RHABDOVIRUSES

Volume II

Editor

David H. L. Bishop

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FOREWORD

The Rhabdoviridae is a diverse family of enveloped RNA viruses (comprising 70 or more known serotypes). Member viruses infect homeothermic or poikilothermic vertebrates, invertebrates, or plants. Particular viruses are discussed in Volumes I and III in this series. Although some of the viruses are antigenically related to each other (so far only three genera of rhabdoviruses have been recognized), for many, no antigenic cross reactivities can be detected using the techniques commonly employed to categorize viruses.

A striking feature of the Rhabdoviridae family is the diversity of the host species that are susceptible to virus infection. Some rhabdoviruses are arboviruses while others have no arthropod host or vector (Volume I). Certain members of the Rhabdoviridae family cause severe diseases of man (e.g. rabies, see Volume I), domestic animals (e.g. bovine ephemeral fever virus, Volume III), fish (e.g. spring viremia of carp virus, Volume III), or plants (Volume III). Many other rhabdoviruses appear to be innocuous in their primary host (e.g. sigma virus, Volume III).

Due to their economic and disease importance, a considerable amount of research into the molecular biology of rhabdoviruses has been undertaken in many countries of the world over the past decade. While there are several important gaps in our understanding, what is known about virus structure (Volume I), the viral infection processes, assay systems, growth potential, molecular biology, and genetics (Volume II) of representative viruses are described in this series.

The process of interference of rhabdovirus replication by defective interfering virus particles has been a feature of rhabdoviruses which has received significant attention over the last few years. The subject is still under investigation and although the developments of the last few months are not covered in this series, background information on defective particle generation, replication and interference capabilities are discussed in Volume II. Other features of rhabdovirus infections which are discussed in this series are their ability to kill cells, form pseudotypes, and establish persistent infections (Volume III).

No book series on rhabdoviruses would be complete without a discussion of virus vaccines and other possible therapeutic processes. The advancements made in rabies vaccine development over the past years are described in Volume III. The development of vaccines for other rhabdoviruses will presumably be governed by the importance of the diseases they cause.

The goal of this book series has been to provide an overview of rhabdovirology as a whole (including an appraisal of current research findings), suitable for students, teachers, and research workers. To realize this goal I asked many of the research leaders in the different disciplines of rhabdovirology to contribute chapters. Only a few were not able to particpate due to prior commitments; most of those asked responded with articles which I believe do justice to what is known about the subject.

A final point, the four International Rhabdovirus Symposia that have been held every two years since 1973 have brought together rhabdovirologists from different parts of the world and from different disciplines of the field; the Symposia have been a major factor in the development of the subject and the communication that exists among the research scientists. This book series is therefore dedicated to those who had the foresight to initiate these Symposia, the participants of the meetings, and to the contributors who have given their time and energies to the compilation of this work.

David H. L. Bishop
October 1979

THE EDITOR

David H. L. Bishop, Ph.D., is Professor of Microbiology in the Medical Center of the University of Alabama in Birmingham, Alabama. He is also a Senior Scientist in the Medical School Comprehensive Cancer Center and Diabetes Research and Training Center.

Dr. Bishop was graduated from the University of Liverpool, England, with a B.Sc. (Hons.) degree in Biochemistry in 1959. He received a Ph.D. (Biochemistry) in 1962 also from the University of Liverpool. After a postdoctoral year sponsored by a Research Fellowship at the Centre Nationale de la Recherche Scientifique, Gif-sur-Yvette, France, Dr. Bishop was a Research Associate from 1963 to 1966 in the Department of Zoology, Edinburgh University, a Research Fellow from 1966 to 1969 in the Department of Microbiology, University of Illinois, Assistant Professor (1969 to 1970) then Associate Professor (1970 to 1971) at Columbia University College of Physicians and Surgeons, Department of Human Genetics and Development. Before joining the faculty as a Professor of Microbiology at the Medical Center of the University of Alabama in Birmingham in the fall of 1975, Dr. Bishop was an Associate Professor (1971 to 1975), then Professor (1975) at the Waksman Institue of Microbiology, Rutgers University.

Dr. Bishop has published more than 100 research papers over his career and has been on the Editorial Board of the *Journal of Virology*, since 1974, and *Virology* since 1979. In addition to being active in the American Society of Microbiology and the American Society of Tropical Medicine and Hygiene, Dr. Bishop is Chairman of the Bunyaviridae Study Group of the International Committee for Taxonomy of Viruses as well as a member of its Rhabdoviridae Study Group. He has also been a member of the National Cancer Institute Scientific Review Committee from 1975 to 1979.

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Chapter 1

ADSORPTION, PENETRATION, UNCOATING, AND THE IN VIVO mRNA TRANSCRIPTION PROCESS

Patricia Repik*

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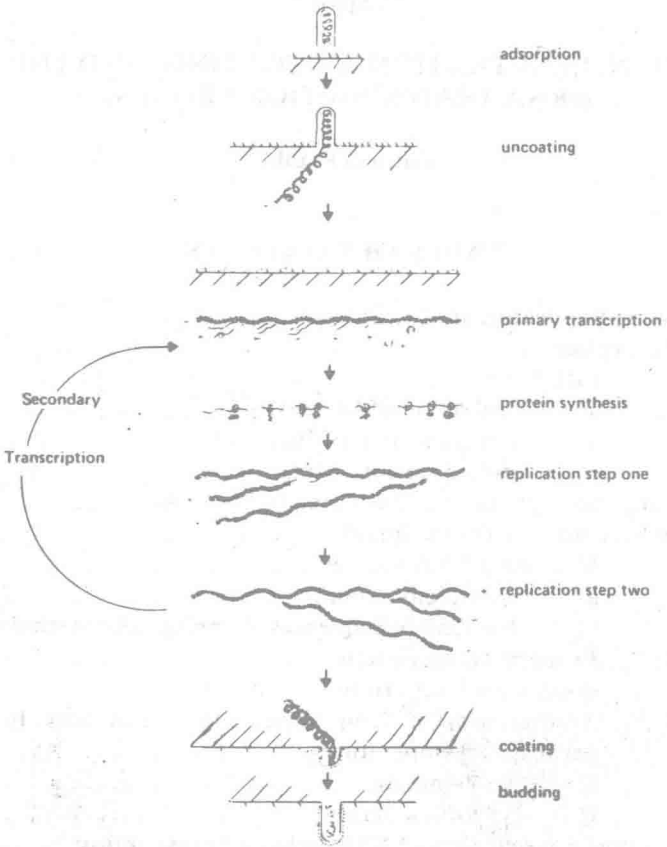

FIGURE 1. Schematic representation of a productive infection of cells by VSV-Indiana. (From Bishop, D. H. L. and Smith, M. S., *The Molecular Biology of Animal Viruses*, Vol. 1, Nayak, D. P., Ed., Marcel Dekker, New York, 1977, 176. With permission.)

I. RHABDOVIRUS REPLICATION IN CELL CULTURE

Although many rhabdoviruses have been isolated from different sources, only VSV-Indiana has been characterized well enough to allow us to draw conclusions concerning its replication cycle. Infection with rhabdoviruses can result either in a productive infection whereby the majority of progeny virions are infectious, or in an abortive infection which results in the majority of progeny virions being defective (noninfectious). The latter type of infection can be caused by the presence of defective interfering particles or by nonpermissive conditions during the replication cycle and will be discussed in greater detail in succeeding chapters. One should be aware that both infectious and defective progeny virions can be produced simultaneously within the same replication cycle.

A productive infection of cells by VSV-Indiana can be divided into several steps as shown schematically in Figure 1. These are

1. Adsorption or attachment of the virus to the cell membrane, followed by penetration into the cell and uncoating of the virion to expose the viral nucleocapsid
2. Transcription of the viral genome into mRNA species by the transcriptase enzymes present in infecting virions (primary transcription)

3. Synthesis of viral proteins (including more transcriptase enzymes)
4. Replication (probably occurring in two steps) of progeny genomes from the infecting parental genome
5. Further rounds of transcription from progeny viral-like RNA (secondary transcription), protein synthesis, and replication
6. Progeny virus assembly and release by budding

A. Adsorption

Attachment of rhabdoviruses to susceptible cell membranes involves an initial association between the viral glycoprotein spikes and some as yet undefined host cell receptor(s). The viral G protein is the sole constituent of viral glycoprotein surface projections (spikes) which stud the surface of the viral envelope. Studies have been conducted which show that the spikes are iodinated by oxidation with lactoperoxidase or chloramine T,[1,2] confirming the fact that most of the G protein is exterior to the envelope. Electron micrographs taken of cells infected with VSV and rabies viruses clearly show that the virions attach to the cell surface via their glycoprotein spikes,[3-6] with attachment noted to occur on all surfaces of the virion — the rounded end, the flat end, or on either side.

The number of adsorbed rhabdoviruses increases linearly with time during the first 30 min of infection at 37°C, and is also able to occur at 4°C.[4,6-8] Vesicular stomatitis virus adsorbs to cells inefficiently and, after adsorption, washed monolayers of infected cells desorb 20 to 40% of their virions within 30 min at 37°C.[7-9] There is also evidence that viruses differ in their efficiencies to adsorb to or desorb from different cell types. By calculating the amount of RNase resistance acquired by ^{3}H-labeled infecting VSV genomes, it was found that VSV adsorbs better to BHK cells than to mouse L cells; adsorption was least efficient on chicken (CEF) cells. By the same token, desorption was found to be less pronounced from BHK or L cells than from CEF cells.[7-10]

1. Infectivity

Since the viral glycoprotein spikes play a key role in the attachment of the virion to the host cell, anything that can alter their conformation or destroy their integrity can affect the process of adsorption and, therefore, the infectivity of the virus.

a. Reduction of Infectivity

Treatment of intact virions (Figure 2) with proteolytic enzymes removes all but a very small fragment of the G protein (fragment mol wt of approximately 7,000 daltons[11,12]) resulting in the production of spikeless virus particles (Figure 3) which retain only a small fraction of the original infectivity.[13-15] It has been found that different proteolytic enzymes have different effects on various rhabdoviruses. Using several strains of VSV, Bussereau et al.[15] studied the effects of trypsin and chymotrypsin on the infectivity, morphology, and antigenic properties. Each enzyme reduced the infectivities of VSV-Indiana and VSV-New Jersey by roughly 10,000-fold, but did not affect the infectivities of VSV-Brazil and Argentina and had minimal effect on the infectivity of Cocal (Table 1). Electron microscopy of the enzyme-treated virions and polyacrylamide gel electrophoresis of their viral polypeptides demonstrated that the enzymes removed all the glycoprotein spikes of VSV-Indiana; however, approximately one third of the spikes of VSV-Brazil were resistant to enzyme action. Similarly, it has been observed that Chandipura retains most of its infectivity after treatment with bromelain,[144] although this same virus loses all its G protein and essentially all its infectivity upon treatment with Pronase® (Table 2).

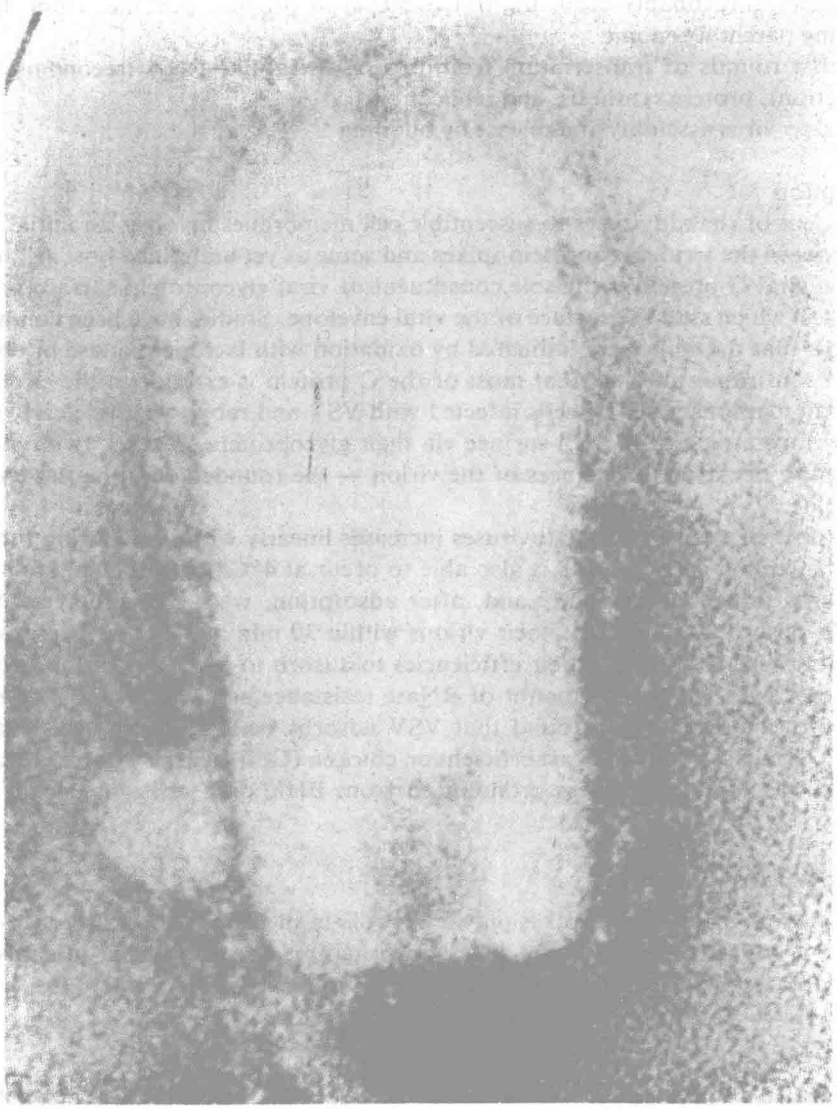

FIGURE 2. Electron micrograph of a VSV-Indiana whole virion negatively stained with sodium phosphotungstate. The particle is approximately 180 nm long, 65 nm wide, and is covered with spikes 10 nm in length. The sample was stained and photographed by Dr. R. W. Compans, University of Alabama Medical Center. Scale: 1 cm = 15 nm.

The infectivities of such spikeless preparations of rhabdoviruses can be restored to varying degrees by homologous and heterologous glycoprotein extracts (Table 2). The glycoprotein extracts contain the viral lipids and appear to be composed of mixed micellar structures (containing both lipids and protein),[16] the lipid portion forming the vesicle and the glycoproteins studding the surface (Figure 4). No infectivity could be detected among any of these extracts. As seen in Table 2, the greatest increases in infectivity resulted from mixing spikeless particles and glycoprotein extracts generated from rhabdoviruses of the VSV subgroup (VSV-Indiana, VSV-New Jersey, or Chan-

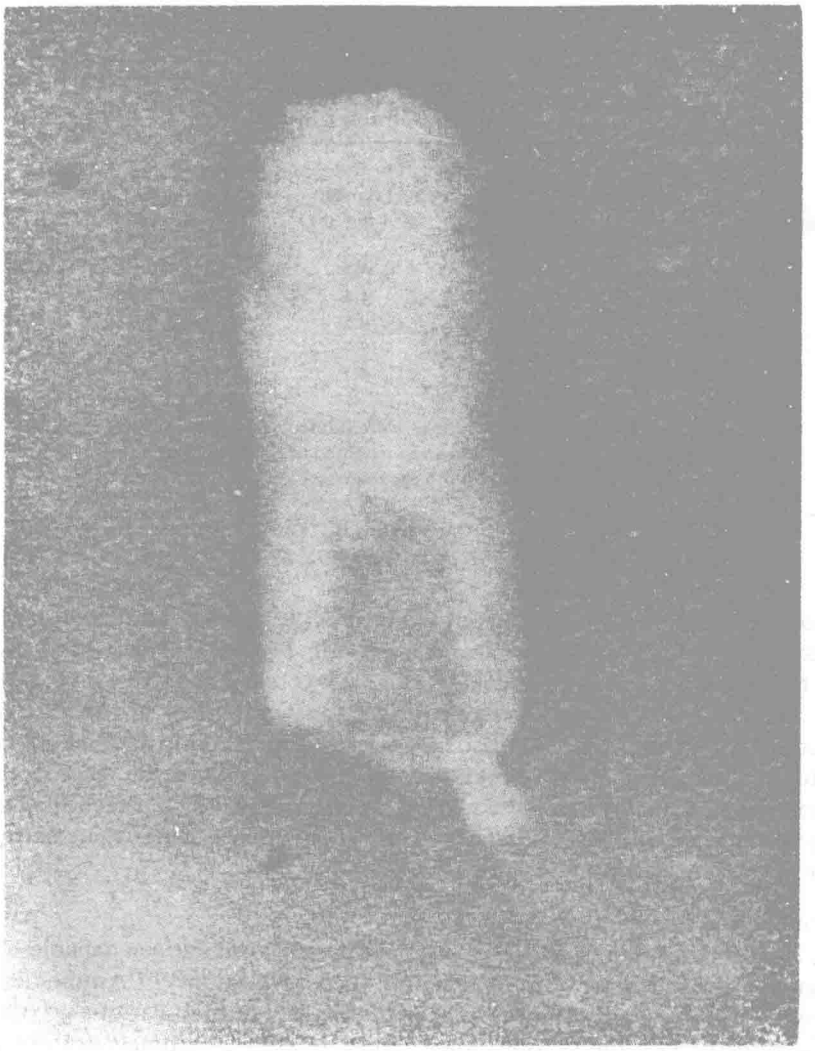

FIGURE 3. Electron micrograph of a VSV-Indiana spikeless particle. Spikeless particles were obtained by incubating purified virus samples in the presence of either bromelain or Pronase® and subsequently resolving them in a glycerol gradient as described by Bishop et al.[14] and Repik.[17] The sample was negatively stained with sodium phosphotungstate and photographed by Dr. R. W. Compans, University of Alabama Medical Center.

dipura spikeless particles plus VSV-Indiana or New Jersey glycoprotein extracts). The fact that the Chandipura glycoprotein extract appeared to be much less efficient in restoring infectivity was most likely due to the very low protein concentration in this particular extract — 0.07 mg protein per mℓ, as compared with approximately 0.3 mg protein per mℓ of most other extracts assayed. The glycoprotein extract of rabies virus strikingly increased the infectivity of VSV-Indiana spikeless particles, although it is serologically unrelated to VSV-Indiana. A substantial increase in infectivity was also observed in the mixture of VSV-Indiana glycoproteins and the spikeless particles of a DNA virus, vaccinia. Where tested, all infectivities obtained were abolished by antiserum prepared against the viruses used to generate glycoprotein extracts.

TABLE I

Effect of Trypsin and Chymotrypsin on Five Strains of Vesicular Stomatitis Virus

Virus strain	Infectivity (log ID_{50}/0.03 mℓ) Untreated	Trypsin* (0.1 mg/mℓ)	Chymotrypsin* (0.5 mg/mℓ)
New Jersey	7.9	4.5	3.3
Indiana	8.5	4.1	4.3
Brazil	8.3	8.5	8.3
Argentina	8.5	8.7	8.7
Cocal	7.5	6.1	6.1

* The unfractionated viruses were diluted 100-fold in 0.04 *M* phosphate, pH 7.6, and incubated at 37°C for 30 min with 0.1 mg trypsin or 0.5 mg chymotrypsin per mℓ.

From Bussereau, F., Cartwright, B., Doel, T. R., and Brown, F., *J. Gen. Virol.*, 29, 189, 1975. With permission.

"Reconstituted" particles negatively stained with sodium phosphotungstate and subjected to electron microscopy are presented in Figure 5. These particles do not contain the proper (original) arrangement of glycoprotein (compare with Figure 2) and, as such, they are *not* technically "reconstituted." Instead, it appears that the glycoprotein micellar structures randomly associate with the surface of the spikeless particles. The nature of this association is unknown. The infectivities of the reconstructed particles are probably mediated by the glycoprotein micelles which attach the complexes to surface receptors on susceptible cells. How such particles enter the cell (by phagocytosis or by fusion) is not known.

b. Neutralization of Infectivity

The glycoprotein spike of rhabdoviruses is the only viral antigen capable of binding virus neutralizing antibodies and of eliciting their formation.[18,19] Antibodies may inhibit the adsorption or penetration steps in a virus infection in various ways.[20,21] They can react directly with the virions either before or after attachment to host cells and prevent the attached virion from continuing with the normal infection sequence (penetration, etc.). They may also bring about the elution of the virion from the host cell. In some cases, the virion-antibody complexes may be taken into the host cell by phagocytosis although the subsequent steps of penetration are then usually blocked. Antibodies may also react directly with the surface of the host cell to prevent attachment of virus to specific host receptors. In any case, the end result of a virus-antibody complex is the prevention of or a reduction in the number of virions able to undergo replication in the host cell.

Antibody to G protein neutralizes the infectivity of whole virions. Mice injected with either purified VSV G protein or with antiserum specific to VSV G protein were rendered immune to subsequent challenge with infectious VSV virions.[22] Similarly, immunities to rabies and Duvenhage virus (a rabies-like virus) infections were conferred in mice injected with the homologous glycoprotein antiserum,[19,23] although some degree of cross-protection against these two virus infections was also noted.[23]

c. Enhancement of Infectivity

The infectivity of many types of viruses, rhabdoviruses included, is greatly affected