RHABDOVIRUSES

Volume III

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 participate 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

THE INTERFERON SYSTEM AND RHABDOVIRUSES

Philip I. Marcus

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I. INTRODUCTION

The interferon system appears as a formidable means of preventing the dire consequences of infection by rhabdoviruses,[1-3] attesting to the acute sensitivity of this family of viruses to interferon action. The prototype rhabdovirus, vesicular stomatitis virus (VSV), with its ubiquitous range of hosts and host cells, is used as an agent *par excellance* for the detection and biological assay of interferon.[4] The rhabdoviruses are perhaps less well recognized for their prowess as inducers of interferon, despite the significant levels of interferon observed in animals injected with rabies virus or certain rabies vaccines,[1,5] or in rabies virus-infected cultures of cells competent for the interferon system.[6] The capacity of rhabdoviruses to induce interferon seems worthy of reexamination in light of these observations and the recent discovery that defective interfering particles of VSV which contain covalently linked, self-complementary (±) RNA[7,8] are extraordinarily efficient inducers of interferon,[9, 10] as are certain *ts* mutants and wild-type revertants of this virus.[11-14,31]

In this chapter, we will examine the role of the interferon system in rhabdovirus infections, considering, in turn, rhabdoviruses as inducers of interferon and as infectious agents sensitive to its action. Studies concerned with the molecular basis of interferon induction and of interferon action encompass many virus-cell systems; we will consider only briefly those concerned with rhabdoviruses. Finally, we will examine the prospects of interferon as an antiviral agent in diseases of rhabdovirus etiology and consider the concept that the prophylactic efficacy of vaccines for rabies may depend, in part, on their content of interferon-inducing particles.

II. VESICULAR STOMATITIS VIRUS

A. Interferon Induction: In Vitro

Vesicular stomatitis virus is generally considered a poor inducer of interferon.[15-17] Either an interferon inducer moiety in sufficient amount is not produced during virus replication and sensed by the cell, or the rapid inhibition of cellular macromolecular synthesis — a hallmark of VSV infection — precludes transcription of interferon mRNA and/or its translation.[17-22,30] However, Wertz and Youngner[17] observed that low levels of interferon can be induced by stocks of VSV under certain conditions. Thus, while stocks of a large-plaque wild-type VSV produced no interferon in mouse L cells, a small-plaque mutant produced significant levels. Stocks of both small- and large-plaque virus induced interferon in monolayers of chick embryo cells, albeit at low levels. These studies provided evidence that actual production of interferon may be related inversely to the efficiency of the viruses at inhibiting cellular macromolecular synthesis. Presumably, if viral-induced inhibition of cellular macromolecular synthesis is delayed long enough, interferon production will ensue. Clearly, the potential to induce interferon resides in stocks of VSV, but generally is not expressed. Is this potential in the form of a preformed inducer moiety intrinsic to the virion of VSV, or must a new molecular species be synthesized? Studies with a special type of defective-interfering (DI) particle of VSV provided part of the answer.

Lazzarini et al.[7] described a defective-interfering (DI-011) particle of VSV (IND) which contained a single strand of covalently linked self-complementary (±) RNA which, upon deproteinization, assumed a helical form (double-stranded, dsRNA) which was 90% resistant to ribonuclease. DI particles with (±) RNA are widely distributed in nature,[8,23] increasing the probability that they may be present as adventitious agents in some, if not many, stocks of wild-type VSV. Marcus and Sekellick[9] tested their capacity to induce interferon, and discovered that a single (±) RNA DI particle, under nonreplicating conditions, could induce a quantum yield of interferon — thus

defining their activity as an interferon-inducing particle (IFP). The IFP capacity of (±) RNA DI-011 particles is very resistant to inactivation by heat (50°C) or UV radiation,[9] an attribute consistent with the preexistance of an interferon-inducer moiety in the particle. This inducer is presumed to be a single molecule of dsRNA formed, at least in part, upon entry of the DI particle into the cell.[9]

The reports of interferon production in cells persistently infected with VSV,[24] but presumably free of, or low in, DI particles, prompted us to measure the interferon-inducing particle capacity of VSV *ts* mutants; especially since Youngner and colleagues[25] had shown that *ts* mutants were the predominant species selected for during the establishment of persistent infection. We found that all *ts* mutants of VSV which failed to inhibit cellular protein synthesis (ts^{PSI-}) or kill cells (ts^{CKP-}) at a nonpermissive temperature (40°C)[21] were excellent inducers of interferon — a single particle usually sufficed to induce a quantum yield of interferon[11-13] (see Table in Chapter 4 of this volume)[24] — such mutants were designated phenotypically as $ts^{PSI-,CKP-,IFP+}$. Some ts^{IFP+} mutants induced over 25,000 VSV-PR_{50} units of interferon when monolayers containing 10^7 "aged" chick-embryo cells[27,39] were infected with an average of one plaque-forming particle(PFP) per cell. Under these same conditions wild-type VSV induced only 300 units. In contrast to the characteristics of (±) RNA DI-011 particles with their preformed inducer, upon heating (50°C), the interferon-inducing particle activity of the ts^{IFP+} mutant G11(I) is lost at the same rate as infectivity (plaque-forming particle activity)[31] — a rate set by the lability of the virion transcriptase,[28] and an indication that primary transcription is a requisite event for IFP activity. Our preliminary results with UV inactivation of *ts*G11(I) *ifp* activity suggest that perhaps only one or two genes need be expressed to form the interferon inducer.[31] If subsequent experiments confirm these preliminary findings, then the genes and virion functions required for expression of the IFP phenotype may be the same as those necessary for the expression of cell-protein synthesis inhibition and killing.[21,28,29]

Most VSV mutants which turned off cell-protein synthesis and killed cells rapidly at 40°C produced less than 1% the level of interferon as did the ts^{IFP+} mutants, and, hence, qualified as $ts^{PSI+,CKP+,\ IFP-}$ [10-12] However, the third category of mutants was lethal to cells, but nonetheless induced significant levels of interferon. Conceivably, these mutants turn off cell macromolecular synthesis slow enough to permit expression of the *ifp* phenotype. Also in this category is the VSV non-*ts* revertant, R1, of Stanners et al.,[30] which appears to manifest a delay in the expression of the *psi* phenotype, such that early after infection, the rate of cellular protein synthesis is high, relative to that in cells infected with wildtype virus. We have found that revertant R1 induces high levels of interferon,[31] confirming a report by Francoeur et al.,[14] and, hence, would classify that virus and these mutants as psi^+ (delayed expression), ckp^+ (delayed expression), and ifp^+. Francoeur et al.[14] also noted that interferon induction by revertant R1 appears to be responsible for the small plaques produced by this virus in hamster embryo fibroblasts[29] — an explanation seemingly equally applicable (perhaps, in general, to many small-plaque formers) to the production of small plaques by VSV mutants described earlier by Wagner et al[16] and by Wertz and Youngner.[17] In support of this thesis, Stanners[75] and Sekellick and Marcus[31] both have observed that the R1 revertant of VSV produces only large plaques on GMK-Vero cells — a line genetically incompetent in its response to inducers of interferon.[9,10,24,32] In addition, Vilček et al.[26] showed that preparations of wild-type VSV (Ind.) were capable of inducing, in human cells, significant levels of interferon, as assayed on the hyperresponsive trisomic 21 GM-258 cells.

These observations provide convincing evidence to support the thesis advanced by Wertz and Youngner[17] that differences in the rate or efficiency of cell macromolecular synthesis determine (at least in part) whether interferon is produced in a given virus-

cell encounter. We conclude that VSV is potentially an extremely efficient inducer of interferon and that that potential is realized only when the virus is defective or delayed in its expression of cell-protein synthesis inhibition or killing.[21,31] As discussed in a section below, rabies virus inherently may possess these same attributes.

B. Interferon Induction: In Vivo

In view of the efficient interferon-inducing capacity of certain DI particles, *ts* mutants, and revertants of VSV revealed by in vitro studies, it perhaps is not surprising (in retrospect) to read reports of interferon induction by VSV in vivo. Youngner and Wertz[33] found that wild-type VSV and a small-plaque mutant both induced several thousand units of newly synthesized interferon in mice within 12 hr after intravenous inoculation. The higher the inoculum, the higher the amount of interferon produced. Significantly, the wildtype virus was as good, or better, an inducer in vivo than the small-plaque mutant, the latter being the better inducer in vitro.[33] The induction of interferon in vivo by viruses which are relatively poor inducers in vitro may be more the rule than the exception. The studies of Gresser et al.[34] provide another example: they showed that wild-type VSV (and other viruses, some which were relatively poor inducers of interferon in vitro), when inoculated into mice treated with anti-interferon serum, produced an exaggerated expression of disease and shortened onset of death — indicating that these "poor" inducers were producing biologically significant levels of interferon in vivo. It is equally clear from other studies of Gresser et al.[35] that exogenously added interferon can protect mice from the lethal effects of VSV or delay their demise, even when administered some 4 days after infection.

It is not immediately apparent why stocks of wild-type virus should be more efficient in inducing interferon in vivo than they are in vitro. Conceivably, for cells in vivo, the rate of macromolecular synthesis inhibition by some viruses is significantly less than that observed in vitro, thus permitting the interferon-inducer moiety of VSV to be expressed. It is also possible that small numbers of interferon-inducing DI particles[9,10] may be expressed in vivo more readily as the large inocula of wild-type virus are distributed (diluted out) within the body. Clearly, the situation is complex and requires more experimentation for its resolution.

Two other studies in animals warrant consideration with regard to the possible role of interferon in the course of rhabdovirus (VSV) infection. Doyle and Holland[36] described the prophylactic use of VSV DI particles in mice to protect against intracerebral infection by wild-type VSV. They showed that large numbers of purified DI particles (5×10^{10}) could protect mice from the lethal action of hundreds of infectious particles inoculated at the same time. These DI particles failed to protect mice against the lethal action of a heterologous VSV(NJ) and NWS influenza virus, suggesting that only homotypic interference was operative under their test conditions. In keeping with the apparent absence of interferon-mediated heterologous interference, we note that the purified DI particles used by Doyle and Holland[36] contained an RNA strand about one third the size of the wild-type (IND) genome and, hence, presumbly represented what we have termed conventional (−)RNA DI particles.[9] Using similar purified preparations of a one-third size (−)RNA DI particle, we showed that they do not induce interferon ($\leqslant$ 10 units per 10^7 cells) in vitro under conditions where a one-fifth genome size (±)RNA DI-011 particle induces over 30,000 units.[9]

The results of Doyle and Holland[36] contrast significantly with those from a related study by Crick and Brown.[37] The latter investigators demonstrated that VSV (IND) DI particles induced a heterologous interference [against VSV(NJ) and (Brazil), rabies, and foot and mouth disease virus]. However, from our view, the two studies differed in at least one important aspect: Crick and Brown used DI particles which had been treated with acetyl ethyleneimine (AEI) to render the preparation biologically inactive

for homotypic interference. Full-size wild-type virions inactivated similarly and inoculated in equally large numbers (10^{10} per mouse), also induced a heterologous interference of lasting duration.[37] In spite of the absence of demonstrable levels of circulating interferon in mice, it is conceivable that the AEI-inactivated preparations of DI or infectious particles contained enough (±)RNA DI particles to induce significantly high levels of interferon locally to produce an antiviral state, yet go undetected in the circulation. In this context, we know that IF → DI particles can produce very high levels of a heterologous antiviral state (in human FS4 cells) in the absence of detectable interferon.[9] Indeed, Dianzani et al.[38] have presented evidence that the local concentration of interferon in a tissue mass may be very much higher than that measured in the circulation. Also, we know that the homotypic interfering capacity of (±)RNA DI-011 particles can be inactivated with high doses of UV radiation which leave virtually unaffected the IFP activity of the preparation.[9] Therefore, to account for the different results in these two studies, we suggest that the DI particles used by Doyle and Holland[36] were of the (−)RNA type (*ifp*⁻ phenotype), and the protection observed was due to homotypic interference; whereas those used by Crick and Brown[37] may have contained some (±)RNA type (*ifp*⁺ phenotype) and, hence, could produce a heterologous interferon-mediated interference and protection.

Questions concerning the possible role of interferon in animal protection experiments, which show heterologous interference induced by inactive virus, might be resolved readily through the use of specific antiinterferon serum[34] and a test of the inactivated virus for its IFP activity in a hyperresponsive system, such as primary chick embryo cells "aged" in vitro.[9,27,39]

C. Sensitivity to Interferon

There is general agreement that VSV is relatively sensitive to interferon action, ranking perhaps close to the highly sensitive togaviruses, and on a par with vaccinia virus in many test systems.[40] However, the host cell may influence the sensitivity; thus, two strains of VSV were inhibited to different degrees by interferon acting in mouse L cells, but showed the same sensitivity in chicken cells.[41] Furthermore, individual clones from an uncloned population of wildtype VSV (IND) were observed to vary as much as seven fold in sensitivity, with the absolute sensitivity also dependent upon the host cell used for assay, but not the method of assay.[42]

D. Interferon Action

The sensitivity of VSV to interferon action and its broad host range, coupled with recent insights into its molecular biology — attested to by the contributions in this monograph, make VSV an excellent subject for the study of interferon-mediated interference. We will consider only briefly its contribution to our understanding of interferon action.

Following the discovery of a viron-associated transcriptase in VSV and its demonstration in vitro by Baltimore et al.,[43] Marcus et al.[44] described an assay for the in vivo accumulation of transcription products in which inhibitors of protein synthesis, such as cycloheximide, were used to restrict transcripts to those originating from the parental genome, i.e., primary transcripts. With that procedure, they demonstrated that chick embryo cells manifesting interferon-mediated interference accumulated, in a dose-dependent manner, fewer viral transcripts than mock-treated cells also infected in the presence of cycloheximide.[44] These observations were confirmed and extended by Manders et al.,[45] using VSV in human muscle skin fibroblasts. They showed that full-size transcripts accumulated in the interferon-treated cells, albeit fewer in number. Under the same conditions, i.e., when cycloheximide was present, Repik et al.[46] also observed a significant decline in the rate of VSV transcript accumulation in mouse L

cells treated with interferon, as did Baxt et al.[47] in LLC-MK2 monkey cells and U human amnion cells. Results similar to the earlier study by Marcus et al.,[44] with chick embryo cells have also been obtained by Thacore[48] in human cells. Thacore[48] has also presented evidence that the species of host cell may be important in dictating the extent to which VSV primary transcription is affected by interferon action. Thus, in different laboratories, using several cell species, interferon action was shown to have an adverse effect on the accumulation of VSV virion-derived transcripts in vivo, as measured in the presence of cycloheximide. Recognizing that cycloheximide might produce aberrant effects on the regulation of viral transcription in different virus-cell systems, Marcus and Sekellick[49] turned to the use of a temperature-sensitive mutant, VSV *ts*G41 (IV),[50,51] to examine the effect of interferon action on primary transcription in green monkey kidney-Vero cells, in the absence of inhibitors of protein synthesis — a condition made possible by the failure of this mutant to amplify RNA synthesis at a nonpermissive temperature (40°C), thus restricting transcription solely to that associated with the virion transcriptase, i.e., to primary transcription. Their studies showed that in Vero cells, the initial rate of virion-associated (primary) transcription increased linearly for 1 to 2 hr after infection, and that interferon acted to reduce this rate (approximately four fold with 50 units per milliliter interferon), irrespective of the presence or absence of cycloheximide.[49] In addition, they showed that the VSV mRNA transcripts synthesized in mock- or interferon-treated cells were equal in size[45] and had an equivalent half-life of 17 hr at 40°C. Thus, it seems likely that once transcription is initiated in interferon-treated cells, it is completed successfully. Furthermore, since interferon reduces the rate of VSV primary transcript synthesis to below that achieved in the presence of cycloheximide, Marcus and Sekellick[49] concluded that interferon had an effect on transcription beyond that attributable solely to protein synthesis inhibition, and postulated that interferon decreased the probability of initiating viral transcription. They also pointed out that viral mRNA escaping this facet of interferon action may then encounter other facets, such as post-transcriptional modification and/or inhibition of translation.[47,48,52] However, it is important to note that the mandatory sequence of primary transcription → primary translation for negative-strand viruses like VSV (and other rhabdoviruses) dictates that the overall inhibitory effect of interferon on translation would derive in part from a prior inhibition of transcription.[49]

A more complete discussion of interferon action as it relates to the transcription and translation of VSV mRNA is considered in papers by Marcus and Sekellick[49] and by Oxman.[53]

III. RABIES VIRUS

A. Interferon Induction: In Vitro

Although early in vitro studies appear equivocal regarding rabies virus as an inducer of interferon,[6] it now seems evident that most genetically competent cell types can produce interferon upon overt infection with most preparations of rabies virus. For example, Wiktor and Clark[54] demonstrated the production of a few hundred units of interferon from primary rabbit-kidney cell cultures infected at plaque-forming particle multiplicities (m_{PFP}) ≥ 10 of the HEP (Flury) strain of rabies virus. At $m_{PFP} \simeq 1$, less than ten units of interferon were produced, but the monolayer of infected cells challenged after an overnight incubation period was capable of reducing by ten fold the plaquing efficiency of VSV, suggesting an interferon-mediated antiviral state was extant — even in the absence of readily demonstrable interferon. In this study, these same preparations of rabies virus, inactivated by high doses of UV radiation or β-propiolactone, were essentially negative as interferon-inducers. Rabies virus (HEP) did

induce significant levels of interferon in primary human embryonic fibroblasts, but, as with primary rabbit kidney cells, only active preparations were effective.

In addition to interferon induction in cells overtly infected with rabies virus, cultures of interferon-competent cells persistently infected with rabies also appear to produce interferon and/or develop heterologous interference, presumably reflecting an interferon-mediated antiviral state.[54] Thus, Wiktor and Clark[54] reported that a line of embryonic hamster cells (Nil-2) persistently infected with rabies virus (Pasteur or HEP - Flury strains) acquired a cyclical heterologous antiviral state, with peaks of resistance to challenge by VSV occurring about 1 day after rabies virus reached maximal levels of infectivity. Interferon, characterized as such, was demonstrable at 100 units or more during the time of maximal resistance to VSV. Viper cell cultures infected with rabies virus also developed a cyclical appearance of infectious virus and resistance to superinfection with VSV, and, although no interferon was demonstrable, the heterologous nature of the interference suggests an interferon-mediated antiviral state may have been induced.[54] Wiktor and Clark[54] also reported that BHK-21 cells persistently infected with rabies virus (HEP) manifested a heterologous antiviral state (as measured by resistance to VSV) which persisted over many cell passages, whether low levels of interferon were demonstrable or not. These results contrast with those of Kawai et al.,[55] who found no evidence for a heterologous antiviral state (as measured by resistance to VSV) or the presence of interferon in their cultures of BHK persistently infected with HEP-Flury rabies virus. Instead, Kawai and his colleagues accounted for the cyclical appearance of rabies virus and/or antigen by the presence of homologous DI particles[56] with the special property of suppressing rabies-virus induced cytopathic effects (CPE).[57] Perhaps the BHK-21 line used by Wiktor and Clark[54] is unusual, with respect to its capacity to respond to inducers of interferon; since most investigators fail to demonstrate interferon production or the development of a heterologous antiviral state in their lines of BHK cells,[55,58,59] even when highly efficient inducers of interferon are used.[9] Perhaps these studies emphasize the value of working with cell systems fully competent for the interferon system when extrapolation to animal systems is desired, and where interferon competence is the rule rather than the exception. Further studies seem warranted to place the interferon-inducing capacity of rabies virus and its defective particles on as firm a quantitative basis as that established for VSV.[9-11,17,26,31]

B. Interferon Induction: In Vivo

An impressive record has been amassed as to the effectiveness of rabies virus and certain rabies vaccines in inducing interferon in vivo, and to the sensitivity of the virus to interferon action in animals.[1,3,5,60] We will assume, with others,[61] that the early presence, and/or stimulation and appearance of significant levels of rabies-immune antibody may be a critical determinant in the final outcome of any prophylactic treatment of rabies, and we might add, particularly so in the absence of the interferon system. In this context, we will attempt to place in perspective a special role that the interferon system may assume in rabies-virus infection and prophylaxis. In a section below, we will emphasize, in particular, a view propounded perhaps first by Stewart and Sulkin,[62] that "—interferon production may be involved in the classical post-exposure antirabies prophylaxis (Pasteur treatment)."

C. Sensitivity to Interferon

Rabies virus is sensitive to the action of interferon as tested in vitro. Thus, treatment with homologous exogenous interferon has been shown to protect the following cell species from rabies virus infection: human,[63] canine,[64] mouse,[65] hamster,[54,63] rabbit,[63] and chicken.[66] With the finding of approximately equal sensitivities of VSV and rabies

virus to interferon action,[63] we might hope that other rhabdoviruses of concern as disease entities in humans and animals will prove equally sensitive.

IV. PERSPECTIVES: THE INTERFERON SYSTEM AS AN ANTIVIRAL AGENT IN RABIES PROPHYLAXIS

It is clear that the interferon system can prevent death from infection by rabies virus. Several types of animals, including primates, can be protected fully from the lethal effects of rabies virus if homologous interferon or interferon inducers are administered shortly before or after the time of infection.[1,2,5] Indeed, the protective effect of some rabies vaccines may be related to their interferon-inducing capacity.[63,67-69] Furthermore, vaccines lacking this capacity can be made more efficacious if interferon or an interferon-inducer is included as part of the prophylactic treatment.[69,70] However, one study with monkeys indicates that interferon may not be the only factor acting to enhance the effectiveness of rabies vaccines.[68] Two observations from this study by Wiktor et al.[68] warrant further consideration: (1) the level of virus-neutralizing antibody stimulated by the vaccine, i.e., its antigenicity, did not correlate with the protection observed and (2) the vaccine used, and which induced interferon, had been inactivated with β-propiolactone. In another animal study where the interferon-inducing capacity of live, and UV- and β-propiolactone-inactivated, virus were compared, active virus induced the highest levels of serum interferon, but the two preparations of inactive virus also induced significant levels.[63] An important experiment in that study by Wiktor et al.[63] showed that live virus did not induce interferon in animals vaccinated 2 weeks earlier and already immune to rabies. These results indicate that the interferon-inducer (actual or potential) resides in a particle with the specificity of rabies virus, and that its expression can be blocked by neutralization with antibody. (Anti-VSV serum acted similarly to block interferon induction in vitro by the VSV (±)RNA IF → DI particle).[9]

Interferon production in animals injected with UV- or β-propiolactone-inactivated rabies virus raises an interesting question regarding the nature of the interferon inducer in rabies vaccines, and in this context, two findings from our studies with VSV seem pertinent. We have preliminary data which show that some degree of primary transcription from the virion of VSV is required to produce an interferon-inducer moiety[31] and UV- and heat-inactivated (±)RNA IF → DI-011 particles still function as excellent inducers of interferon.[9] These results, taken in concert with the observations of interferon induction by some rabies vaccines, suggest that the interferon-inducing moiety in live-virus vaccines may be produced in cells in vivo following virion-associated (primary) transcription; whereas inactivated vaccines may already contain preformed (potential) inducer in the population of particles making up the vaccine. Since the existing evidence points to the induction of interferon by rabies vaccine as having a beneficial effect on prophylaxis, it behooves us to identify the interferon-inducing moiety in vaccines and enrich for it. Based on the discovery of a (±)RNA IF → DI particle in VSV stocks,[9] we suggest a similar particle may be responsible for, or contribute to, the interferon-inducing capacity of both active or inactivated rabies vaccines. This suggestion requires a qualification, since it is conceivable that a sufficient portion of the rabies virus genome in full-size particles survives functionally, following inactivation by UV radiation or β-propiolactone, and can provide the primary transcripts required to form an interferon-inducer moiety.[11-13,21,28,71] The discovery of rabies virus DI particles[56,57,72,73] provides a possible candidate for the inactivation-resistant interferon-inducer moiety in some rabies vaccines. The presence of 18S RNA material extractable from purified preparations of the Pittman-Moore strain of virus[74] suggests the candi-