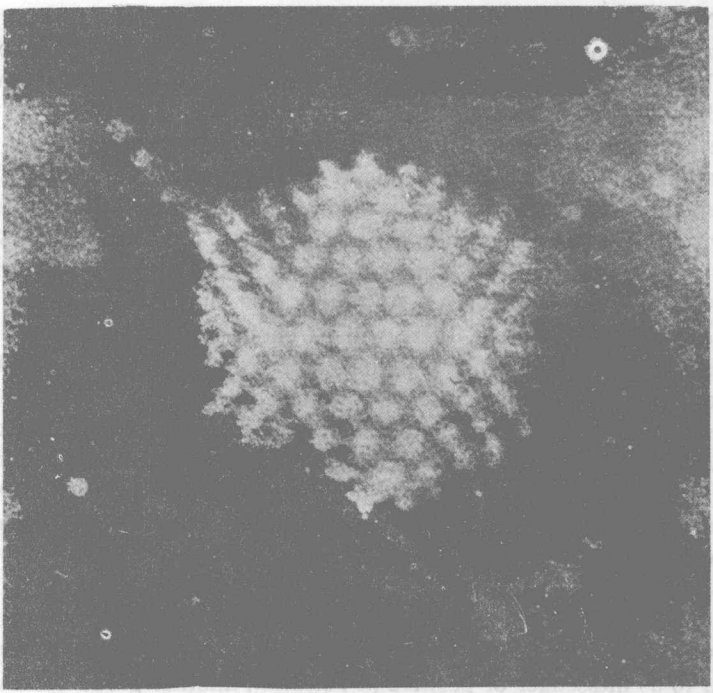

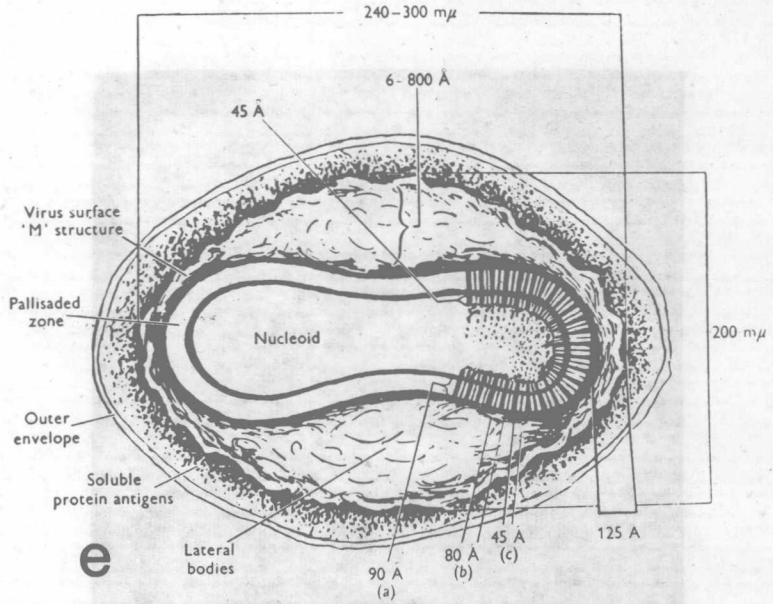

FIG. 2. E: Diagram of poxvirion. The structure labeled "nucleoid" is the viron core containing DNA. (From Westwood et al., ref. 71, with permission.)

Both RNA and DNA can be present in either *single-stranded* form or *double-stranded* form. However, with the exception of Parvoviridae, all DNA-containing viruses have double-stranded DNA. 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 viruses are found in Parvoviridae (molecular weight 2×10^6 d, single-stranded DNA) and Picornaviridae ($2–3 \times 10^6$ d, single-stranded RNA). Assuming that the entire genomes in these viruses can code for protein, there will be enough genetic formation to code for proteins with total molecular weights of approximately 220,000 d and 270,000 d, respectively. This conclusion stems from the *coding ratio:* the molecular weight of single-stranded nucleic acid divided by the molecular weight of coded protein = 9. The largest viruses, Poxviridae, have double-stranded DNA genomes with molecular weights up to 2×10^8 d; 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 d (or about 500 different average-size proteins).

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 con-

TABLE 1. *Most important structural characteristics 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)	75	I	Penton fibers
Herpetoviridae	Herpes simplex viruses	DNA(DS)	200	I	Envelope
Poxviridae	Smallpox 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 or 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)	120–180	H	Envelope
Rhabdoviridae	Rabies virus	RNA(SS)	75 × 180	H	Bullet-shaped, envelope
Arenaviridae	Lymphocytic choriomeningitis virus	RNA(SS)	80–120	H	Envelope
Coronaviridae	Infectious bronchitis viruses	RNA(SS)	80–120	H	Envelope
Retroviridae	Avian leukosis viruses	RNA(SS)	100–150	H (?)	Envelope
Reoviridae	Rotaviruses	RNA(DS)	70	I	

[a] According to Fenner (18).
[b] SS = single stranded; DS = double stranded.
[c] I = icosahedral; H = helical.