THE BIOLOGY OF ANIMAL VIRUSES

SECOND EDITION

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Preface

In the years that have elapsed since the first edition of this book was published there has been a very substantial growth in our knowledge of animal viruses, especially in the areas of molecular biology and tumor virology. In the Acknowledgments of the first edition the senior author, who was then the sole author, suggested that "to review critically as broad and as rapidly changing a field as animal virology is a bold, perhaps a foolhardy, enterprise." It is now an impossible enterprise, and this edition is the result of the collaborative efforts of five erstwhile colleagues from the Department of Microbiology, John Curtin School of Medical Research, who are now located in three continents: Australia, Europe, and North America. Each of us has taken prime responsibility for particular chapters, but all have read and criticized the whole text, with the senior author acting as editor where coordination was needed.

Several chapters of the first edition were almost completely rewritten, in two instances two chapters were consolidated into single chapters (now Chapters 3 and 7), two chapters (Chapters 5 and 8 of the first edition) were eliminated as separate chapters, and a new Chapter 2 was added for the benefit of scientists moving into animal virology from other fields.

We have endeavored to select references best suited to guide the newcomer to the appropriate literature, and have not always attempted to give due notice to those whose contributions have priority. This function is best fulfilled by histories or specialist review articles. We have provided article titles in the Bibliography as a valuable guide to readers wishing to consult original papers.

Because of the existence today of an International Committee on Nomenclature of Viruses that embraces all viruses and the realization that "animal" embraces both vertebrates and invertebrates, an apology is perhaps needed for retaining the words "animal viruses" in the title. We plead only that the term is widely used and was used in the first edition to designate the viruses of vertebrates.

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Preface to First Edition

During the last twenty years virology has developed into an independent science. It is now growing so rapidly that two new journals of virology (in English) were launched this year. Four major works on viruses of vertebrate animals have been published recently, which deal with the viral diseases of man (Horsfall and Tamm, 1965) and his domestic animals (Betts and York, 1967–1968), with techniques in virology (Maramorosch and Koprowski, 1967–1968), and provide an encyclopedic description of the viruses of vertebrates (Andrews, 1964). However, none of these books deals in a comprehensive way with the broader biological principles of animal virology, which is the aim of this two-volume work. It began as an attempt to revise Burnet's "Principles of Animal Virology," but changes in knowledge and emphasis made a revision impossible.

Since the publication of the second edition of Burnet's book in 1960, animal viruses have become major objects for study by molecular biologists, and the knowledge so gained has greatly clarified our understanding of the structure and composition of the virions and their interactions with animal cells. Volume I deals chiefly with the molecular biology of animal viruses and the cellular biology of viral infections. In Volume II a detailed description is given of viral infections at the level of organism, i.e., the pathogenesis of disease and the specific and non-specific reactions of vertebrates to viral infections, including those due to the oncogenic viruses. Finally, the ecology of animal viruses is discussed in relation to their spread through populations of vertebrates.

Only the viruses of warm-blooded vertebrates have been considered. One of the great simplifications of the last decade has been the realization that nearly all of the 500 viruses of warm-blooded vertebrates now recognized can be allocated to one of about a dozen groups. In each of these groups the viruses are very like each other in many properties, but often strikingly different from viruses of other groups. In the opening chapter of Volume I the basis for this classification and the properties of the major groups are discussed in detail, and in subsequent chapters different aspects of virology are discussed in terms of model viruses selected from some or all of these groups.

This work was written primarily for virologists and molecular biologists, including teachers, research workers, and graduate students in these fields. It should also prove useful to doctors and veterinarians interested in either clinical or public health aspects of the viral diseases of man or his domestic animals. Each chapter ends with a summary so that casual readers can rapidly orient themselves. In order to keep the text reasonably short no historical account is given of the development of virology; the reader is referred to the first chapter of Burnet's "Principles of Animal Virology" for such information.

The selection of references presented a problem. Rather than attempt to observe priority of discovery over so wide a field, the references were selected mainly for their potential value in providing the reader with an effective entry into the scientific literature of the subject under discussion. References to abstracts were retained only when no later paper on the topic could be found.

FRANK FENNER

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Annals of the New York Academy of Sciences (Fig. 9–10)
Archives für gesamte Virusforschung (Fig. 11–5, Plate 3–7)

Australasian Annals of Medicine (Figs. 11–2, 11–3)
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British Journal of Experimental Pathology (Fig. 9–7)
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Cold Spring Harbor Laboratory (Figs. 1–2, 5–5)
Journal of Cell Biology (Plates 3–9, 5–1)
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Journal of General Virology (Plate 3–7, Figs. 3–2, 3–4)
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"Fundamental Techniques in Virology" (Academic Press) (Plate 2–3)
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"Myxomatosis" (Cambridge University Press) (Fig. 11–1)
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CHAPTER 1

The Nature and Classification of Animal Viruses

INTRODUCTION

Virology began as a branch of pathology, the study of disease. At the end of the nineteenth century, when the microbial etiology of many infectious diseases had been established, pathologists recognized that there were a number of common infectious diseases of man and his domesticated animals for which neither a bacterium nor a protozoan could be incriminated as the causal agent. In 1898, Loeffler and Frosch demonstrated that the economically important disease of cattle, foot-and-mouth disease, could be transferred from one animal to another by material which could pass through a filter that retained the smallest bacteria. Following this discovery such diseases were tentatively ascribed to what were first called "ultramicroscopic filterable viruses," then "ultrafilterable viruses," and, ultimately, just "viruses."

Independently, the plant pathologist Beijerinck (1899) recognized that tobacco mosaic disease was caused, not by a conventional microorganism, but by what he called a "contagium vivum fluidum" (i.e., "contagious living fluid"). Many economically important diseases of domestic plants were subsequently found to be caused by viruses. Years later, the bacteriologists Twort (1915) and d'Hérelle (1917) recognized that bacteria also could be infected by viruses, for which d'Hérelle coined the name "bacteriophages." Insect viruses were not recognized as such until the 1940's (Bergold, 1958), and even more recently viruses have been recovered from fungi (Hollings, 1962), from blue-green algae (Schneider *et al.*, 1964; review: Brown, 1972), from free-living mycoplasmas (Gourlay, 1971), and from protozoa (Diamond *et al.*, 1972).

THE NATURE OF VIRUSES

From the practical viewpoint of the plant pathologist and the public health worker, it is convenient to regard the viruses that cause disease as pathogenic microorganisms (Burnet, 1945). However, the question arose as to whether viruses, whatever their host, might have common properties that distinguished them from microorganisms. Lwoff has cogently argued (Lwoff, 1957; Lwoff and Tournier, 1966) that all viruses show some properties that distinguish them from any microorganism. Exceptions to some of Lwoff's generalizations have since been discovered, but two still apply: (a) unlike even the smallest microorganisms (chlamydiae), viruses contain no functional ribosomes or other cellular organelles, and (b) in RNA viruses the whole of the genetic information is encoded in RNA, a situation unique in biology. Other distinctions apply to some but not all viruses, e.g., the isolated nucleic acid of viruses of several genera is infectious (i.e., the virus can be generated intracellularly from a single molecule of nucleic acid), and viruses of most genera contain either no virus-coded enzymes, or one or more enzymes that belong to particular classes (neuraminidases and nucleic acid polymerases).

It is impossible to define viruses satisfactorily in a sentence or even a paragraph, bearing in mind both their intracellular states and the extracellular particles or virions. Virions consist of a genome of either DNA or RNA enclosed within a protective coat of protein molecules, some of which may be associated with carbohydrates or lipids of cellular origin. In the vegetative state and as "provirus" (see Chapter 5), viruses may be reduced to their constituent genomes, and the simplest "viruses" may be transmitted from one host to another as naked molecules of nucleic acid, possibly associated with certain cellular components. At the other extreme, the largest animal viruses, e.g., the poxviruses and the leukoviruses, are relatively complex.

Lwoff's concept that "viruses are viruses" has had important theoretical and practical consequences; on the one hand, it emphasized their similarities irrespective of the nature of the host (animal, plant or bacterium), and, on the other hand, it led to the possibility of freeing viruses from the rules of bacteriological nomenclature. However, the operational division of viruses made according to type of host continues to be used by the majority of virologists most of the time, and it is significant that the International Committee on Nomenclature of Viruses (ICNV), although dedicated to a universal classification, operates through Subcommittees on Bacterial, Invertebrate, Plant, and Vertebrate Viruses (Wildy, 1971).

THE CHEMICAL COMPOSITION OF ANIMAL VIRUSES

The simpler viruses consist of nucleic acid and a few polypeptides specified by it. More complex viruses usually also contain lipids and carbohydrates; in the great majority of viral genera these chemical components are not specified by the viral genome but are derived from the cells in which the viruses multiply. In exceptional situations, cellular nucleic acids or polypeptides may be built into viral particles.

Nucleic Acids

Viruses contain only a single species of nucleic acid, which may be DNA or RNA. Viral nucleic acid may be single- or double-stranded, the viral genome may consist of one or several molecules of nucleic acid, and if the genome consists of a single molecule this may be linear or have a circular configuration.

As yet, no animal viral nucleic acid has been found to be methylated, or to contain novel bases of the type encountered in bacterial viruses or mammalian transfer RNA's, but some virions contain oligonucleotides rich in adenylate, of unknown function. The base composition of DNA from animal viruses covers a far wider range than that of the vertebrates, for the guanine plus cytosine (G+C) content of different viruses varies from 35 to 74%, compared with 40 to 44% for all chordates. Indeed, the G+C content of the DNA of viruses of one genus (*Herpesvirus*) ranges from 46 to 74%.

The molecular weights of the DNA's of different animal viruses varies from just over 1 to about 200 million daltons; the range of molecular weights of viral RNA's is much less, from just over 2 to about 15 million daltons. The nucleic acid can be extracted from viral particles with detergents or phenol. The released molecules are often fragile but the isolated nucleic acid of viruses belonging to certain genera is infectious. In other cases, the isolated nucleic acid is not infectious even though it contains all the necessary genetic information, for its transcription depends upon a virion-associated transcriptase without which multiplication cannot proceed.

All DNA viruses have genomes that consist of a single molecule of nucleic acid, but the genomes of many RNA viruses consist of several different molecules, which are probably loosely linked together in the virion. In viruses whose genome consists of single-stranded nucleic acid, the viral nucleic acid is either the "positive" strand (in RNA viruses, equivalent to messenger RNA) or the "negative" (complementary) strand. Preparations of some viruses with genomes of single-stranded DNA consist of particles that contain either the positive or the complementary strand.

Viral preparations often contain some particles with an atypical content of nucleic acid. Host-cell DNA is found in some papovaviruses, and what appear to be cellular ribosomes in some arenaviruses. Several copies of the complete viral genome may be enclosed within a single particle (as in paramyxoviruses) or viral particles may be formed that contain no nucleic acid ("empty" particles) or that have an incomplete genome, lacking part of the nucleic acid that is needed for infectivity.

Terminal redundancy occurs in the DNA of some vertebrate viruses, but most sequences are unique. The largest viral genomes contain several hundred genes, while the smallest carry only sufficient information to code for about half a dozen proteins, most of which are structural proteins of the virion.

Proteins

The major constituent of the virion is protein, whose primary role is to provide the viral nucleic acid with a protective coat. As predicted by Crick and Watson (1956), from a consideration of the limited amount of genetic information carried by viruses, the protein shells of the simpler viruses consist of repeating protein subunits. Sometimes the viral protein comprises only one sort of polypeptide chain, although, more commonly, there are two or three different polypeptides. The proteins on the surface of the virion have a special affinity for complementary receptors present on the surface of susceptible cells. They also contain the antigenic determinants that are responsible for the production of protective antibodies by the infected animal.

Viral polypeptides are quite large, with molecular weights in the range 10,000–150,000 daltons. The smaller polypeptides are often but not always internal, the larger ones often but not always external. There are no distinctive features about the amino acid composition of the structural polypeptides of the virion, except that those intimately associated with viral nucleic acid in the "core" of some icosahedral viruses are often relatively rich in arginine.

Viral envelopes usually originate from the cellular plasma membrane from which the original cellular proteins have been totally displaced by viral peplomers and a viral "membrane protein" (see Fig. 1–1). The peplomers consist of repeating units of one or two glycoproteins, the polypeptide moiety of which is virus-specified while the carbohydrate is added by cellular transferases. In many enveloped viruses, the inside of the viral envelope is lined by a viral protein called the membrane or matrix protein.

Not all structural viral proteins are primary gene products, since with many viruses the viral mRNA is translated into a large polypeptide that is enzymatically cleaved to yield two or more smaller virion proteins. Cleavage is often one of the terminal events in the assembly of the virion and it can occur *in situ* after most of the proteins are already in place.

Although most virion polypeptides have a structural role some have enzymatic activity. Many viruses contain a few molecules of an internal protein that functions as a transcriptase, one of the two kinds of peplomers in the envelope of myxoviruses has neuraminidase activity, and a variety of other enzymes are found in the virions of the larger, more complex viruses.

In addition to polypeptides that occur as part of the virion, a large part of the viral genome (most of it, with the large DNA viruses) codes for polypeptides that have a functional role during viral multiplication but are not incorporated into viral particles. Few of these "nonstructural viral proteins" have been characterized.

Lipid and Carbohydrate

Except for the large and complex poxviruses, which constitute a special case, lipid and carbohydrate are found only in viral envelopes and are always of cellular origin. The lipids of viral envelopes are characteristic of the cell of origin, though minor differences between the viral envelope and the normal plasma membrane may be demonstrable. About 50 to 60% of the lipid is phospholipid and most of the remainder (20–30%) is cholesterol. Some of the viral carbohydrate occurs in the envelope as glycolipid characteristic of the cell of origin, but most of it is part of the glycoprotein peplomers that project from the viral envelope.

THE STRUCTURE OF ANIMAL VIRUSES

During the 4 years that followed the introduction of negative staining for the electron microscopic study of viruses (Brenner and Horne, 1959), a general