Comprehensive Virology

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4

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Foreword

The time seems ripe for a critical compendium of that segment of the biological universe we call viruses. Virology, as a science, having only recently passed through its descriptive phase of naming and numbering, has probably reached that stage at which relatively few new—truly new—viruses will be discovered. Triggered by the intellectual probes and techniques of molecular biology, genetics, biochemical cytology, and high-resolution microscopy and spectroscopy, the field has experienced a genuine information explosion.

Few serious attempts have so far been made to chronicle these events. This comprehensive series, which will comprise some 6000 pages in a total of about 22 volumes, represents a commitment by a large group of active investigators to analyze, digest, and expostulate on the great mass of data relating to viruses, much of which is now amorphous and disjointed and scattered throughout a wide literature. In this way, we hope to place the entire field in perspective as well as to develop an invaluable reference and sourcebook for researchers and students at all levels. This series is designed as a continuum that can be entered anywhere but which also provides a logical progression of developing facts and integrated concepts.

The first volume contains an alphabetical catalogue of almost all viruses of vertebrates, insects, plants, and protists, describing them in general terms. Volumes 2–5 deal primarily, though not exclusively, with the processes of infection and reproduction of the major groups of viruses in their hosts. Volume 2 deals with the simple RNA viruses of bacteria, plants, and animals; the togaviruses (formerly called arboviruses), which share with these only the feature that the virion's RNA is able to act as messenger RNA in the host cell; and the reoviruses of animals and plants, which all share several structurally singular features, the most important being the double-strandedness of their multiple RNA molecules. This grouping, of course, has only

slightly more in its favor than others that could have been or indeed were considered.

Volume 3 addresses itself to the reproduction of all DNA-containing viruses of vertebrates, a seemingly simple act of classification, even though the field encompasses the smallest and the largest viruses known.

The reproduction of the larger and more complex RNA viruses represents the subject matter of Volume 4. These share the property of lipid-rich envelopes with the togaviruses included in Volume 2. They share as a group, and with the reoviruses, the presence of enzymes in their virions and the need for their RNA to become transcribed before it can serve messenger functions.

Volume 5 attends to the reproduction of DNA viruses in bacteria, again ranging from small and simple to large and complex.

Aspects of virion structure and assembly of many of these viruses will be dealt with in the following series of volumes, while their genetics, the regulation of their development, viroids, and coviruses will be discussed in subsequently published series. The last volumes will concentrate on host–virus interactions, and on the effects of chemicals and radiation on viruses and their components. At this juncture in the planning of *Comprehensive Virology*, we cannot foresee whether certain topics will become important aspects of the field by the time the final volumes go to press. We envisage the possibility of including volumes on such topics if the need arises.

It is hoped to keep the series at all times up to date by prompt and rapid publication of all contributions, and by encouraging the authors to update their chapters by additions or corrections whenever a volume is reprinted.

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CHAPTER 1

Reproduction of Rhabdoviruses

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1. DESCRIPTIVE BIOLOGY

1.1. Definition

The rhabdoviruses are ubiquitous, highly infectious agents of animal and plant disease and are generally transmitted by arthropods. Assignment of viruses to the taxon rhabdoviruses (rod-shaped viruses) was originally based entirely on morphology. This classification has turned out to be fortuitously fortunate because later biochemical studies have revealed remarkable uniformity among these structurally similar viruses isolated from extremely diverse hosts. It is perhaps not farfetched to postulate a common ancestor for all the rhabdoviruses of plants, arthropods, and vertebrates. Classification of a virus as a rhabdovirus should be based on the following most important characteristics:

1. Rhabdoviruses are rod-shaped particles, varying considerably in length (60–400 nm) but of a reasonably consistent width (60–85 nm).
2. Animal rhabdoviruses tend to be bullet-shaped in appearance, flat at one end and a tapered sphere at the other. Plant rhabdoviruses are usually bacilliform in shape, quite elongated and with two round ends.
3. All rhabdoviruses appear to be surrounded by a membranous

envelope with protruding spikes. All these viruses probably contain lipids and are, therefore, susceptible to disruption by ether and detergents.

4. Wound inside the envelope of rhabdoviruses is a ribonucleocapsid (RNC) core which gives the appearance of striations when viewed by electron microscopy. All rhabdoviruses examined to date contain one molecule of single-stranded RNA, which is not by itself infectious and does not serve as messenger. Therefore, rhabdoviruses are generally classified along with the myxoviruses and paramyxoviruses as "negative-strand viruses," in contradistinction to the "positive-strand" picornaviruses and togaviruses (Baltimore, 1971).

5. Many, if not all, rhabdoviruses contain an RNA-dependent RNA polymerase (transcriptase) as part of the nucleocapsid which renders it infectious in the absence of the envelope.

6. A common characteristic of animal rhabdoviruses, conceivably also applicable to plant rhabdoviruses, is the frequent occurrence of defective truncated (T) virions which are noninfectious because a considerable segment (one-third to two-thirds) of the RNA genome is deleted.

A reliable overview of the rhabdoviruses is available in the then-topical review of Howatson (1970), which provides important information on the biology and morphology of these viruses and their distribution in nature. Much of the older literature on rabies is available in the review by Matsumoto (1970). The excellent, very recent review by Knudson (1973) is recommended reading for a true understanding of comparative rhabdovirology, as is the even more recent and more comprehensive review by Francki (1973). Some rhabdoviruses are important causative agents of human disease, particularly rabies, and others are of considerable economic importance as serious infectious agents of disease in animals, both livestock and wild animals. Probably the greatest economic impact is caused by the rhabdoviruses that infect a great variety of plants. The extensive literature on the pathogenesis of rhabdoviral infections will not be covered in this chapter. Highly recommended for the interested reader is the excellent article on the pathogenesis of infection with vesicular stomatitis virus by Miyoshi *et al.*, (1971), as is the review of the natural history of vesicular stomatitis by Hanson (1952). Very good articles and reviews of the literature on the comparative pathogenesis of rabies and rabieslike viruses have recently been written by Murphy *et al.* (1973*a*,*b*); also recommended is "Natural History of Rabies" edited by Baer (1973).

1.2. Morphology

1.2.1. Vesicular Stomatitis Virus

1.2.1a. Infectious B Virions

Vesicular stomatitis (VS) virus, the prototype of rhabdoviruses, has been examined by electron microscopy in many laboratories (Chow *et al.*, 1954; Reczko, 1960; Howatson and Whitmore, 1962; Bradish and Kirkham, 1966; McCombs *et al.*, 1966; Simpson and Hauser, 1966; Nakai and Howatson, 1968). Only minor variations can be discerned in the morphological characteristics of other rhabdoviruses, and they will not be described here in detail. The reader is referred to the reviews of Howatson (1970) and Knudson (1973) for comparative analyses of the electron microscopy of rhabdoviruses. Figures 1 and 2 illustrate the general structure of VS virus as viewed by negative staining and thin-section electron microscopy; Fig. 3 shows a schematic representation of the infectious virion. The typical infectious virion is a bullet-shaped (B particle) cylinder, 180 ± 10 nm in length and 65 ± 10 nm in diameter at the blunt end. Most intact virions are planar at one end and hemispheric at the other; occasional particles can be seen to be round at both ends, but they are rarely, if ever, flat at both ends, unless broken. The B-virion cylinder appears to have a hollow center that is penetrable by phosphotungstic acid (PTA) to a varying extent, up to almost its whole length in some virions. PTA almost invariably penetrates through the planar (blunt) end of the virion, a finding which has given rise to the concept that the virion envelope is weaker or structurally defective at the planar end. This structural weakness is consistent with the observation that the virion is invariably pinched off at the blunt end as it buds from the plasma membrane; the resealed membrane that forms the virion envelope is, therefore, presumed to be more permeable at the blunt end. A distended piece of membrane, often seen ballooning from the blunt end of the virion, is thought to be a distinctive feature by some investigators.

Any structural model of the VS virion must consider that the virion is composed of two separate and distinct components: (1) the RNC and (2) the envelope (see Fig. 3). When the envelope is stripped off completely with detergents such as deoxycholate, the RNC structure remains (Fig. 4). The RNC has a buoyant density in CsCl of about 1.31 g/ml and contains only RNA and protein (Wagner *et al.*, 1969*b*; Kang and Prevec, 1969). When viewed by PTA negative-

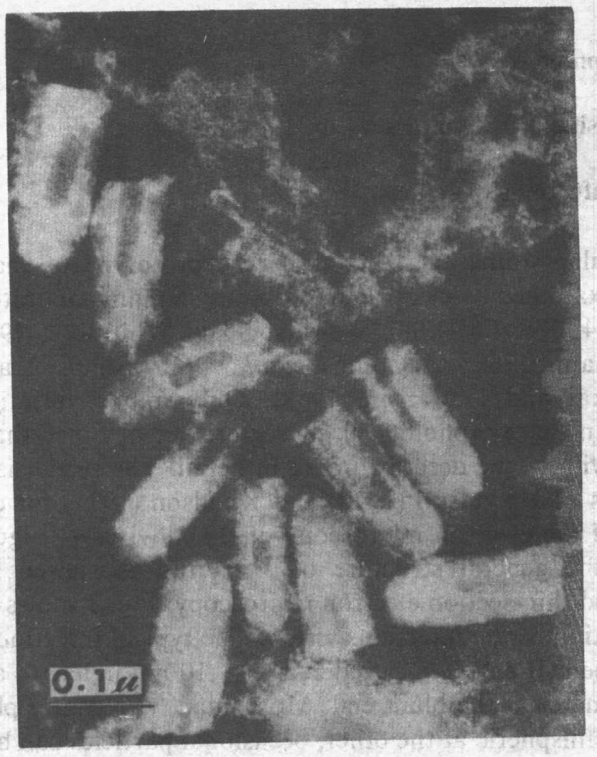

Fig. 1. Electron micrograph of unfixed L cells infected with VS virus and negatively-stained with phosphotungstic acid. Reprinted by permission from Printz and Wagner (1971).

staining electron microscopy, the RNC of infectious B virions appears as a partially or completely extended coil, about 3.5 μm in length. When the RNC is wound up in the virion envelope, it assumes a helical configuration of 35 $\pm$ 1 turns with a total of about 1000 subunits (undoubtedly protein) of dimensions $\sim 9 \times 3 \times 3$ nm; the long axis of the subunits appears to be oriented radially (Nakai and Howatson, 1968; Howatson, 1970). The intact RNC, completely devoid of envelope, is infectious, but at a considerably lower order of efficiency than the whole virion (Brown *et al.*, 1967*b*; Szilágyi and Uryvayev, 1973). The RNA without protein is not infectious (Huang and Wagner, 1966*b*).

The outer surface of the VS virion is composed of a lipoprotein membrane that surrounds and envelopes the nucleocapsid (Howatson, 1970; Nakai and Howatson, 1968; Cartwright *et al.*, 1972). Morphologically, the envelope comprises two distinct structural units. The more internal component is a membrane that has all the morphological

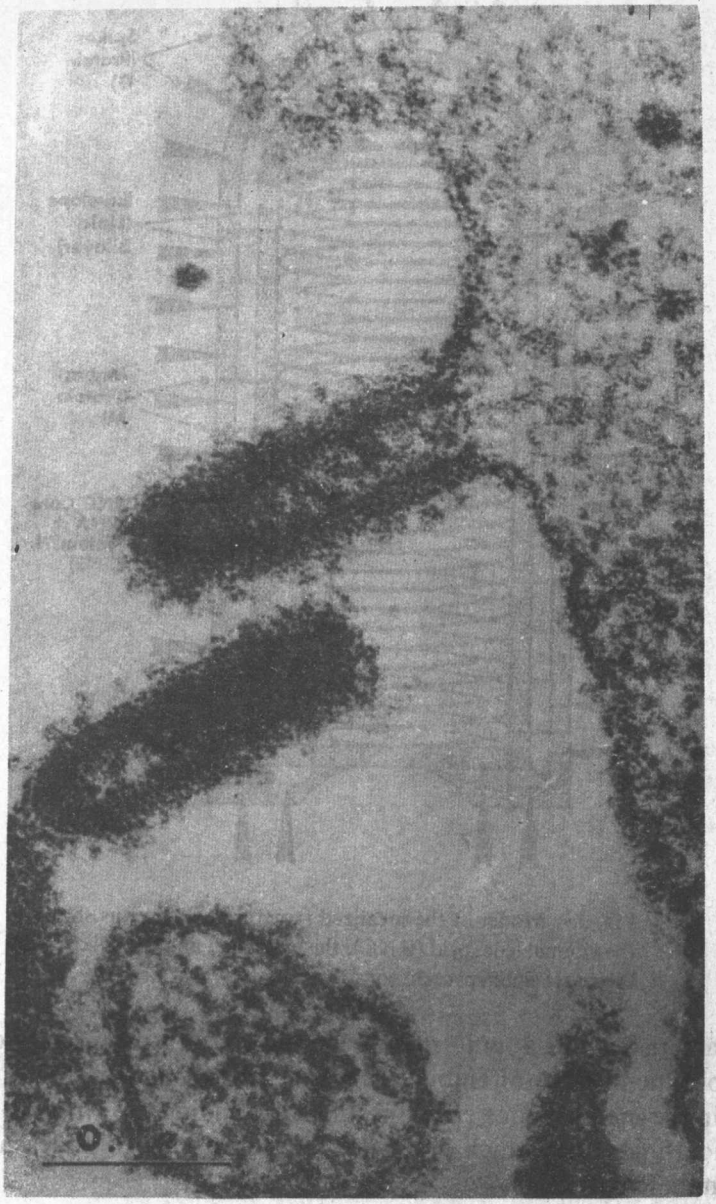

Fig. 2. Thin-section electron micrograph of L cell infected with VS virus, fixed with glutaraldehyde and OsO_4, and stained with uranyl acetate and lead.

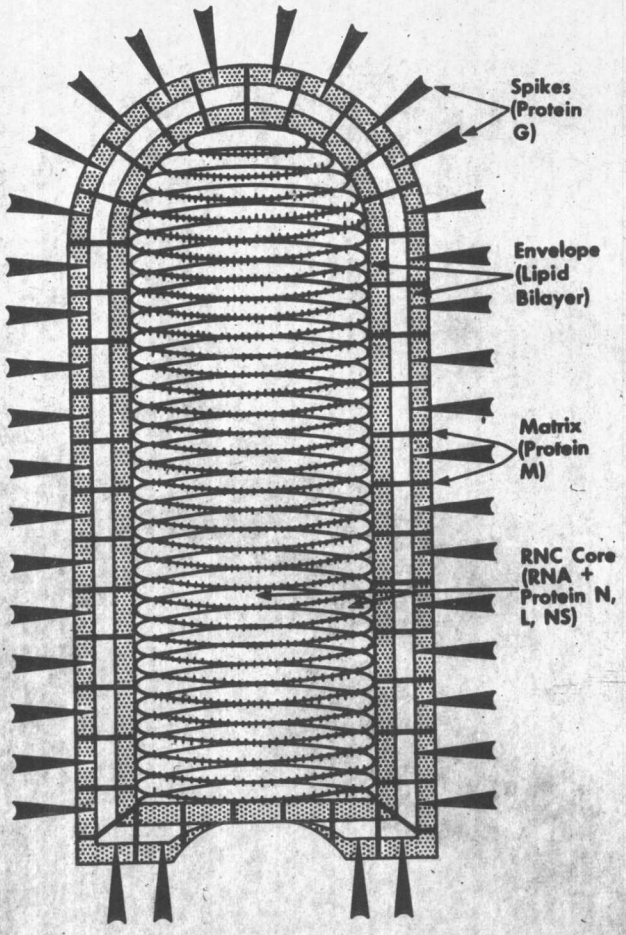

Fig. 3. Model of the idealized structure of VS virus showing the ribonucleocapsid (RNC), the envelope, and the spikes with their associated protein constituents.

characteristics of a unit membrane derived from plasma or other smooth membranes of animal (or plant) cells (Knudson, 1973). Embedded in the membranous envelope and surrounding the virion is an array of radiating spikes which protrude from the surface and measure about 10 nm in length.

1.2.1b. Defective Truncated (T) Virions

The concept of defective rhabdovirus virions dates from the observation of Cooper and Bellett (1959) of a transmissible (T)

component in VS viral preparations that interferes with VS viral infectivity. Hackett (1964) demonstrated a possible morphological basis for this autointerference phenomenon which was subsequently proven by definitive identification and purification of a truncated (T) particle in undiluted-passage preparations of VS virus (Huang *et al.*, 1966; Huang and Wagner, 1966*a*; Brown *et al.*, 1967*c*). When such preparations are examined by PTA negative-staining electron microscopy, the predominant virions are about one-third the length (about 65 nm) of

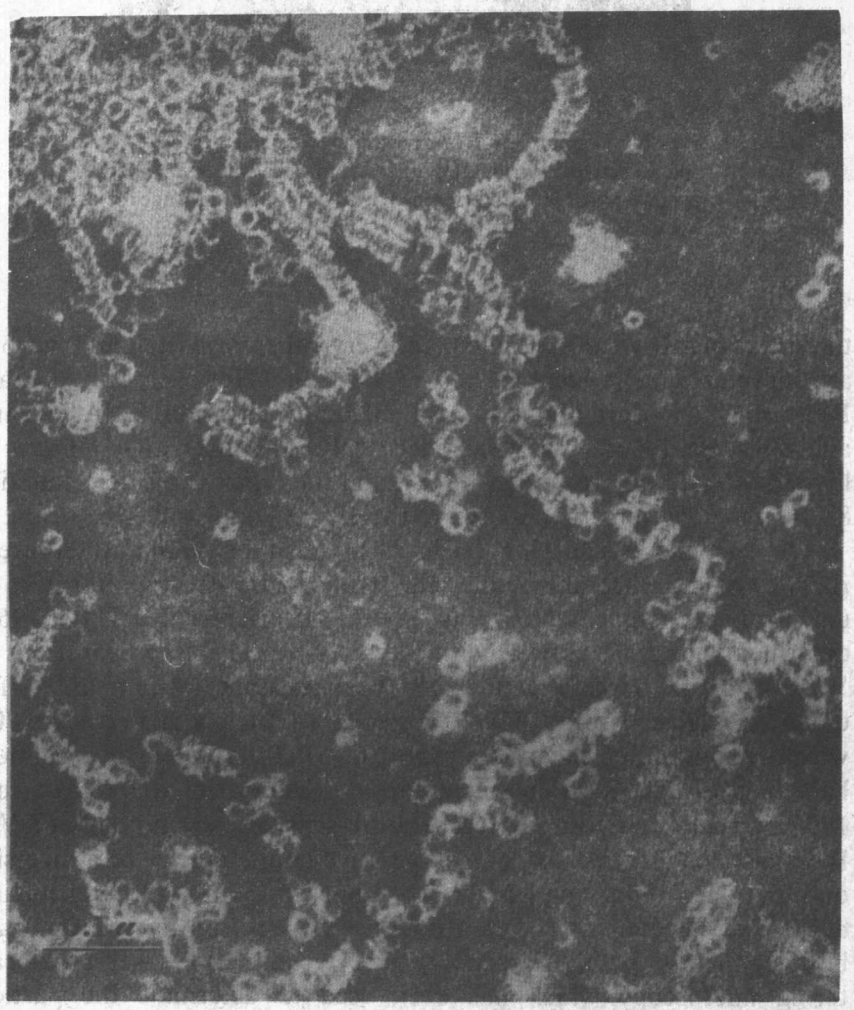

Fig. 4. RNC coils released from VS virions by exposure to Triton X-100 in 0.74 M NaCl and negatively stained with PTA. Reprinted by permission from Emerson and Wagner, (1972).

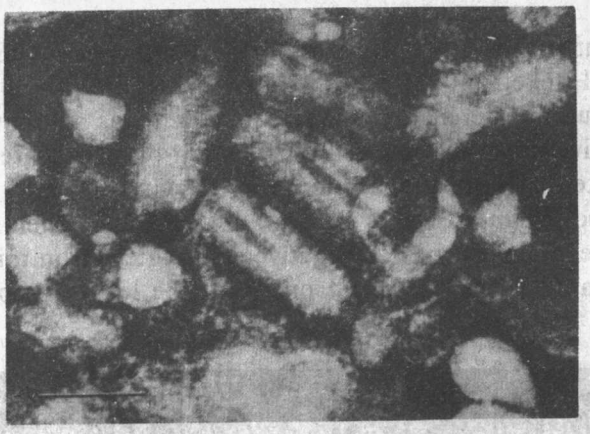

Fig. 5. Mixture of PTA negatively stained defective T and infective B virions obtained from cells infected under conditions of autointerference with undiluted passage of VS virus.

the infectious B virions (Fig. 5). In every other respect, the T virions are morphologically identical to the B virions. However, they contain RNA only one-third the length of infectious B-virion RNA (Huang and Wagner, 1966*c*), their extended RNC measures only 1.1 μm in length (Nakai and Howatson, 1968), and the intact T particle contains 8–10 cross-striations, as opposed to the 35 $\pm$ 1 in the infective virion.

T particles of varying lengths can also be found in different rhabdoviruses, suggesting that the truncated, defective form is a universal phenomenon for this group of viruses. Hackett (1964) described a longer T particle with 14 cross-striations in preparations of VS-New Jersey virus, and the HR (heat-resistant) variant of VS-Indiana virus gives rise to "long" T particles (Petric and Prevec, 1970), which contain a nucleocapsid strand 1.6 $\pm$ 0.2 μm in length, or almost half that of the B-virion nucleocapsid (Schincariol and Howatson, 1970). In addition, various temperature-sensitive (*ts*) mutants of VS-Indiana virus give rise, even at permissive temperatures, to a diversity of T particles of varying length, depending on the mutant (Reichmann *et al.*, 1971). Many of the data obtained by electron microscopy have been confirmed by the interesting new technique of laser light-scattering microscopy which has been used to determine the particle weights of VS virus and its defective particles (Ware *et al.*, 1973). The origin, chemistry and physiology of T particles will be discussed later.