Advances in

VIRUS RESEARCH

VOLUME 11

616.988
S 663a
1965
J. 11
e. 1

CONTRIBUTORS TO THIS VOLUME

R. Bercks
J. Brandes
Francis O. Holmes
Herbert S. Hurlbut
Irving Rappaport
Irving R. Schneider
Albert Siegel
J. T. Slykhuis
Kenneth M. Smith
Peter K. Vogt

Advances in VIRUS RESEARCH

Edited by

KENNETH M. SMITH
Department of Botany
University of Texas
Austin, Texas

MAX A. LAUFFER
Department of Biophysics
University of Pittsburgh
Pittsburgh, Pennsylvania

VOLUME 11

1965
ACADEMIC PRESS
NEW YORK AND LONDON

Reg 590
c 1

Copyright © 1965, by Academic Press Inc.
ALL RIGHTS RESERVED.
NO PART OF THIS BOOK MAY BE REPRODUCED IN ANY FORM, BY PHOTOSTAT, MICROFILM, OR ANY OTHER MEANS, WITHOUT WRITTEN PERMISSION FROM THE PUBLISHERS.

ACADEMIC PRESS INC.
111 Fifth Avenue, New York, New York 10003

United Kingdom Edition published by
ACADEMIC PRESS INC. (LONDON) LTD.
Berkeley Square House, London W.1

Library of Congress Catalog Card Number: 53-11559

PRINTED IN THE UNITED STATES OF AMERICA

Academic Press 9-9-1968 US $14.50

CONTRIBUTORS TO VOLUME 11

Numbers in parentheses indicate the pages on which the authors' contributions begin.

R. Bercks, *Biologische Bundesanstalt für Land- und Forstwirtschaft, Institut für Virusserologie, Braunschweig, Germany* (1)

J. Brandes, *Biologische Bundesanstalt für Land- und Forstwirtschaft, Institut für landwirtschaftliche Virusforschung, Braunschweig, Germany* (1)

Francis O. Holmes, *The Rockefeller Institute, New York, New York* (139)

Herbert S. Hurlbut, *Naval Medical Research Institute, Bethesda, Maryland* (277)

Irving Rappaport, *Department of Microbiology, New York Medical College, New York, New York* (223)

Irving R. Schneider, *Plant Virology Laboratory, Crops Research Division, U.S. Department of Agriculture, Beltsville, Maryland* (163)

Albert Seigel, *Department of Agricultural Biochemistry, University of Arizona, Tucson, Arizona* (25)

J. T. Slykhuis, *Plant Research Institute, Canada Department of Agriculture, Ottawa, Canada* (97)

Kenneth M. Smith,* *Department of Biophysics, University of Pittsburgh, Pittsburgh, Pennsylvania* (61)

Peter K. Vogt, *Department of Pathology, University of Colorado Medical School, Denver, Colorado* (293)

* Present address: Cell Research Institute, Department of Botany, University of Texas, Austin, Texas.

ESCUELA DE MEDICINA
UNIVERSIDAD DE CHILE
Valparaíso
BIBLIOTECA

CONTENTS

CONTRIBUTORS TO VOLUME 11 v

Gross Morphology and Serology as a Basis for Classification of Elongated Plant Viruses

J. BRANDES AND R. BERCKS

I. Introduction 1
II. Relationship and Classification 2
III. Morphology of Elongated Plant Viruses 3
IV. Serology of Elongated Plant Viruses 7
V. Combination of Morphological Data and Serological Results 12
VI. Additional Characteristics and Classification 18
VII. Taxonomic Assessment and Nomenclature 20
VIII. Conclusions 22
References 22

Artificial Production of Mutants of Tobacco Mosaic Virus

ALBERT SIEGEL

I. Introduction 25
II. Techniques for Detecting and Isolating Variants 26
III. Induction of TMV Mutants 31
IV. The TMV Genome 44
V. TMV Mutants and the Genetic Code 45
VI. Discussion and Summary 55
References 58

Plant Virus-Vector Relationships

KENNETH M. SMITH

I. Introduction 61
II. Mechanical Vectors 62
III. The Vector-Relationships of Tobacco Mosaic Virus 64
IV. The Problem of Aphid-Virus Relationships 67
V. Biological Transmission 79
VI. Vectors Outside the Insecta 87
VII. Discussion 91
References 93

Mite Transmission of Plant Viruses

J. T. SLYKHUIS

I. Introduction 97
II. Characteristics of Mites That Transmit Plant Viruses 98

III. Virus Transmission by Eriophyidae 102
IV. Virus Transmission by Tetranychidae 129
V. Conclusions 131
References 134

Genetics of Pathogenicity in Viruses and of Resistance in Host Plants

FRANCIS O. HOLMES

I. Introduction. 139
II. Natural Selection for the Most Favorable Level of Pathogenicity in Viruses 140
III. Natural Selection for Increased Resistance in Host Plants 143
IV. Viral Diseases of Plants for Which Single or Multiple Genes Have Been Found to Confer Resistances 146
V. Viral Diseases for Which Resistances Have Been Noted without Disclosure of Genic Controls 156
References 158

Introduction, Translocation, and Distribution of Viruses in Plants

IRVING R. SCHNEIDER

I. Scope of Review 163
II. Problems in Studying the Translocation of Viruses 164
III. Introduction of Viruses into Different Regions and the Effect on Distribution 166
IV. Intracellular Movement and Distribution of Viruses 175
V. Transport from Cell to Cell 184
VI. Distribution. 202
VII. Summary 213
References 215

The Antigenic Structure of Tobacco Mosaic Virus

IRVING RAPPAPORT

I. Introduction. 223
II. Morphology of TMV 224
III. TMV as an Antigen 227
IV. TMV as an Infectious Particle 255
References 271

Arthropod Transmission of Animal Viruses

HERBERT S. HURLBUT

I. Introduction. 277
II. Arthropod Infection 278

III. Vertebrate Infection 282
IV. Effects of Alternation of Hosts 284
V. Interhost and Environmental Relationships 285
References 289

Avian Tumor Viruses

PETER K. VOGT

I. Introduction. 294
II. The Elementary Particles 295
III. Characteristics of Virus Synthesis and Release 312
IV. The Defectiveness of *RSV* 336
V. Principles Governing Avian Tumor Virus Infection *in Vivo* 352
VI. *RSV* in Mammalian Hosts 364
VII. Conclusion 372
References 374

AUTHOR INDEX 387

SUBJECT INDEX 404

CUMULATIVE SUBJECT INDEX VOLUMES 1–11 411

GROSS MORPHOLOGY AND SEROLOGY AS A BASIS FOR CLASSIFICATION OF ELONGATED PLANT VIRUSES

J. Brandes and R. Bercks

Biologische Bundesanstalt für Land- und Forstwirtschaft, Institut für landwirtschaftliche Virusforschung and Institut für Virusserologie, Braunschweig, Germany

I. Introduction 1
II. Relationship and Classification 2
III. Morphology of Elongated Plant Viruses 3
A. Fine Structure, Shape, and Diameter 3
B. Length of the Virus Particles 5
IV. Serology of Elongated Plant Viruses 7
A. Significance of Serological Relationship 7
B. Some Aspects of Investigations on Serological Relationships of Plant Viruses 9
V. Combination of Morphological Data and Serological Results . . . 12
A. Preliminary Remarks 12
B. Description of Virus Groups 14
VI. Additional Characteristics and Classification 18
VII. Taxonomic Assessment and Nomenclature 20
VIII. Conclusions 22
References 22

I. Introduction

We have no intention of giving an historical review of the problems of virus classification or of entering into discussion of controversial issues. Essentially we want to emphasize that any classification should start "at the bottom"; this means that we must first regard all viruses as being unrelated and then try to unite within groups only those whose relationships and common origin are beyond doubt. Thus, the title of this article should have made it clear that we have confined ourselves to viruses which have elongated (rod-shaped, threadlike) particles because we have made a particular study of the relationships of these viruses.

Apart from earlier suggestions of several authors, Brandes and Wetter (1959) were the first to make an attempt to classify elongated plant viruses on the basis of particle morphology, using experimental results obtained by comparison of many different viruses of this kind. The ideas put forward in that paper were intended as a stimulus to further experimental work in this field and the results were to act as a framework which could be supplemented and changed if necessary. Brandes and Wetter suggested the existence of a correlation between the dimensions of virus particles and their serological properties and advocated a search for serological cross-reactions between viruses which, on the basis of particle morphology, seemed likely to be related.

In the meantime a number of experimental data have been published which permit extension of the scheme of classification. These results are contained in several papers and summarized by Brandes (1961, 1964).

We cannot present a conclusive arrangement, but among elongated forms we can distinguish groups of related viruses. However, we cannot yet unite these groups into higher taxonomic units because such steps would be merely speculative. We do not wish to imply that all elongated viruses belong to one taxonomic unit and therefore have a common origin.

II. Relationship and Classification

As with higher organisms, a classification of viruses should aim at reflecting the genealogical relationships. This is an idealistic aim, of course, which probably never will be realized but we should strive after it.

To classify viruses according to their phylogenetic relationships it is less necessary to know all details of their nature than it is to recognize their evolution. Proof that some viruses have common ancestry and have evolved divergently should allow of their classification phylogenetically.

Viruses are like higher organisms in important features: they have a specific organization which is reproduced time and again and which is changed by mutations. There are many experimental results proving that mutation occurs among viruses and probably nobody doubts that closely related strains of a virus have common ancestry, even if the mutation or the chain of mutations which led to the strains can no longer be reproduced experimentally.

Virus isolates considered to be strains are usually closely related; they show only small differences in a number of characteristics. In particular, serological relationship is taken as evidence for close relationship. The degree of serological relationship, however, is so small in some cases that it can be demonstrated only under precise experimental conditions. Assuming that serological relationship implies common ancestry, the results of serological experiments strongly suggest that a process of evolution occurs among viruses because all degrees of relationships—from very close to very distant—can be demonstrated.

Any classification should consider as many characteristics as possible, but features which are changed least during the evolutionary process are of greater importance. Because of the fact that those elongated plant viruses which have proved to be serologically related show no, or only minor, differences in particle size, particle morphology is probably a basic criterion, as already suggested by theoretical considerations. Consequently, we then have to consider whether morphological similarities signify true relationship as a rule or whether analogies are possible.

Summing up, we see no reason to doubt that an evolutionary process occurs among viruses and, hence, no reason against attempting to classify them phylogenetically. Hansen (1957) also agrees with the theory of the evolution of the plant viruses but considers that a common origin does not necessarily result in a close relationship, and that true relationship is not dependent on, or indicative of, a common origin. We take the opposite view, namely, that the horizontal relationship between viruses depends on the vertical relationship to their ancestors; two viruses are more closely related to one another the more closely related they are to an imaginary common ancestor.

III. Morphology of Elongated Plant Viruses

A. *Fine Structure, Shape, and Diameter*

Rod-shaped plant viruses can be characterized by size and external shape, which may be called gross morphology, and by internal structure. The type of symmetry of the capsid has already been suggested as a basic criterion for classification by Wildy (1962). Although there is little information on the fine structure of elongated viruses, all are thought to have helical symmetry. We do not know, however, whether similarities in symmetry indicate common ancestry in all cases, nor do we know whether certain groups of elongated viruses have different fine structure. The internal canal has been clearly demonstrated in the particles of tobacco mosaic virus (e.g., Nixon and Woods, 1960), tobacco rattle virus (Nixon and Harrison, 1959), sugarbeet yellows virus (Horne *et al.*, 1959), barley stripe mosaic virus (Gibbs *et al.*, 1963), and soil-borne wheat mosaic virus (Brandes *et al.*, 1964) by negative staining with phosphotungstic acid and other "stains." On the other hand, using the same technique, it is apparently more difficult to present any data on the fine structure of some other elongated viruses (Nixon and Woods, personal communication; Brandes, unpublished work).

It is obvious that different symmetry of the capsid probably indicates lack of relationship, thus suggesting its importance in any scheme of classification but, until now, lack of sufficient experimental results does not permit the use of common symmetry as a criterion for relationship, so far as elongated plant viruses are covered.

These comments also refer to the external shape of the virus particles. Brandes and Wetter (1959) grouped elongated viruses into four categories based on particle shape: (*1*) rigid, (*2*) rigid to slightly flexible, (*3*) flexible, and (*4*) very flexible. These are subjective terms but are of value in separating groups of viruses. They cannot be regarded as basic because some groups of viruses with common shape can be further separated by the diameter and the length range of the particles. So far there is no evidence that tobacco rattle virus and tobacco mosaic virus, for instance, are related although both have similarly shaped particles (rigid rods) but different diameters. Possibly the two groups of viruses characterized by flexible particles in the length ranges 480 to 580 mμ and 700 to 760 mμ are no more closely related to one another than to other groups despite their common shape.

The diameter of elongated plant viruses ranges from about 10 to about 22 mμ. This small variation limits the application of particle diameter as a useful criterion, as it is extremely difficult or impossible with many viruses to establish any differences by electron microscope measurements. Many data in the literature on particle diameter must be regarded with caution because in most cases no careful comparison of different viruses has been made, so that apparent differences actually are due to different techniques.

In some cases, however, reliable differences could be demonstrated and these differences combined with the shape of the particles, fortunately permit the definition of groups of viruses with related mean lengths. The diameters of virus particles with mean lengths up to about 220 mμ are indubitably larger (about 22 mμ) than those of the particles of tobacco mosaic virus and its relatives (about 18 mμ); these again are thicker than all other elongated viruses with mean lengths of 480 mμ and more (10 to 15 mμ). Further differences of the diameter have been suggested but so far have not been established with absolute certainty. Probably potato virus X and related viruses as well as sugar-beet-yellows virus are a little thinner than the other rod-shaped viruses.

There seems no reason to doubt that the morphological features so far mentioned are intrinsic characters of a virus and there is no good evidence that they vary with the nature of the host plant in which the virus has been propagated.

B. Length of the Virus Particles

1. The "Normal Length"

Work at this Institute (summarized by Brandes, 1964) has shown that each of the viruses so far investigated is characterized by a value for particle length which is consistently reproducible. This value has been called the "normal length" and is evaluated as the arithmetic mean of the main maximum of the length-distribution curve.

Usually a high percentage of all particles forms the main maximum when the preparation is made from crude sap according to the exudate or dip method (see Brandes, 1964). Sometimes subsidiary peaks have been found which usually correspond to about half or double the normal length, with the exception of tobacco rattle virus (Paul and Bode, 1955; Harrison and Nixon, 1959) and soil-borne wheat mosaic virus (Brandes *et al.*, 1964; Brakke, personal communication).

The proportions of particles with lengths other than the main maximum vary somewhat for reasons largely unknown, although some results indicate that the age of infection is of some importance (Bercks and Querfurth, 1956; Brandes, unpublished work). Therefore it seemed illogical to take the mean length of all particles present in a preparation as a characteristic of these viruses.

Naturally, there is a minor variation of lengths within the main maximum and we do not know whether this is a true variation in particle length or results from the mode of preparation used, but it has been shown that in some cases main peaks were not normal but excessive distributions (Brandes and Wetter, 1959; Wetter *et al.*, 1962; Brandes, unpublished). Nevertheless, the mean of the main maximum in every case was reproducible.

2. Constancy of the Normal Length

Brandes and Wetter (1959) stated that the normal length of a given virus was not dependent on the host plant but invariable except for the errors resulting from electron microscopy and the technique of measurement. This statement is reinforced by many additional results from recent work.

On the other hand, Johnson (1951) was quoted as an exponent of the claim for variable lengths (Pirie, 1957, 1962). Johnson, however, said in his remarkable paper that he did not prove "any consistent influence of the host on the size, shape, or structure of the rod-shaped particles," nor did he publish any results of measurements. It can be assumed from the photographs published by Johnson that variation in the frequency of double lengths and still longer aggregations account for his comment that he found longer particles of tobacco mosaic virus in certain plants than in others. As we stated, however, constancy of mean lengths refers to the main maximum only and not to the whole range of all lengths present.

The accepted values of mean particle lengths of some viruses have changed during the years; tobacco mosaic virus, for example, was previously thought to be 280 mμ and now is believed to be 300 mμ long. But this likewise is no argument for variability and does not disturb anyone familiar with the techniques of electron microscopy.

It is more difficult than is generally thought to determine true magnification in an electron microscope and to reproduce this magnification consistently for comparison of different material. We have paid particular attention to this last point because we feel that the absolute size of a virus is less important for characterization than are differences between different viruses. Therefore, when measuring a "new" virus, we take at least one—usually several—known viruses and compare the lengths of all by parallel preparation, photography, and measurements. Thus it is possible to get more reliable results than by relying on customary determinations of the magnification, such as the inclusion of polystyrene latex spheres, as a standard.

Many results of different authors, indicating apparent variation of mean lengths, actually can be explained by the fact that only one virus was measured at a particular time without sufficient regard to variation in the magnification of the microscope.

3. *Identification of Elongated Viruses by Electron Microscopy*

Our determinations of normal lengths are based on preparations from crude sap made according to the exudate or dip method, but purified preparations of a number of elongated viruses have also been used. In most cases, however, the lengths of particles were not very uniform after purification, thus making it difficult or impossible to determine any valid mean lengths. On the other hand, special purification techniques with several viruses yielded evidence that infectivity was correlated with particles of specific lengths and these always corresponded to the normal lengths determined from crude sap preparations. By analogy it seems reasonable to assume that this is true in every case. There is, for instance, abundant evidence that infectivity of tobacco mosaic virus is correlated with the 300 mμ rods; this has recently been demonstrated again by Steere (1963). It is desirable to know more about these correlations, but it seems especially difficult to purify some

of the longer flexible viruses and to separate different fractions from them. Such knowledge, however, should not be an essential prerequisite for morphological classification of elongated viruses, since the normal length can be taken as an invariable characteristic associated with the virus. Similar comments can be made as to whether odd lengths other than the main maximum are actually present in the plant or have been formed during the preparation process, and as to whether the particles seen in the electron microscope represent "the virus" or a special resting or sporelike state.

The experienced electron microscopist is unlikely to confuse the particles of elongated plant viruses with other cell components because they have specific and clearly recognizable sizes and shapes. If these specific particles can be transmitted from one plant to another and increase therein, then it seems to be needlessly pedantic—in the present state of knowledge—to deny that they are correlated with virus.

Certainly, it is most important to exclude the possibility of mixed infection by the customary techniques of using differential host plants, dilution series, and single-lesion isolates. Otherwise, even purification can lead to wrong results if a mixed infection with two different viruses of the same or very similar size is being investigated.

Assuming that we are able to identify an elongated plant virus by electron microscope tests from crude sap, a second problem is to provide evidence that this virus is correlated with a special disease and to make crucial tests by serological and other means to establish its identity. In this process electron microscope observations on crude sap are of great value for rapid identification, as emphasized by Brandes and Paul (1957) and Brandes (1961, 1964).

IV. Serology of Elongated Plant Viruses

A. *Significance of Serological Relationship*

Until a few years ago investigations on serological relationships among plant viruses had been performed primarily to demonstrate identity or strain relationships. Positive serological reaction was taken as evidence that the virus studied was identical or at least a strain of the serological partner, and usually reinforced other kinds of evidence indicative of a close connection.

Difficulties arose with the discovery of serological relationships between tobacco mosaic virus and cucumber viruses 3 and 4 (Bawden and Pirie, 1937; Knight, 1955) and, to a lesser extent, with those between common bean mosaic virus and bean yellow mosaic virus (Beemster and van der Want, 1951). However, these results, formerly regarded as exceptional, and the many additional results in recent years pose the question whether serology is a valid and useful means of determining close as well as remote relationships among viruses. This is worthy of comment, although we believe that most virologists hold views about serology similar to those expressed by Cadman and Lister (1962): "At present, serology provides the best evidence of relationship between viruses and this must take precedence over other kinds of evidence if any logical classification of viruses is to evolve."

It has been known for a long time that correlations between the serological reaction and the chemical constitution of the antigen exist (e.g., Landsteiner, 1947). This has been demonstrated again in a recent bacteriological study of the genus *Salmonella* (Kauffmann, 1961). After chemical identification of the sucrose components of the polysaccharides acting as antigens, the latter have been arranged as so-called chemotypes according to the combination of the sucrose components. As a result it has become evident that close correlations exist between classification of serogroups according to the Kauffmann-White scheme and that of chemotypes according to the chemical composition of the antigenic determinants.

With plant viruses, however, only few experiments in this direction have been done. Moreover, they have been restricted primarily to investigations of closely related virus strains. Knight and Stanley (1941) compared several relatives of tobacco mosaic virus, including the cucumber viruses 3 and 4 and the ribgrass isolate found by Holmes (1941), by serological tests and parallel determinations of amino acids. Five strains of tobacco mosaic virus which were serologically closely related showed no differences in amino acid composition within the margin of error. The aberrant ribgrass isolate and the cucumber viruses 3 and 4, which were only distantly related serologically to tobacco mosaic virus, differed distinctly from it in amino acid composition. In further studies with some distinctive strains of tobacco mosaic virus Knight *et al.* (1962) found differences in the serological behavior, amino acid composition, and sequence of nucleotides. Price (1954) examined the effect of potassium iodide on tobacco mosaic virus and observed a considerable alteration in serological behavior when the tyrosine of the virus was transformed to diiodine tyrosine. After treatment with carboxypeptidase tobacco mosaic virus was dethreoninated and then showed serological differences from untreated virus (Harris and Knight, 1955).

Recently, the first special investigations on chemical characterization of antigenic determinants of a plant virus have been performed by

Anderer (1963).* This author showed that a synthetic antigen, with the C-terminal hexapeptide of the polypeptide chain of tobacco mosaic virus as determinant group, produced an antiserum which precipitated and neutralized tobacco mosaic virus. This antiserum did not react, however, with the dahlemense strain which had a similar C-terminal sequence.

All results suggest that immune sera possess very high specificity and may therefore serve as ideal reagents in the analysis of chemical structure (Westphal and Lüderitz, 1963).

As there is no qualitative difference between strong and weak serological reactions, it is not possible to differentiate between close and distant relationships. Therefore it would be illogical to concede to systematic serology pride of place only in instances where relationship is evident for other reasons. Boyden (1953, p. 23), for example, said: "If the precipitin tests give results in accord with known genetic relationships then we have a right to assume that they apply equally to species whose genetic and systematic relationships are not sufficiently established." This is not to underestimate the problems raised by the possibility of convergence. On this matter Boyden (1953, p. 28) commented thus: "It is a serious problem for the student of animal evolution. As far as serological convergence is concerned we have as yet no proven cases for protein antigens. As further work is undertaken they may appear but it is unlikely that they will be frequent. Divergence and parallelism rather than convergence seem to be rules for protein evolution, as far as the serological characters of protein antigens are concerned."

We believe that the last sentence can be applied also to plant viruses. It is unreasonable to take the view that only distant relationships may exist as a result of convergence because there is a complete gradation between close and distant relationship. If serological reactions provide conclusive evidence of identity then they must also provide conclusive evidence for the determination of more or less distant relationships. Even if a weak serological reaction may be due exceptionally to convergence, this does not mean that all distant serological relationships are open to question.

B. *Some Aspects of Investigations on Serological Relationships of Plant Viruses*

The requirements for serological investigations depend on whether

* Analogous to the work of Anderer, Benjamini *et al.* (1964) studied the immunological relationships of the tryptic peptides of tobacco mosaic virus protein to the whole protein.

the relationships between the viruses being studied are close or distant. It is common knowledge that low-titered antisera are sufficient for the determination of close relationships, whereas high-titered ones are necessary for the detection of distant relationships. In addition, it is known that antisera prepared against the same antigen may differ. These differences are reflected not only in the height of the titer against the homologous antigen but also in the behavior of the sera with heterologous antigens.

If there is a close correlation between the antigen that was used for production of the antiserum and the antigen used in the test, most of the antibodies will react with the antigen. In this case, therefore, a low-titered antiserum that contains relatively few antibodies is sufficient to detect relationship. In the case of distant relationships, however, only a relatively small proportion of antibodies is able to react with the heterologous antigen. If the absolute amount of antibodies in an antiserum is low then the number of antibodies necessary for a noticeable heterologous reaction may be insufficient, whereas corresponding high-titered antisera may contain enough antibodies for a reaction to be detectable.

High-titered antisera are useful and necessary not only for their high content of antibodies but also because the proportion of antibodies which can also react heterologously is higher than with low-titered antisera. On the other hand, this is the reason that high-titered antisera are less useful for differentiation of closely related viruses which share many antigenic groups.

Individual differences among antisera play a subordinate role in the determination of close relationships; on the other hand, they can lead to wrong conclusions if distant relationships are being tested.

Problems of this kind with plant viruses have received little attention until recently. Using antisera against two strains of turnip yellow mosaic virus, Matthews (1957) found that the proportion of strain-specific antibodies was distinctly dependent on the titer of the antisera; the higher the titer, the smaller was the proportion of strain-specific antibodies. It is, therefore, widely believed that high-titered antisera are less specific than low-titered ones. The term "specific," however, is not correct according to the conceptions of Augustin (1959). This author suggested that extended immunization and correlated increase in titer lead not to lower specificity but to the production of antibodies against an increasing proportion of the determinants present on the particle. This means that a low-titered antiserum does not yet contain antibodies against all the determinant groups of an antigen, and that only during the course of immunization do those antibodies which can react also with a related antigen originate in a sufficient number.

Regarding individual differences among antisera, Kleczkowski (1941) mentioned that differences between his results and those of other authors on differentiation of tobacco mosaic virus strains possibly were due to qualitative differences among antisera produced against the same antigen. A similar explanation was proposed in a later work on the serological behavior of tobacco mosaic virus and its protein fragments (Kleczkowski, 1961).

Using extremely high-titered antisera (Bercks, 1960b, 1960–61), we were able to demonstrate distant relationships between several viruses previously considered as unrelated. In these papers and in some subsequent ones we have pointed out that probably many other instances of distant relationship would escape detection unless antisera of titers as high as possible were used. This observation was also borne out by the fact that in some earlier investigations relationship was not detected when low-titered antisera were used (Zimmer and Brandes, 1956; Bos *et al.*, 1959; Pratt, 1961), but was detected when high-titered sera were used (Bercks, 1960b; Bercks and Brandes, 1961, 1963).

On the other hand, further investigations have shown that sometimes fairly low-titered sera give similar reactions to high-titered ones and that antisera with similar titers sometimes differ greatly in ability to react with heterologous antigens. For example, tests with three antisera against bean yellow mosaic virus showed that they had homologous titers of 1:128,000, 1:2,048, and 1:256, respectively. Their titers toward the same preparation of potato virus Y were 1:128, 1:2, and 0, respectively (Bercks, 1960–61). In another study we tested three antisera against white clover mosaic virus obtained from the same rabbit at three different intervals after immunization. Over this period the titers decreased from 1:8192, 1:4096, to 1:1024. Correspondingly the heterologous titers to potato virus X decreased from 1:32, 1:16, to 1:8, respectively (Bercks and Brandes, 1961). A low-titered antiserum against potato virus X which failed to react with white clover mosaic virus did so when the homologous titer was increased by concentrating the antibodies (Bercks and Brandes, 1961).

As a rule, apparently, the heterologous reaction is dependent on the height of the homologous titer, but early investigations on distant relationships revealed differences among antisera with the same or very similar homologous titer.

Of two antisera against white clover mosaic virus of the titer of 1:8000, only one reacted with *Hydrangea* ringspot virus (Bercks and Brandes, 1961). Two antisera prepared against potato virus Y had titers of 1:32,000 and 1:64,000, respectively; yet the first of these gave better heterologous reactions with bean yellow mosaic virus than did the second (Bercks, 1960–61).

Correlations between homologous and heterologous titer as well as individual differences among antisera have been described in a study where 55 antisera against the homologous potato virus X and the heterologous partners clover yellow mosaic virus, cactus virus X, *Hydrangea* ringspot virus, and white clover mosaic virus were tested (Bercks, 1963). Some examples of these results may be mentioned here.

The antibodies of 35 antisera, of which only 11 reacted heterologously with clover yellow mosaic, were concentrated. Thereupon, not only did the homologous titer increase, but all except one then reacted with clover yellow mosaic virus. Striking differences between antisera were also observed in this test. Of two antisera with the same homologous titer of 1:4096 one reacted heterologously with the viruses mentioned above at dilutions of 1:4, 1:8, 1:8, 1:4, whereas the second one reacted at 1:32, 1:256, 1:256, and 1:64, respectively. So far, all results definitely indicate that one high-titered antiserum is sometimes not sufficient for the assessment of distant relationship, and that where one antiserum yields a negative result others should be tested.

Another result of our investigations is that heterologous reactions of one antiserum do not allow of trustworthy statements on the degree of relationship. This is an additional reason for working with several antisera. In the case of very close relationship, however, such difficulties are unlikely greatly to affect the result.

The previous general comments on serology are of course also valid for isometric viruses. It is relevant to mention that MacLeod and Markham (1963), studying relationships between turnip yellow mosaic virus and wild cucumber mosaic virus, referred to the importance of high-titered antisera for the determination of distant relationships. Their results have, however, been criticized for technical reasons by van Regenmortel (1963).*

V. Combination of Morphological Data and Serological Results

A. *Preliminary Remarks*

Reliable determinations of normal lengths are limited by the accuracy of the techniques of electron microscopy and measurement. Usually

* Using relatively high-titered antisera, Agrawal and Maat (1964) demonstrated distant serological relationships among several isometric plant viruses. Their results suggest that similar conditions exist among isometric viruses as with elongated viruses with regard to different degrees of relationship between morphologically related viruses.

the normal length of one virus is reproducible within a range of about 10 mμ. Thus we are able, with comparative measurements, to distinguish between viruses the normal lengths of which differ by more than 10 mμ (e.g., Bercks and Brandes, 1961). In the case of lower uniformity or low virus concentration this range possibly must be extended to about 20 mμ. So far as our tests go virus strains exhibiting close serological relationship have not shown any differences in their normal lengths. In addition, no differences in normal lengths have been found between several distantly related viruses or among others whose serological relationships have not yet been established. This is not to imply that there may not be minor differences undetectable with the techniques used.

Several elongated viruses have proved to have slightly different normal lengths but no recognizable differences in shape and diameter. Serological relationships permit the grouping of some of these viruses together, as already suggested by Brandes and Wetter (1959). Groups can now be established containing viruses of the same and slightly different normal lengths. Serological relationship was found only within these groups and even between viruses of different lengths, suggesting that slight changes of the normal lengths can take place during evolution. Consequently, it is of no value taxonomically whether two viruses differ slightly in their lengths or whether they have the "same" normal length, i.e., normal lengths which cannot be distinguished (van Regenmortel *et al.*, 1962).

The next section, (V,B) briefly summarizes the results of morphological characterization of virus particles combined with information on distant serological relationship. Further details can be found in a recent compilation by Brandes (1964). All the measurements quoted have been carefully made, although exact comparisons of the particle lengths of all the viruses mentioned here are still lacking. However, as this is not of vital importance, for the reasons just given, these gaps can no doubt be filled in later. So far there is apparently no significant correlation between the degree of these slight differences in length and the degree of serological relationship. But additional investigations of this kind possibly may help to elucidate the problem of how to distinguish between viruses and strains.

It has already been mentioned that it is difficult to establish the degree of serological relationship in many cases. Therefore, the present results are partly incomplete. Preliminary experiments with some viruses, e.g., legume viruses with normal lengths of about 750 mμ, suggest that between some of these viruses, previously considered distinct, rather close relationships exist, so that they must possibly be considered as strains of one virus. On the other hand, some isolates which are nowadays regarded as strains show only fairly distant relationships. Thus we want to emphasize that we do not lay claim to group together all the viruses mentioned.

According to the present morphological and serological results, many elongated plant viruses can, with conviction, be arranged in six different groups:

1. Tobacco rattle virus group
2. Tobacco mosaic virus group
3. Potato virus X group
4. Potato virus S group
5. Potato virus Y group
6. Beet yellows virus group

For convenience, the name of one well-known representative has been used to characterize each group, but this does not imply that these particular viruses are to be regarded as typical of the group. So far there is no experimental evidence that true relationship exists between the several groups. Therefore, the order, from shorter to longer viruses, is not of any taxonomic significance.

The description of the groups that follows also includes viruses which have not yet been investigated with regard to distant serological relationship. Those viruses which may tentatively be assigned to particular groups on the basis of particle size and shape are shown in parentheses. These placings are speculative and are made to encourage further experimentation. In some cases morphological similarity may be due to convergence. Some viruses have been noted as related in spite of lack of corresponding publications because preliminary and unpublished results suggest serological relationship. In addition, it should be mentioned that within a group not all possible combinations of partners have been tested for serological relationship. Potato virus A and bean yellow mosaic virus are examples, yet these can be regarded as related because both viruses are related to potato virus Y.

As a matter of course we also performed a large number of cross-reaction tests between viruses of different groups, including some isometric viruses. In neither case did we ever observe any reaction.

B. *Description of Virus Groups*

1. *Tobacco Rattle Virus Group*

180 mμ, tobacco rattle virus
210 mμ, pea early browning virus
160 mμ (soil-borne wheat mosaic virus)
130 mμ (barley stripe mosaic virus)

The viruses of this group are characterized by short rigid rods of a diameter of about 20 to 22 mμ. Distant serological relationship between pea early browning virus (Bos and van der Want, 1962) and tobacco rattle virus was recently described by Maat (1963). Tobacco rattle virus includes also strains from potato (potato stem mottle virus), but the latter name is less often used.

It is possible that the speculative placing of barley stripe mosaic virus and soil-borne wheat mosaic virus is wrong in spite of the similar shape and diameter of the virus particles. The normal length of barley stripe mosaic virus is somewhat different from that of the other viruses and soil-borne wheat mosaic virus is characterized by a longer subsidiary length (about 300 mμ), whereas tobacco rattle and pea early browning viruses have shorter subsidiary lengths (about 70 and 100 mμ, respectively).

2. *Tobacco Mosaic Virus Group*

300 mμ, tobacco mosaic virus
300 mμ, *Odontoglossum* ringspot virus
315 mμ (Sammons' *Opuntia* virus)

The particles of these viruses are rigid rods with a diameter of about 18 mμ. Since exact comparative measurements of these viruses are still lacking, minute differences in the normal lengths may exist. Cucumber green mottle mosaic virus (cucumber viruses 3 and 4), the ribgrass isolate of Holmes (1941), a virus from cowpea (Bawden, 1958), and "Dolichos enation mosaic virus" from India (Badami, 1963), which have been regarded as aberrant strains of tobacco mosaic virus, also belong to this group. Preliminary serological tests by Wetter also suggest distant relationships between *Odontoglossum* ringspot virus with other viruses of this group.

3. *Potato Virus X Group*

480 mμ, white clover mosaic virus
500 mμ, *Hydrangea* ringspot virus
515 mμ, potato virus X
520 mμ, cactus virus X
540 mμ, clover yellow mosaic virus
490 mμ, *Cymbidium* mosaic virus
580 mμ (potato aucuba mosaic virus)

The diameter of the flexible virus particles of this group is about 13 mμ. Comparative serological and electron microscope investigations among the first five viruses of this group have been made in detail (Bercks and Brandes, 1961, 1963; Brandes and Bercks, 1962–63; Bercks, 1963). Occasional cross-reactions between *Cymbidium* mosaic virus and potato virus X suggest distant relationship (Wetter, 1960, unpublished work; Bercks, 1963, unpublished work). According to current tests, potato aucuba mosaic virus also may be distantly related to some of these viruses.

4. Potato Virus S Group

620, mμ, pea streak virus	650 mμ, "Cactus virus 2"
650 mμ, red clover vein mosaic virus	650 mμ, (*Freesia* mosaic virus)
650 mμ, *Passiflora* latent virus	650 mμ, ("hop virus")
650 mμ, carnation latent virus	670 mμ, (poplar mosaic virus)*
650 mμ, potato virus S	700 mμ, (wheat streak mosaic virus)?
650 mμ, potato virus M	700 mμ, (ryegrass mosaic virus)?
690 mμ, *Chrysanthemum* virus B	

The virus particles may be described as being rigid to slightly flexible. The particles, which are often curved to one side, have a diameter of about 15 mμ. Serological and morphological relationships between carnation latent virus, potato viruses S and M, *Chrysanthemum* virus B, and *Passiflora* latent virus have been described in several papers (Kassanis, 1956; Rozendaal and van Slogteren, 1958; Wetter and Brandes, 1956; Bagnall *et al.*, 1959; Brandes *et al.*, 1959; Hakkaart *et al.*, 1962; Brandes and Wetter, 1963–64). It may be noted that separation of some of these viruses is still disputed. Kassanis (1961b) considered potato viruses S and M and carnation latent virus as so-called serotypes of potato leaf rolling mosaic virus (see Chapter VII).

Relationships between several isolates of pea streak virus and red clover vein mosaic virus have been described by Wetter and Quantz (1958) and Wetter *et al.* (1962). This was the first reported case of serological relationship between viruses with distinctly different normal lengths. We placed pea streak virus and red clover vein mosaic virus in this group although sufficient evidence on relationship with other viruses of the group is still lacking. Distant relationship, however, between these two viruses and those mentioned in the former paragraph seems probable according to preliminary results of Wetter and Paul (1961–62).

* Berg (1964) recently reported that poplar mosaic virus was serologically unrelated to other viruses of the potato virus S group although the virus would fit the group morphologically. We believe, however, this negative result does not exclude a very distant relationship which can be demonstrated with very high-titered antisera only.

It is doubtful whether wheat streak mosaic virus and ryegrass mosaic virus (Mulligan, 1960) can be placed in this group at all. The normal lengths lie between those of the potato virus S group and the potato virus Y group; according to their shape they might belong to the former group. However, both viruses are the only elongated viruses so far known which are transmitted by mites.*

5. Potato Virus Y Group†

730 mμ, potato virus Y	750 mμ, mosaic virus from *Vigna* (formerly cowpea mosaic virus)‡
730 mμ, potato virus A	750 mμ, Bagnall's SB 29 virus
730 mμ, beet mosaic virus	750 mμ, lettuce mosaic virus
730 mμ, tobacco etch virus	750 mμ, turnip mosaic virus
730 mμ, henbane mosaic virus	750 mμ, sugarcane mosaic virus
730 mμ, watermelon mosaic virus	750 mμ, (cocksfoot streak virus)
740 mμ, *Eucharis* mosaic virus	740 mμ, (tulip mosaic virus)
750 mμ, bean yellow mosaic virus	760 mμ, (asparagus virus 1)
750 mμ, bean common mosaic virus	790 mμ, (papaya ringspot virus)?
750 mμ, pea mosaic virus	
750 mμ, soybean mosaic virus	

Viruses of this group, of which probably more exist than those listed above, have flexible particles and a diameter of about 15 mμ. Relationships among bean yellow mosaic virus, potato virus Y, beet mosaic virus, and watermelon mosaic virus have been described by Bercks (1960b, 1960–61) and van Regenmortel *et al.* (1962). Beemster and van der Want (1951) reported on the relationship between bean yellow mosaic virus

* The speculative placing of wheat streak mosaic virus and ryegrass mosaic virus becomes more doubtful as the result of some recent studies in our institute. We found that the shape of these viruses which in length lie midway between the potato virus S group and the potato virus Y group, is not recognizable definitely because the particles appear to be sometimes more rigid (as viruses of the potato virus S group) and sometimes more flexible (as viruses of the potato virus Y group).

† The size and shape of some viruses which can be placed tentatively in the potato virus Y group have been described recently. Onion yellow dwarf virus (normal length 772 mμ) and plum pox virus (normal length 764 mμ) may be mentioned (Schmidt and Schmelzer 1964; Kegler *et al.* 1964). Papaya ringspot virus (Herold and Weibel 1962), the placing of which is doubtful, apparently is not the only virus in the length range near 800 mμ, since Kitajima *et al.* (1962) described the normal length of the *Malva* vein clearing virus as being about 830 mμ. All these viruses have not been investigated so far for serological relationships with other elongated viruses.

‡ Agrawal (1964) claimed that the name "cowpea mosaic virus" is valid only for the isometric beetle-transmissible virus. The aphid-transmissible elongated mosaic virus from *Vigna* (normal length about 750 mμ), which formerly was also called cowpea mosaic virus, should have another name. Further work on this virus is in progress (Lovisolo, personal communication).

and bean common mosaic virus and this was confirmed by Bercks (1960a). One legume virus isolate which may be regarded as pea mosaic virus was also related to these viruses, thus supplementing the results of Goodchild (1956). Further preliminary tests suggest that relationships exist between other legume viruses (soybean mosaic virus, mosaic virus from *Vigna*) and the viruses mentioned above (Bercks, quoted by Quantz, 1961–62, and unpublished work). According to Bartels (1963–64) relationships of different degrees exist among potato viruses Y and A, tobacco etch virus, and henbane mosaic virus. Further preliminary results suggest distant relationships between turnip mosaic virus, sugarcane mosaic virus, lettuce mosaic virus, and several other viruses of this group, although strong evidence is still lacking. A virus from *Solanum phureja* recently described by Bagnall (1963) and named "SB 29 virus" was shown to be distantly related to potato virus Y and not identical with potato virus A.

6. *Beet Yellows Virus Group*

Until recently, beet yellows virus was the only elongated virus which exhibited morphological properties entirely different from those of other elongated viruses. It has very long particles (normal length 1250 mμ; Brandes and Zimmer, 1955) of a very flexible nature and a diameter of about 10 mμ. However, we now have reports of viruses of the same shape but still longer normal lengths; these include a virus from *Festuca* (Schmidt *et al.*, 1963) and citrus tristeza virus (Kitajima *et al.*, 1963). Since serological work is lacking we have to wait and see whether these viruses can be grouped with beet yellows or whether it is necessary to set up new groups.

VI. Additional Characteristics and Classification

When we consider other characteristics of the viruses and compare them with the classification described here, it can be stated first of all that there are apparently no correlations with regard to symptoms and host range. As this is a matter of common observation (e.g., Hollings, 1959) it seems unnecessary to discuss this question further. It seems worth mentioning, however, that there are several examples of related viruses having no known common host.

Properties such as thermal inactivation point and concentration of virus in the plant are, however, correlated with our classification scheme. We do not have reliable and comparable values for all viruses but the approximate data compiled from the literature and given in Table I may serve as illustration.

TABLE I
Some Comparable Values for Virus Groups

Virus group	Thermal inactivation point (°C.)	Concentration
Tobacco rattle	65–80	Medium
Tobacco mosaic	85–95	High
Potato virus X	60–80	High
Potato virus S	60–75	Medium to high
Potato virus Y	50–65	Low to medium
Beet yellows	—	Medium

However, values of thermal inactivation point and dilution endpoint of different viruses lie within a rather continuous range and show overlapping between different groups, apart from some exceptions which do not fit the groups very well. This indicates that these two features are not of basic importance but can contribute a certain degree of support to a classification based on natural relationships. Among other features they will probably play a role in the separation of viruses and strains. Even more striking are correlations of the groups with the mode of transmission, the feature most favored in earlier attempts of classification.

In the tobacco rattle virus group tobacco rattle and pea early browning viruses have been shown to be transmitted by nematodes (Sol *et al.*, 1960; van Hoof, 1962). This kind of transmission has so far not been observed with viruses of other groups of elongated viruses.

Viruses of the tobacco mosaic virus and potato virus X groups are transmitted only mechanically, but it must be mentioned that for two viruses of the latter group a special type of aphid transmission has been described. According to Kassanis (1961a), potato aucuba mosaic virus can be transmitted by aphids if it is present in a mixed infection together with potato virus Y or potato virus A. Goth (1962) reported successful aphid transmission of white clover mosaic virus only when a special clone of Ladino white clover and extremely short feeding times were used (Goth, personal communication).

In the potato virus S group several viruses are known to be transmitted by aphids. But *Passiflora* latent virus and potato virus S have so far been transmitted only mechanically. It does not seem illogical to assume that the common ancestor of the serologically related viruses of the group was aphid-transmissible and that some descendants lost this ability during prolonged vegetative propagation of the host. Regarding potato virus S Wetter and Völk (1960) referred to such an explanation. This assumption is confirmed by earlier reports on loss of insect transmissibility by probable selection of vectorless mutants, such as Black's (1953) work on potato yellow dwarf virus.

The odd position of wheat streak mosaic and ryegrass mosaic viruses, which are transmitted both mechanically and by mites, has already been noted. It would be most interesting to study the serological relationships between these and other elongated viruses.

So far as they have been studied, viruses of the potato virus Y group are transmitted by aphids.

Beet yellows virus differs from all the viruses previously mentioned not only because of its size and shape but also because it is a "semipersistent" virus.

Correlations between the mode of transmission and the serological grouping of related viruses support the general idea of our classification. On the other hand, it is probable that the mode of transmission is not of importance at higher levels. Viruses of different and possibly unrelated groups have similar modes of transmission; even among isometric viruses some are transmitted by aphids and others by nematodes. This situation requires reserved assessment of the value of the mode of transmission for classification. At the present time we have no grounds for assuming that all viruses transmitted by aphids or all viruses transmitted by nematodes have a common origin.

VII. Taxonomic Assessment and Nomenclature

Although classification should reflect phylogenetic relationships, artificial steps are necessary to arrange different entities within taxonomic groups. The levels of grouping should be chosen in such a way that the result is a reasonable and useful scheme. These levels are determined by the terms "strain," "species," "genus," and so on, and we see no reason not to use them. Regarding the preceding proposals the question arises as to the rank which should be attributed to the present six groups of related viruses. We believe that it is impossible to retain the former general conception that serologically related viruses are "strains" of one virus "species," since we have considerable variation in degree of relationships.

On the basis of the present results it would be reasonable to use the term "genus" to designate the groups which include several serologically distantly related "species." The term "strain" should be restricted to those viruses which are serologically closely related and which show no or only slight differences in intrinsic properties.

In this way it would be easy to define the genera as groups of viruses with related morphological and serological properties. We do not know whether they can be combined in the future into higher taxonomic groups such as families; at present they have to stay as independent groups. However, below the generic level, it seems very difficult to find any obligatory definition of species and strain, because all transitions between the two exist. Possibly "subspecies" must be taken in account.

Kassanis (1961b) tried to overcome these difficulties by introducing the term "serotype" which he proposed for "viruses that share a few of their antigens in contrast to strain for viruses that have most of their antigens in common." According to Kassanis the degree of difference distinguishing strains and serotypes should be defined later. This indicates that the difficulties of separation remain the same and Kassanis merely prefers to use the term "serotype" for entities which we would call "species" or, if necessary, "subspecies." The term "serotype" is borrowed from microbiology. The most detailed knowledge on serological relationships comes from work on the genus *Salmonella,* and within the Kauffmann-White scheme, serotypes are now treated as equivalent to species (Kauffmann, 1961). Serotype would imply only differences of serological properties. But one has to consider that differences of several other characteristics are also present.*

This is also valid with regard to plant viruses. It is a fact that several elongated viruses which have only few common antigens differ in many important respects so that separation into different species would be preferable. Where closer relationships exist, it may be useful to arrange several strains within taxonomic units below the species. In this case we would prefer to use the term "subspecies" to clarify the taxonomic status. However, we want to emphasize that it would be premature to make any hard and fast proposals. Classification cannot be considered as absolute but must comply with pragmatic needs and be settled by agreement among experts.

Because classification is only in its early stages there is no urgent need to settle such problems of nomenclature. For the future we favor binomial Latin nomenclature, and for the present time we suggest that the common names (as quoted in *Rev. Appl. Mycol.*) be retained as a provisional arrangement. Newly described viruses should be given an English common name for uniformity, even if described in another language. However, it may be stressed that in every case clear distinction should be made between the virus disease and the virus. This distinction would also be advantageous with respect to priority and would facilitate

* It is interesting to note that Bawden (1964) recently criticized the term "serotype" and that he took into consideration that a genus might contain several serologically related viruses as formerly suggested by Brandes and Wetter (1959) and emphasized in this paper. We do not agree with Bawden's interpretation that the twelve morphological groups of viruses in the classification scheme of Brandes and Wetter represent distinct species.

decision on the correct names of viruses. Many early descriptions of viruses are in fact descriptions of virus diseases. For the naming of a virus, however, it should be a necessary condition that the virus itself can be identified by intrinsic properties.

VIII. Conclusions

The present results on comparative electron microscope and serological investigations justify confidence in proceeding with these studies, and this concerns not only completion of the present groups. Atypical strains should be examined in greater detail, because it might be possible to establish links between distantly related viruses, thus supplementing our knowledge on evolution. We should also try to determine the relations between different groups.

The exact degree of relationship can be estimated in a few cases only. Corresponding investigations are needed, although they are protracted and require intensive study.

Comparative investigations on chemical characterization of viruses which are serologically distantly related or unrelated are also desirable. We should also take into consideration that, in the future, other features for objective characterization of a virus may be found.

In every case it is necessary that more virologists participate in these studies on relationships. There would then be no difficulty in predicting that future results would allow a better appraisal of the question of true relationship.

These comments are not made with the object of relegating classification to a nebulous future. We believe that the present experimental results are sufficient to suggest a general framework for classification of elongated viruses. With regard to the details we agree with Richard von Wettstein, who fifty years ago said, in effect: "We should consider that the systematic units are not units defined by nature, but that they are abstractions. Hence it follows that in the conception of the units much latitude must be allowed for personal views, effects of tradition, as well as scientific judgment."

Acknowledgment

The authors are greatly indebted to Dr. C. H. Cadman for critical comments and his help with the English language.

References

Agrawal, H., (1964). *Meded. Landbouwhogeschol Wageningen* **64**, 1.
Agrawal, H, and Maat, D. Z. (1964). *Nature* **202**, 674.
Anderer, F. A. (1963). *Biochim. Biophys. Acta* **71**, 246.
Augustin, R. (1959). *Immunology* **2**, 148.
Badami, R. S. (1963). *In* "Symposium on Plant and Animal Viruses," pp. 166–193. Natl. Inst. Sci. of India, New Dehli.

Bagnall, R. H. (1963). *Phytopathology* **53**, 546.
Bagnall, R. H., Wetter, C., and Larson, R. H. (1959). *Phytopathology* **49**, 435.
Bartels, R. (1963–64). *Phytopathol. Z.* **49**, 257.
Bawden, F. C. (1958). *J. Gen. Microbiol.* **18**, 751.
Bawden, F. C. (1964). "Plant Viruses and Virus Diseases," 4th ed. Ronald Press, New York.
Bawden, F. C., and Pirie, N. W. (1937). *Brit. J. Exptl. Pathol.* **18**, 275.
Beemster, A. B. R., and van der Want, J. P. H. (1951). *Antonie van Leeuwenhoek J. Microbiol. Serol.* **17**, 285.
Benjamini, E., Young, J. D., Shimizu, M., and Leung, C. Y. (1964). *Biochemistry* **3**, 1115.
Bercks, R. (1960a). *Phytopathol. Z.* **39**, 120.
Bercks, R. (1960b). *Virology* **12**, 311.
Bercks, R. (1960–61). *Phytopathol. Z.* **40**, 357.
Bercks, R. (1963). *Phytopathol. Z.* **47**, 301.
Bercks, R., and Brandes, J. (1961). *Phytopathol. Z.* **42**, 45.
Bercks, R., and Brandes, J. (1963). *Phytopathol. Z.* **47**, 381.
Bercks, R., and Querfurth, G. (1956). *Phytopathol. Z.* **26**, 35.
Berg, Th. M. (1964). *Mededel. Landbouwhogeschool, Wageningen* **64**, 1.
Black, L. M. (1953). *Phytopathology* **43**, 466.
Bos, L., and van der Want, J. P. H. (1962). *Tijdschr. Plantenziekten* **68**, 368.
Bos, L., Delevič, B., and van der Want, J. P. H. (1959). *Tijdschr. Plantenziekten* **65**, 89.
Boyden, A. (1953). *Systematic Zool.* **2**, 19.
Brandes, J. (1961). *Proc. 4th Conf. Potato Virus Diseases, Braunschweig, 1960* p. 170.
Brandes, J. (1964). *Mitt. Biol. Bundesanstalt Land-Forstwirtsch. Berlin-Dahlem* **110**.
Brandes, J., and Bercks, R. (1962–63). *Phytopathol. Z.* **46**, 291.
Brandes, J., and Paul, H. L. (1957). *Arch. Mikrobiol.* **26**, 358.
Brandes, J., and Wetter, C. (1959). *Virology* **8**, 99.
Brandes, J., and Wetter, C. (1963–64). *Phytopathol. Z.* **49**, 61.
Brandes, J., and Zimmer, K. (1955). *Phytopathol. Z.* **24**, 211.
Brandes, J., Wetter, C., Bagnall, R. H., and Larson, R. H. (1959). *Phytopathology* **49**, 443.
Brandes, J. Phillippe, M. R., and Thornberry, H. H. (1964). *Phytopathol. Z.* **50**, 181.
Cadman, C. H., and Lister, R. M. (1962). *Plant Disease Reptr.* **46**, 770.
Gibbs, A. J., Kassanis, B., Nixon, H. L., and Woods, R. D. (1963). *Virology* **20**, 194.
Goodchild, D. J. (1956). *Australian J. Biol. Sci.* **9**, 231.
Goth, R. W. (1962). *Phytopathology* **52**, 1228.
Hakkaart, F. A., van Slogteren, D. H. M., and de Vos, N. P. (1962). *Tijdschr. Plantenziekten* **68**, 126.
Hansen, H. P. (1957). *Kgl. Vet. Land-Bohojskole Arsskr.*, p. 31.
Harris, T. T., and Knight, C. A. (1955). *J. Biol. Chem.* **214**, 215.
Harrison, B. D., and Nixon, H. L. (1959). *J. Gen. Microbiol.* **21**, 569.
Herold, F., and Weibel, J. (1962). *Virology* **18**, 302.
Hollings, M. (1959). *Ann. Appl. Biol.* **47**, 98.
Holmes, F. O. (1941). *Phytopathology* **31**, 1089.
Horne, R. W., Russell, G. E., and Trim, A. R. (1959). *J. Mol. Biol.* **1**, 234.
Johnson, J. (1951). *Phytopathology* **41**, 78.
Kassanis, B. (1956). *J. Gen. Microbiol.* **15**, 620.

Kassanis, B. (1961a). *Virology* **13**, 93.
Kassanis, B. (1961b). *European Potato J.* **4**, 14.
Kauffmann, F. (1961). "Die Bakteriologie der Salmonella-Species." Munksgaard, Copenhagen.
Kegler, H., Schmidt H. B., and Trifonow, D. (1964). *Phytopathol. Z.* **50**, 97.
Kitajima, E. W., Costa, A. S., and Carvalho, A. M. B. (1962). *Bragantia* **21**, 103.
Kitajima, E. W., Silva, D. M., Oliveira, A. R., Müller, G. W., and Costa, A. S. (1963). *Nature* **201**, 1011.
Kleczkowski, A. (1941). *Brit. J. Exptl. Pathol.* **22**, 44.
Kleczkowski, A. (1961). *Immunology* **4**, 130.
Knight, C. A. (1955). *Virology* **1**, 261.
Knight, C. A., and Stanley, W. M. (1941). *J. Biol. Chem.* **141**, 39.
Knight, C A., Silva, D. M., Dahl, D., and Tsugita, A. (1962). *Virology* **16**, 236.
Landsteiner, K. (1947). "The Specificity of Serological Reactions." Harvard Univ. Press, Cambridge, Massachusetts.
Maat, D. Z. (1963). *Neth. J. Plant Pathol.* **63**, 287.
MacLeod, R., and Markham, R. (1963). *Virology* **19**, 190.
Matthews, R. E. F. (1957). "Plant Virus Serology." Cambridge Univ. Press, London and New York.
Mulligan, T. E. (1960). *Ann. Appl. Biol.* **48**, 575.
Nixon, H. L., and Harrison, B. D. (1959). *J. Gen. Microbiol.* **21**, 582.
Nixon, H. L., and Woods, R. D. (1960). *Virology* **10**, 157.
Paul, H. L., and Bode, O. (1955). *Phytopathol. Z.* **24**, 341.
Pirie, N. W. (1957). *Advan. Virus Res.* **4**, 159.
Pirie, N. W. (1962). *In* "Microbial Classification" (G. C. Ainsworth and P. H. A. Sneath, eds.), pp. 374–393. Cambridge Univ. Press, London and New York.
Pratt, M. J. (1961). *Can. J. Botany* **39**, 655.
Price, W. C. (1954). *Trans. N.Y. Acad. Sci.* **16**, 196.
Quantz, L. (1961–62). *Phytopathol. Z.* **43**, 79.
Rozendaal, A., and van Slogteren, D. H. M. (1958). *Proc. 3rd Conf. Potato Virus Diseases, Lisse-Wageningen, 1957*, p. 30.
Schmidt, H. B., and Schmelzer, K. (1964). *Phytopathol. Z.* **50**, 191.
Schmidt, H. B., Richter, J., Hertzsch, W., and Klinkowski, M. (1963). *Phytopathol. Z.* **47**, 66.
Sol, H. H. van Heuven, J. C., and Seinhorst, J. W. (1960). *Tijdschr. Plantenziekten* **66**, 228.
Steere, R. L. (1963). *Science* **140**, 1089.
van Hoof, H. A. (1962). *Tijdschr. Plantenziekten* **68**, 391.
van Regenmortel, M. H. V. (1963). *Phytopathol. Z.* **48**, 197.
van Regenmortel, M. H. V., Brandes, J., and Bercks, R. (1962). *Phytopathol. Z.* **45**, 205.
Westphal, O., and Lüderitz, O. (1963). *Naturwissenschaften* **50**, 413.
Wetter, C., and Brandes, J. (1956). *Phytopathol. Z.* **26**, 81.
Wetter, C., and Paul, H. L. (1961–62). *Phytopathol. Z.* **43**, 207.
Wetter, C. and Quantz, L. (1958). *Phytopathol. Z.* **33**, 430.
Wetter, C., and Völk, J. (1960). *European Potato J.* **3**, 158.
Wetter, C., Quantz, L., and Brandes, J. (1962). *Phytopathol. Z.* **44**, 151.
Wildy, P. (1962). *In* "Microbial Classification" (G. C. Ainsworth and P. H. A. Sneath, eds.), pp. 145–163. Cambridge Univ. Press, London and New York.
Zimmer, K., and Brandes, J. (1956). *Phytopathol. Z.* **26**, 439.

ARTIFICIAL PRODUCTION OF MUTANTS OF TOBACCO MOSAIC VIRUS*

Albert Siegel

Department of Agricultural Biochemistry, University of Arizona, Tucson, Arizona

I. Introduction 25
II. Techniques for Detecting and Isolating Variants 26
A. General Considerations 26
B. Types of Virus-Host Interactions 27
C. The Background of Spontaneous Mutation 28
D. Isolation of Variants 29
III. Induction of TMV Mutants 31
A. Treatment of Infected Plants 31
B. Treatment of Virus Particles *in Vitro* 33
C. Induced Mutation of Different Strains of TMV 42
D. Direction of Induced Mutation 43
IV. The TMV Genome 44
V. TMV Mutants and the Genetic Code 45
A. Evidence for a Nonoverlapping Code 46
B. Matching Coding Triplets to the Amino Acid Replacements 47
VI. Discussion and Summary 55
References 58

I. Introduction

One of the outstanding problems of biology is the nature of the relationship between gene and gene product. Viruses offer a useful material for attacking this problem because virus particles consist of a small number of genes and one to several gene products. The small ribonucleic acid-containing viruses are particularly useful for this purpose because many of them contain only enough nucleic acid to comprise a small number of genes and only a single species of protein as a gene product. Of the small viruses that are available, tobacco mosaic virus (TMV) seems particularly well suited for study of the relationship between gene and gene product. The reason for this lies, perhaps not in any intrinsic property of the virus or the virus-host system, but rather in the fact that it has been the virus of choice in many previous plant virus investigations. The end result has been that as much, if not more, is known about the chemistry of the *in vitro* TMV particle than about any other species of virus. The facts and speculations about

* Arizona Agricultural Experimental Station Technical Paper No. 864.

several aspects of the chemistry and biology of TMV have been the subject for a large number of review articles (Mundry, 1963; Tsugita and Fraenkel-Conrat, 1963; Siegel and Wildman, 1960; Gierer, 1960a; Wildman, 1959; Caspar, 1963; Anderer, 1963; Kleczkowski, 1963).

The TMV particles consist of two components, ribonucleic acid (RNA) and protein. The nucleic acid exists as a single polynucleotide chain of some 6400 residues, and it has a molecular weight of 2×10^6. The protein consists of 2130 identical molecules or subunits, each of which has a molecular weight of 17,500 and consists of 158 amino acids. The primary structure of the protein has been determined independently in two laboratories. It is the largest protein whose amino acid sequence is known and confirmed. The nucleic acid component of the virus particle can initiate infection by itself when it is freed of the protein subunits. The host tissue responds to infection, initiated by either a nucleic acid molecule or an intact virus particle, by synthesizing intact virus particles. Thus, the nucleic acid part of the virus carries into a host cell the necessary specifications for synthesis of the viral protein, a protein with which it has had no prior experience. The nucleic acid, or some part of it, therefore, is a gene whose gene product is the viral protein.

A question that may be asked is how does the gene specify the gene product. This is a central question in biology today, and many systems are being used in an effort to solve this problem. The approach that has employed TMV, and which has yielded valuable information, has been to modify chemically the viral nucleic acid and to study the consequent perturbations which occur in the viral protein. The nucleic acid can be modified by treating it with mutation-inducing agents whose action is well understood. It is the purpose of this review to describe the methods and results of mutation studies that have been carried out with TMV.

II. Techniques for Detecting and Isolating Variants

A. General Considerations

It is well at the outset to review the techniques that have been developed for distinguishing and isolating biological variants of TMV and to review the methods that are available for determining that these variants are heritable. Mutants of microorganisms are usually detected because they have an altered growth pattern which is reflected in the characteristics of a group of organisms. This is in contrast to mutant

detection in larger organisms where differences between individuals can be readily observed. An additional consideration applicable to obligate parasites is that, generally, differences between clones are detected by their effects on the host and cannot usually be recognized by direct observation of the organisms. Therefore, since viruses are obligate parasites, mutants are generally detected by a unique symptom that they induce in a host. This is not to say that differences in chemical and physical properties have not been observed between virus particles of different strains. Such differences have been observed, but only among strains that were isolated initially because they were detectably different in biological activity. On the other hand, chemical differences have not been found between all strains of virus that have demonstrable differences in biological activity, although such differences are presumed to exist.

B. *Types of Virus-Host Interactions*

A description of the different TMV host-virus interactions will be useful in understanding the techniques which have been employed in detecting and isolating mutants. Infection of a host plant is usually initiated by abrading a leaf in the presence of virus particles. The following reactions ensue.

1. *The Systemic Reaction*

There exists a group of hosts for TMV which synthesize virus first in groups of cells surrounding initially infected cells. Generally, no overt symptoms of disease develop on a mature inoculated leaf, although large quantities of virus particles are synthesized. Virus particles are transported from the inoculated leaf, via the phloem, to many other parts of the host plant where infection is reestablished. In the phloem, virus particles are carried passively with the main flow of sap, part of which goes to the young expanding leaves. When these young leaves become infected, typical disease symptoms develop which consist of some leaf malformation and of a mosaic of light green and dark green areas. This mosaic gives the disease and the causative virus their names. Bawden (1950) provides an excellent introduction to the classical plant virus literature.

The hosts which react in this manner to infection by all strains of TMV will be called *systemic hosts* in this review.

In a particular host, different strains of TMV will induce symptoms which can clearly be distinguished from one another. When comparing symptoms induced by different variants, it is necessary to maintain the infected plants under as nearly uniform conditions as possible, because symptom development is influenced by environmental conditions.

2. The Local Lesion Reaction

Another group of host species and varieties react quite differently to inoculation. The reaction in this case is termed hypersensitive, because virus multiplication, in cells surrounding initially infected cells, results in death of the infected tissue. The dead cells are called local necrotic lesions, and they appear only on inoculated leaves. Virus infectivity can be recovered from the necrotic tissue but not from interlesionary tissue. Lesions grow with time on certain hosts (Rappaport and Wildman, 1957), and if they are measured at a given time, different strains of the virus are found to induce lesions of different sizes. The number of lesions which appear on an inoculated leaf is a function of the virus concentration in the inoculum. This relationship is the basis for the most commonly used plant virus bioassay. Those hosts which react to all strains of TMV with production of local necrotic lesions are called *local lesion hosts.*

3. The Differential Host Response

A third group of hosts reacts to inoculation with some strains of TMV by becoming systemically invaded, and reacts to inoculation with other strains by forming local lesions. A convenient method for quantitating response of TMV to a mutagenic agent is to enumerate the number of local lesions which appear on a differential host after it has been inoculated with a treated systemic strain.

C. The Background of Spontaneous Mutation

One difficulty in conducting induced mutation studies with TMV results from the relatively high level of spontaneous variants in most virus preparations. Considerable evidence exists for the notion that only a single particle participates in the initiation of an infection. If an infection results in the formation of a local necrotic lesion, then all of the virus particles which can be isolated from the lesion are descendants from the single virus particle which initiated the infection. Usually from 50 to 1000 infections can be initiated by the progeny found in a single lesion. Because the number of virus particles which can be isolated from a single lesion are too few for most studies, it has been the general practice to initiate a systemic infection with infectious material from a single lesion in order to increase the amount of virus. The virus particles isolated from a systemic host which had been thus inoculated are, therefore, descendants of the particle which

initiated the formation of the lesion. During the course of systemic infection, massive synthesis of virus particles occurs. It is not unusual, for instance, to recover 10^{14} virus particles from a single leaf of a systemic host. As might be expected, mutation, leading to the appearance of new strains, occurs during the course of this massive synthesis. The types of virus particles that are recovered at the end of this period of synthesis have been subjected to rigorous selection. Several investigators have observed that the progeny of a single virus particle, as recovered from a systemically invaded host, contains a small proportion of virus particles which are unlike the original parent (Jensen, 1933; McKinney, 1926).

Estimates of the proportion of variants in preparations of TMV have ranged from about 0.1 to 2% (Kunkel, 1940, 1947; Knight, 1959). It is well to keep in mind, therefore, that virus preparations, which are used in studies of induced mutation, already contain variant types. The relatively high proportion of variants makes the detection of a low frequency of induced mutants a difficult task.

D. Isolation of Variants

1. Transfer of Local Lesions to a Systemic Host

The methods that are available for the isolation of TMV variants can be readily deduced from the foregoing description of the different host-virus interactions. Because local necrotic lesions only contain the progeny of a single virus particle, a most convenient method for separating virus particles from each other is to inoculate them to a local lesion host. Different strains of a virus will induce lesions which have a different average size, but it is only under rare circumstances (Wu *et al.*, 1960) that strains can be separated from a mixture on the basis of lesion size or other lesion characteristics. The virus particles present in each lesion can be partially characterized, however, by the symptoms which they induce in a systemic host. Following multiplication of an isolate in a systemic host, it generally becomes possible to obtain enough virus particles so that they can be further characterized by additional physical, chemical, and biological criteria.

The proportion of variant virus particles in a preparation can be assessed by first separating virus particles on a local lesion host and then determining the frequency of odd symptoms that they induce in systemic host plants. Several investigators have used this method to quantitate the effect of mutagenic agents on TMV preparations and to isolate variants for further study. One pitfall of this method is the possibility that the "mutagenic treatment" will differentially inactivate virus particles in such a manner that the proportion of spontaneously occurring variants is increased.

2. *Use of the Differential Host*

Another method that has been used for quantitating the effects of a mutagenic treatment is the inoculation of a differential host with a systemically invading strain. The variants which induce local lesions on the differential host can then be readily detected. When a virus preparation is treated with a mutagenic agent for increasing periods of time, the number of lesions it will induce first increases and then decreases. The maximum number of lesions appear when 63% of the particles in the virus preparation are inactivated. Although this method detects only a single type of variant (one that has changed from systemic invasiveness to local-lesion-producing on the differential host), it is useful for determining several parameters of the mutation process. Misleading results can be obtained with this technique if a mutagenic agent is selective in its inactivating capacity. Lesion-producing variants can be isolated from differential hosts by repeatedly passing the virus from an initial local lesion to other differential and local lesion hosts until the parent systemic strain is eliminated. The lesion can then be transferred to a systemic host for massive multiplication. Tsugita and Fraenkel-Conrat (1960), in particular, have employed this technique when isolating mutants for further characterization.

Advantage also has been taken of the differential host for study of the reverse mutation from a local lesion strain to a systemic strain. In this method, a local lesion strain is inoculated to a number of differential-type host plants and observation is made of the number of plants which exhibit systemic symptoms.

3. *Direct Inoculation to Systemic Host Plants*

Another method for isolation of variants consists of the direct inoculation of systemic-type host plants with a virus preparation. When using this method, care must be taken to inoculate under conditions which ensure that many plants are infected with only a single virus particle. This can best be achieved by inoculating with a virus preparation that is diluted so that many (at least half) of the inoculated plants remain uninfected. Although this technique for the isolation of variants is more laborious than the others that have been described, it is useful for isolating particular types of variants. Variants which can only be isolated by this technique are of a type which induce local lesions from which no infectivity can be recovered.

III. Induction of TMV Mutants

A. *Treatment of Infected Plants*

1. *Irradiation*

The earliest reports of attempts to induce mutation of TMV were by Kausche and Stubbe (1939, 1940). They irradiated leaves of tobacco (a systemic host) with X-rays in the dose range 12,000–16,000 r, either before or after they had been inoculated with tobacco mosaic virus. Variants were detected by inoculating a virus preparation obtained from irradiated leaves to a local lesion host. The lesions thus induced were transferred individually to systemic host plants and observation was made for unusual symptom expression. In one sample experiment, only 1 out of 1200 lesions induced by untreated virus produced a variant symptom, whereas 3 out of 400 lesions induced by virus extracted from irradiated leaves yielded symptoms differing from that of the parent strain. In another report, 91 out of 139 attempted transfers from lesions induced by treated virus were successful; of these, one gave altered symptoms. It is doubtful that enough information is given to decide whether these data are significant in indicating the presence of a higher frequency of variants among virus particles extracted from irradiated leaves as compared with that from unirradiated leaves.

Even if it can be clearly demonstrated in future work that irradiation of host plants leads to recovery of an increased frequency of variants, a difficulty of interpretation presents itself. Have the variants been induced by the treatment, or has the treatment altered the intracellular environment of the host in such a manner as to provide more favorable conditions for some of the spontaneously occurring variants? Even though Kausche and Stubbe failed to present a convincing case for the induction of mutation, their work, together with that of Pfankuch (Pfankuch *et al.,* 1940), is classic because of the intensive study they made of the physical and chemical properties of several variants.

2. *Heat*

It has been noted for some time that variant strains of TMV can be readily isolated from the particles found in infected host plants that are maintained at high temperatures. Holmes (1934) found that he could isolate masked strains of TMV from infected tobacco and tomato stems which had been maintained at 36°C. (The designation "masked" is given to a strain that induces no apparent symptom in a systemic host, although the host synthesizes fairly large quantities of virus particles. The presence of virus particles in the systemic host can be detected by subinoculation to a local lesion host where typical necrotic lesions are induced.) Johnson (1947) reinvestigated this phenomenon by inoculating a systemic host with a rigorously purified isolate of the common strain of TMV. After maintaining the host plants at 35°–37°C. for 5 to 13 days, he screened for the presence of variants by first inducing local lesions and then transferring these individually to systemic host plants. He observed that many of the lesions were smaller than usual, and that many of the isolates were variants of a type that gave mild or masked systemic symptoms. Other types were also observed. Johnson's conclusions were stated as follows: "It has been shown that single lesion pure-line strains of the tobacco mosaic virus, normally remaining constant, may be induced to mutate at a relatively high rate by allowing virus multiplication to occur at temperatures of 35°–37°C." The same conclusion has been reached by Sukhov (1956) on the basis of the same type of experimental data.

Mundry (1957a) performed similar experiments, but he studied a different class of TMV variants. This class of variants is easily recognized because it produces bright yellow spots on a background of the typical light green-dark green mosaic induced by the common strain (McKinney, 1926; Jensen, 1933). Mundry observed that the number of yellow spots appearing after inoculation with a rigorously purified isolate of common TMV increased as a function of temperature. For instance, in one experiment there was an average of 3 yellow spots per plant when the infected plants were maintained at 17°C. and 35.5 yellow spots when the plants were maintained at 35°C. After considerable deliberation Mundry concludes that, ". . . it *seems likely* that the observed effect of temperature is not caused by changes in the selection of mutants during plant growth and virus multiplication but by changes in the mutation rate of the virus."

It may be true that the mutation rate of TMV is raised by treatments such as X-irradiation and heat applied to infected plants, but the aforementioned authors have failed to make their case as strong as it might be. It can be argued that the increased frequency of variants results from the selection of spontaneously occurring mutants as a result of an altered intracellular environment induced by the treatments.

As a matter of fact, Kassanis (1957) has demonstrated the likelihood that selection of preexisting variants may explain the increased frequency of attenuated strains when TMV-infected plants are maintained at high temperature. He isolated "avirulent" strains after heat treatment, and he demonstrated that many of them multiplied to a greater concentration than did the parent strain in plants maintained at a high temperature

(36°C.). He also was able to demonstrate that common TMV, which is stable *in vitro* and *in vivo* under moderate conditions, actually became unstable when maintained in plants at a high temperature for relatively long time periods. A serious doubt exists, therefore, that mutation induction has been demonstrated to result from treatment of infected plants.

3. *5-Fluorouracil*

When bacteria or bacterial viruses reproduce in the presence of certain base analogs, their rate of mutation is increased. Tests have been performed to determine whether this may also be true for tobacco mosaic virus. It has been determined that an appreciable amount of TMV RNA uracil is replaced by 5-fluorouracil when virus replicates in host tissue exposed to this analog (Gordon and Staehelin, 1958). Holoubek (1963) could detect no mutants or change in specific infectivity in virus preparations that had up to 29% replacement of uracil by 5-fluorouracil. However, Kramer *et al.* (1964) claimed that virus preparations which have greater fluorouracil content do contain an elevated number of mutants. They observed that virus preparations with 36 and 56% of uracil replaced by fluorouracil had a specific infectivity only half that of control virus preparations. Moreover, the high fluorouracil preparations appeared to have about 5 times the mutant frequency as control preparations. Only 2 of 245 lesions induced by control virus yielded aberrant symptoms on systemic hosts, whereas 11 mutant strains were isolated from the same number of lesions induced by virus containing 56% fluorouracil. Other tests also suggest an increased mutant frequency to be associated with fluorouracil incorporation.

The conclusion of Kramer *et al.* (1964) that mutants are induced as a result of fluorouracil incorporation into TMV RNA is subject to the same type of objection raised for other mutagenic treatments which are applied to the host. It is possible that the increased mutant frequency may result from the selection of spontaneously occurring mutants.

B. *Treatment of Virus Particles in Vitro*

1. *Irradiation*

Kausche and Stubbe (1939) were the first to attempt mutation induction of TMV by X-irradiation *in vitro*. They reported failure to detect mutation, but they employed only very low dosages in the range of 12,000–16,000 r.

Gowen, however, in 1941, reported the presence of an increased frequency of variants among the survivors of X-irradiated virus preparations. He also observed an absolute increase in the number of variants in some treated preparations. Such an increase provides strong evidence for the induction of mutation, as will be described in the next section of this review. Gowen studied the kinetics of appearance of local lesion-inducing strains when he irradiated a strain that was systemic in a differential host. He also studied induction of the reverse type of variant—that producing the systemic reaction when a local lesion-inducing strain was the parent type. When studying induced mutation from a local lesion strain to a systemic strain, Gowen inoculated leaves of the differential host, *Nicotiana sylvestris* Speg. and Comes, with treated and untreated virus preparations, and he noted the number of plants exhibiting systemic symptoms. He assumed that a single mutant was present in the inoculum applied to a plant, if that plant showed secondary symptoms on noninoculated leaves. This type of analysis leads to an error because 2 or more particles capable of initiating systemic infection may have been applied to some plants. The magnitude of this error can be estimated if the reasonable assumption is made that the variants are randomly distributed among the inoculated plants. In this instance, however, the error is impossible to assess because of the paucity of experimental detail. Gowen reports that somewhat less than 2×10^5 lesions of untreated virus gave rise to 295 systemically infected plants, whereas 33 systemic infections were yielded by 3720 lesions that were induced by virus that had received an X-ray dose of 1.36×10^6 r. Intermediate values were obtained when lower dosages were used. The variant frequency has been increased from 0.15 to almost 0.9% by the irradiation.

Mundry (1957b) has attacked the conclusions reached from these experiments because he has been able to demonstrate an increased frequency of variants when infectivity of virus preparations are reduced by dilution as well as by irradiation. The apparent increase of variant frequency upon dilution is attributed to a release of an inhibitory action known to occur between strains of a virus. He cautions, therefore, that the variant frequency found following a treatment which reduces infectivity should be properly compared with a virus preparation diluted to the same infectivity as that found in the treated preparation. Although Mundry's criticism of Gowen's experimental technique appears to be valid, it does not rule out the possibility that mutation induction from a local-lesion strain to a systemic strain may occur upon irradiation. It will require, however, well-controlled experiments to demonstrate that this is so.

Mundry's arguments are not as cogent when applied to the study of the reverse mutation—from the systemic type strain to the local lesion strain. However, even in this case, doubt will exist about Gowen's

experiments until they are repeated under more carefully controlled conditions. It will be necessary, as Mundry points out, to compare the number of apparent variants in a treated preparation with the number in an untreated preparation that has been diluted to the same level of infectivity.

2. *Chemical Mutagenesis*

In contrast to the absence of conclusive evidence for mutagenicity of *in vivo* treatments of the virus-host system or of irradiation of virus preparations *in vitro*, little doubt exists concerning the mutation-inducing power of at least one chemical reagent, nitrous acid. The chief reason for the lack of uncertainty lies in an exceptionally high frequency mutation induction with this reagent.

a. Nitrous Acid. i. *The killing and mutagenic effect of nitrous acid.* The efficacy of nitrous acid as a mutagen for TMV was first demonstrated by Mundry and Gierer (1958) (see also Gierer and Mundry, 1958). The primary method used for the detection of mutation was to observe the number of necrotic local lesions induced on a differential host by a systemic-type strain which had been treated for different periods of time. They employed, as a differential host, Java tobacco, which reacts to their parent strain "vulgare" with chlorotic primary lesions followed by secondary systemic symptoms, and which reacts to certain other strains with the necrotic local lesion response. The fractional survival of treated preparations was indicated by the total number of lesions induced, both chlorotic and necrotic; whereas the proportion of variants was indicated by the number of necrotic lesions. As an adjunct to estimation of survival, assays were also performed using the host "Xanthi" (Takahashi, 1956), which forms necrotic lesions with all strains. Mundry and Gierer consider that three features of their experimental data stand out to indicate mutation induction as a result of nitrous acid treatment. These are:

a. The proportion of infections that are necrotic increased with time. In one experiment this proportion increased from 0.21% at 0 time to 15.5% after treatment of infectious RNA for 96 minutes with $NaNO_2$ at pH 4.8.

b. The number of necrotic lesions induced on Java tobacco by a treated virus preparation is much higher than the number of necrotic lesions induced by a preparation whose infectivity has been reduced to the same level by dilution.

c. The absolute number of necrotic lesions first increases with time and then decreases as the treatment is extended. The maximum number of necrotic lesions is induced after a treatment which leaves 37% survivors. The data are seen to fit fairly well to a theoretical curve relating the number of necrotic lesions to the dose; the theoretical curve having been derived on the basis of two assumptions: (1) a single event or "hit" results in the inactivation of a virus particle, and (2) a single event or "hit" results in mutation. The assumption that inactivation results from a single event is well supported by the experimental finding of a linear relationship between the logarithm of the fraction of survivors and the time of exposure to nitrous acid. This relationship is represented by the equation

$$s = e^{-kt}$$

in which s is equal to the fraction of surviving virus particles, t is equal to the time of treatment, and k is a constant. The assumption that a single event results in a mutation is expressed by the equation

$$m = 1 - e^{-rkt}$$

in which m is the fraction of mutants in the total population and r is the fraction of events which are mutating events. When r is small in relation to kt, m becomes approximately equal to rkt. The fraction of the total population which can be detected as mutants is that fraction, M, which has been mutated but not killed. Thus,

$$M = ms = rkte^{-kt}$$

and

$$\frac{dM}{dkt} = re^{-kt} - rkte^{-kt}$$

from which it can be seen that M will have a maximum value when kt is equal to 1, and

$$\frac{M}{M_{\max}} = kte^{1-kt}$$

Mundry and Gierer found that they did observe a maximum value for M when kt was 1 (the fraction of survivors is 37% when kt is equal to 1), and also that reasonably good agreement was found between experimental values for $M/M_{\max}$ at different kt's and the theoretical values derived from the above equation. They concluded, therefore, that nitrous acid induced mutation by a single event during the course of treatment.

Bawden (1959) took issue with the interpretation that Mundry and Gierer gave to their data, claiming that the results might have a different explanation. He pointed out that the same general type of results would be obtained if the local lesion-inducing variants were more resistant

ESCUELA DE ... UNIVERSIDAD DE CHIL Valparaíso BIBLIOTECA

than the parent strain to the inactivating effect of nitrous acid. That this can be true in qualitative terms was demonstrated by Rappaport and Wu (1962) by experiments in which they irradiated a mixture of two TMV strains with ultraviolet light. One of the strains, VM, produces minute necrotic lesions and is 5 times more sensitive to inactivation with ultraviolet light than the U1 strain which produces normal-sized lesions. Lesion formation by the U1 strain is suppressed as a result of interference (Sadisivan, 1940; Siegel, 1959), when a mixture of the two strains with VM in excess is used as inoculum. When the mixture is irradiated, there is an increase in the number of lesions induced by the U1 strain. This is then followed by a decrease as the dose is further increased. The increase in the number of lesions is attributed to the more rapid inactivation of the radiation-sensitive VM strain which had blocked lesion formation by the U1 strain.

Mundry (1959) admitted that Bawden's arguments might be valid in qualitative terms, but he also pointed out that his quantitative data made it highly unlikely that there could be validity to an interpretation which did not allow for a potent mutagenic effect of nitrous acid.

In addition to the data based on the kinetics of appearance of necrotic lesions on a differential host, there is another type of experimental result which argues strongly for the notion that nitrous acid is a potent mutagen. Among the total number of variants which can be isolated from a nitrous acid-treated virus suspension, those variants that induce lesions on a differential host are in the minority. Other variants are detected by inoculating treated preparations to local lesion hosts and then transferring the resultant lesions individually to systemic host plants. Mutants which induce systemic symptoms different from that of the parent strain can then be isolated. Mundry and Gierer (1958) found that of those virus particles which had survived nitrous acid treatment to 0.5% survival, only 15% induced symptoms which were like those of the parent strain.

Another characteristic by which variants can be recognized is the size of lesion which they induce on a local lesion host. It has been demonstrated (Siegel, 1960) that many of the survivors of nitrous acid treatment induce lesions which are smaller, on the average, than those induced by the parent preparation. Siegel (1960) investigated a TMV population that had been treated with nitrous acid to 10% survival. At least 50% of the survivors proved to be variants which induced altered symptoms, a smaller lesion size, or both. The figure of 50% is probably low because of uncertainty relating to the "miss" phenomenon; a phenomenon which will be discussed later. This *high* frequency of variants makes it unlikely that the variants were present in the parent population and merely selected by the treatment. If this were indeed so, then 5% of the original population would have consisted of variants which were completely resistant to inactivation by nitrous acid. That this cannot be the case is demonstrated by the survival curve of the virus, which is exponential at least to the 0.1% level of survival. An experiment of Rappaport and Wildman (1962) supplies additional evidence against the hypothesis that the variant frequency is increased as a result of selection. They isolated five spontaneously occurring variants and demonstrated that these variants have the same sensitivity to inactivation by nitrous acid treatment as does the parent U1 strain. In the light of this evidence as well as that previously presented, the conclusion is reached that most of the variants appearing following nitrous acid treatment are mutants that have been induced by the treatment. Moreover, it appears that the action of nitrous acid upon TMV is one of the most potent mutagenic effects yet discovered.

ii. *The chemical reaction of nitrous acid with virus nucleic acid.* Schuster and Schramm (1958) investigated the chemistry of the reaction of nitrous acid with TMV RNA. They found that the nitrogen bases were oxidatively deaminated and that the phosphodiester linkages were left undisturbed. During nitrous acid treatment the nucleic acid residue, adenine, is converted to hypoxanthine, guanine is converted to xanthine, and cytosine is converted to uracil. The purines, adenine and guanine, are converted to bases which are not found in TMV RNA, whereas the pyrimidine, cytosine, is converted to the fourth naturally occurring base, uracil. The purines, adenine and guanine, were found to react about 50% faster than the pyrimidine, cytosine. Because the loss of the reacting residues could be almost completely accounted for by the appearance of the deaminated residues, Schuster and Schramm concluded that very little in the way of side reaction was occurring. In later work with improved methods, Schuster and Wilhelm (1963) reported that although loss of adenine and cytosine could be quantitatively accounted for by the appearance of hypoxanthine and uracil, only half the loss of guanine could be accounted for by the appearance of xanthine. What happens to the rest of the missing guanine is unknown.

We have already discussed the finding that an exponential survival curve is observed when TMV or TMV RNA is treated with nitrous acid. It follows from this, and from the foregoing discussion of the chemical reaction of nitrous acid with TMV RNA, that a single deamination is sufficient to inactivate an infectious unit. By relating the rate of deamination to the rate of inactivation, Schuster and Schramm (1958) have concluded that deamination of any one of approximately half of the bases of the 6400 residue polynucleotide chain leads to inactivation.

It should be pointed out, however, that this conclusion is based on the assumption that the kinetics of deamination is uniform for a long period of time. The kinetics of inactivation is deduced from results collected during the first 60 minutes of the reaction, whereas in order to determine the kinetics of deamination, the reaction is run for at least an order of magnitude longer.

iii. *Comparison of the effect of nitrous acid on TMV and TMV RNA.* Nitrous acid has both an inactivating and a mutagenic effect on intact virus particles as well as on the free, infectious nucleic acid. The intact virus, however, is inactivated at a rate 5 times slower than is the free nucleic acid. A difference in the chemical reaction of the two infectious agents with nitrous acid has been described by Schuster and Wilhelm (1963). In contrast to the reaction of nitrous acid with free nucleic acid in which adenine, guanine, and cytosine residues are altered, in the reaction with intact virus particles, the guanine residues are somehow prevented from reacting. At the same time, cytosine is deaminated 1.8 times faster than adenine in the reaction with intact virus particles; whereas in the reaction with free nucleic acid adenine is deaminated 1.4–1.5 times faster than cytosine.

Although direct critical experiments have not been performed to test the relative mutagenic efficiency of nitrous acid for the two infectious agents (TMV and TMV RNA), a comparison of the data of Mundry and Gierer (1958) and of Siegel (1960) indicates that this efficiency can not be too dissimilar. In one laboratory the frequency of symptom variants was determined among survivors of free nucleic acid inactivated to 0.5 to 1% survival, and in the other laboratory the mutant frequency was determined for intact virus particles inactivated to the same level of survival. Of 60 lesions induced by treated nucleic acid, in one case 7 and in another case 12 proved to yield systemic infections with unaltered symptoms. Of 50 lesions induced by treated intact virus particles, 11 were isolates with unaltered symptoms.

Schuster and Wilhelm (1963) have pointed out the importance of a precise determination of the ratio of mutating to killing deaminations when the two infectious agents are treated with nitrous acid. Such precise determination of the ratio might yield information relative to the biological effect of guanine alteration, because this base is deaminated and otherwise altered only when free nucleic acid, not intact virus, is treated with nitrous acid.

iv. *The total number of mutants induced by nitrous acid treatment.* Of the several properties of TMV that have been examined, the two that are most frequently altered are symptom expression in a systemic host and the size of necrotic local lesions in a local lesion host (Siegel, 1960). When survivors of nitrous acid-treated virus are screened for alteration in both of these properties, it is found that 1 mutating deamination occurs for each 2 to 3 killing deaminations. Schuster and Schramm (1958) calculated that a deamination is lethal if it occurs to any one of about 3000 of the base residues contained in an infectious nucleic acid molecule, and there is reason to believe that this number is similar when the intact virus is treated. We have discussed the observation that only cytosine and adenine are deaminated when the intact virus is treated. These two bases account for 3000 residues and there would seem to be no additional residues whose deamination could lead to mutation. However, the number 3000 is only a crude approximation which permits the presence of the 1000 to 1500 bases whose deamination would result in mutation. The high frequency of mutating deaminations indicates that essentially all deaminations of TMV nucleic acid can be recognized either because they lead to inactivation or to mutation. Further, it seems that almost all mutations can be recognized either by an altered symptom or by a reduction in lesion size. It is surprising that few or no alterations of the viral nucleic acid are neutral in their biological effect. Although the validity of these conclusions depends on more precise measurements of the parameters used in their derivation, it is unlikely that they can be changed in a qualitative manner. There is a necessity, however, for postulating that a fraction (perhaps 16–20%) of the deaminations results in no biological effect. The reason for this will be presented in a later section.

v. *The "miss" phenomenon.* When a nitrous acid-treated virus preparation is inoculated to a local lesion host and the consequent lesions are transferred individually to systemic host plants, it is observed that some of the lesions yield no infection upon transfer. This is in sharp contrast to the transfer of lesions induced by untreated virus, in which case there is high efficiency of infection transfer. One third of the lesions fail to transfer when they are induced by either nucleic acid or virus preparations which have been 99% inactivated by nitrous acid. Uncertainty presents itself in deciding whether the particles which induce sterile lesions are mutant or normal particles because these particles cannot be further classified. Two reports emphasize that it is highly likely, however, that the sterile lesions represent mutant particles. Veldee and Fraenkel-Conrat (1962) found that many nitrous acid-induced mutants have very low productivity in the sense that lesion transfer to another local lesion host results in the induction of very few lesions, whereas a lesion induced by the parent strain yields many lesions upon such transfer. It becomes likely, therefore, that many of the sterile lesions may represent strains with extremely low productivity. Another

possible cause for the "miss" phenomenon was discovered by Siegel *et al.* (1962), who isolated two strains of TMV following nitrous acid treatment that consistently produce sterile lesions. These strains are defective in their capacity to induce the formation of functional virus protein in a host. The infectivity present in the host plant is only in the form of free nucleic acid; intact virus particles are absent. This situation makes transfer of infection from host to host difficult for two reasons: the specific infectivity of free nucleic acid is several orders of magnitude lower than that of nucleic acid incorporated into virus particles, and host cell nucleases degrade the infectivity of free nucleic acid when host cells are disrupted during the transfer procedure. It is possible to transfer infectivity of defective strains from systemic host to systemic host and from systemic host to a local lesion host, but it is rarely possible to recover infectivity from a local lesion induced by a defective strain. The defective strains have been isolated from a nitrous acid-treatment virus preparation by direct inoculation to systemic seedlings at limit dilution, under conditions where many of the seedlings become infected by a single virus particle. It is probable that the phenomena of defective strains and strains with low productivity can account for the high frequency of misses encountered when transferring lesions induced by nitrous acid-treated virus.

b. Other Chemical Mutagens Effective on TMV or TMV RNA. Although nitrous acid is by far the most potent mutagen yet discovered for TMV, other agents have also been reported to be effective. The action of these other agents is less well understood than is the action of nitrous acid. It should also be mentioned that the mutagens to be discussed in this section have been reported to be effective in only a single laboratory, whereas nitrous acid has been shown to be mutagenic in at least three laboratories.

i. *Dimethyl sulfate.* Fraenkel-Conrat (1961) tested several alkylating agents (iodoacetate, ethylene oxide, propylene oxide, dimethyl sulfate, and diethyl sulfate) and found that they were all effective in inactivating both TMV and TMV RNA. Of these, only dimethyl sulfate proved to be mutagenic. The appearance of local lesions on a differential host following treatment of a systemic strain was used as a test for mutagenicity. Although few experimental details are given, it appears that the number of lesions induced by a treated virus preparation were compared with the number induced by a nontreated preparation diluted to the same survival level.

One of the consequences of the reaction of dimethyl sulfate with TMV RNA is the conversion of many of the guanine residues to 7-methylguanine.

ii. *N-Bromosuccinamide.* Fraenkel-Conrat (1961) has reported that bromination of TMV RNA with the reagent *N*-bromosuccinamide is mutagenic, but experimental details are lacking.

iii. *Hydroxylamine.* Schuster and Wittmann (1963) have reported a mutagenic effect of hydroxylamine on TMV RNA. It is of interest that hydroxylamine has both an inactivating and mutagenic effect only on free nucleic acid and apparently has no effect on intact virus particles. The enumeration of lesions appearing on a differential host and the observation of symptoms induced in systemic host plants by particles passed through local lesions were used as tests for the presence of variants. Hydroxylamine inactivates TMV RNA when the reaction is carried out at both pH 6.1 and 9.1, but there is a mutagenic effect only at pH 6.1.

Schuster (1961) has determined that the pyrimidine residues of RNA react with hydroxylamine in such a manner that uracil is destroyed and cytosine is modified to a compound(s) that may act like uracil in its base-pairing properties. At pH 9.1 uracil reacts with hydroxylamine at least 8 times faster than it does with cytosine, whereas at pH 6.1 the reaction with cytosine is at least 30 times faster than with uracil. Thus, it appears that it is the reaction of hydroxylamine with cytosine that leads to mutation. Hydroxylamine acting on RNA has approximately $\frac{1}{2}$ to $\frac{1}{3}$ the mutagenic efficiency of nitrous acid acting on intact virus particles according to the limited available data.

C. *Induced Mutation of Different Strains of TMV*

This review has so far dealt with mutation experiments that have utilized strains of TMV that are dominant in tobacco. These strains, maintained in many laboratories, are probably quite similar to each other and are variously designated common, vulgare, and U1. Many of the mutants induced by nitrous acid are less virulent than the common strain either as measured by type of symptom, by amount of virus produced in a host, or by lesion size. It is interesting to note, therefore, that the mutagenic efficiency of nitrous acid is lower for laboratory strains of TMV with low virulence than it is for the common strain (Siegel, 1960). For instance, when suspensions of the U1, U2, and U4 strains were inactivated to 1% survival with nitrous acid, 23 out of 50 U1 lesions gave rise to symptom variants, whereas only 14 out of 54 U2 lesions and 5 out of 50 U4 lesions produced variants. The parent strains are such that U1 induces the typical mosaic, U2 a mild mosaic, and U4 is symptomless, although host plants support the multiplication of a considerable quantity of virus particles. The reason for the difference of the three strains in mutation response to nitrous acid

is unknown, but it is possible that the mild and masked symptoms of strains U2 and U4 are epistatic for many genetic changes which would lead to symptom alteration in other genetic backgrounds.

Dahl and Knight (1963) isolated mutants of the Y-TAMV strain of tobacco mosaic virus after nitrous acid treatment. The Y-TAMV strain was originally found in field-grown tomatoes (Miller, 1953). Two characteristics of this strain were especially sensitive to alteration as a result of nitrous acid-induced mutation. These were size of lesion on Xanthi and ability to induce necrotic local lesions on tobacco. The 12 mutants that these authors isolated were selected because they induced smaller lesions on Xanthi than did the parent strain. One of the mutants induced necrotic local lesions on tobacco and on all other hosts that were tested except tomato, which was completely resistant to this isolate.

D. *Direction of Induced Mutation*

The material in the preceding section suggests that nitrous acid may alter certain phenotype characters of the virus unidirectionally. This notion is supported by a study of the lesion size character. All of the strains isolated after nitrous acid treatment which have an altered lesion size have a *smaller* lesion size than that of the parent-treated strain. This is true even when the parent strains themselves induce lesions of different sizes. For instance, the masked strain, U4, induces a smaller lesion size than the U1 strain. Nevertheless, of the 6 isolates, obtained from a treated U4 preparation which had an altered lesion size, all 6 induced a smaller lesion than did U4. More extensive investigation of treated U1 preparations revealed that of 22 isolates with altered lesion size, all 22 had a smaller lesion size than the parent (Siegel, 1960). A similar phenomenon is observed when Newcastle disease virus is treated with either nitrous acid or hydroxylamine (Thiry, 1963). These agents induce the formation of small and minute plaque variants but fail to induce reversion to the wild type.

It is an odd observation that both spontaneous and induced mutants of TMV are extremely stable in regard to back mutation. The very nature of isolating mutants, however, ensures that only variants that are stable will be isolated. Under ordinary glasshouse conditions the common strain will predominate in tobacco when placed in competition with any of the many other TMV strains. This predominance of the common strain has been investigated (Cohen *et al.*, 1957). Many variant strains have been isolated initially in a local lesion and then increased in a systemic host where ample opportunity occurs for revertants to become predominant. It would thus appear that mutants which did have a tendency to revert would seldom, if ever, be isolated. The fact that many, if not all, of the nitrous acid-induced mutants can be recognized provides strong evidence for an essential absence of reversion. One may question why the situation regarding reversion is different for nitrous acid-induced TMV variants from that of nitrous acid-induced bacteriophage variants, which revert spontaneously and also can be induced to revert with nitrous acid (Bautz-Freese and Freese, 1961). The answer may lie in the fact that the genetic material in one case is single-stranded and in the other case is double-stranded. It would be of interest to test this notion by treating the postulated double-stranded replicative form of TMV RNA should it prove to be infectious (Mandel *et al.*, 1964; Shipp and Haselkorn, 1964; Burdon *et al.*, 1964).

IV. The TMV Genome

The simple plant and animal viruses are useful in studies relating the gene to the gene product. On the one hand, it is possible to isolate the genome in undamaged infectious form (Gierer and Schramm, 1956; Fraenkel-Conrat and Williams, 1955), on the other, at least one gene product, the virus protein, can be readily isolated and characterized. The primary structure of the TMV protein has been determined (Tsugita and Fraenkel-Conrat, 1963; Anderer and Handschuh, 1962), and the secondary and tertiary structures of this protein are subjects for intensive study (Caspar, 1963). One relationship between the genome and the viral protein becomes clear upon a comparison of the number of building blocks of which each is composed. The genome contains about 6300 nucleotides and the viral protein is composed of 158 amino acids. These figures yield a ratio of 40 nucleotides for each amino acid. Current theory, however, postulates that a sequence of only three nucleotides codes for each amino acid (Crick, 1963). It becomes likely, therefore, that much of the viral genome is not concerned with specifying the viral protein. The fact that many naturally occurring and chemically induced variants have proteins that are the same as the parent strain demonstrates that the viral genome has, in addition, other functions. Two rather crude estimates have been made of the fraction of the viral genome that is concerned with coding the viral protein (Siegel, 1961; Wittmann, 1962). These estimates were derived from a determination of the number of nitrous acid-induced mutants which have protein alterations. They are in agreement in indicating that only a relatively small portion of the viral genome (from $\frac{1}{30}$ to $\frac{1}{10}$) is responsible for specifying the viral protein. The values for the coding ratio (the number of nucleotides which specify an amino acid) derived from these estimates are not incompatible with the theoretical and experimental value of 3 (Crick, 1963), although they are too imprecise to confirm this number.

If only a portion of the viral nucleic acid has an assigned function, we may inquire if any part of it is dispensable. Several lines of evidence indicate that much of the nucleic acid is required to initiate infection. Although Sugiyama and Fraenkel-Conrat (1963) have recently shown that the terminal few nucleotides are dispensable, other workers (Ginoza, 1958; Gierer, 1957) have shown that essentially every break of the polynucleotide chain is lethal. A large number of spontaneous and induced mutants have no protein alteration. These mutants presumably have suffered an alteration in a part of their nucleic acid which performs a function not associated with specifying the viral protein. At the present time not enough is known about the TMV life cycle to attempt to enumerate the other functions of the nucleic acid. However, by analogy to RNA animal and bacterial virus-host systems, it may be presumed that TMV RNA must at least code for an enzyme that is necessary for its own biosynthesis (Baltimore and Franklin, 1963; Weissman *et al.*, 1963, Haruna *et al.*, 1963). Most likely there are also other functions.

V. TMV Mutants and the Genetic Code

Useful information concerning the genetic code can be obtained from an analysis of the types of mutants which appear following a mutagenic treatment. The changes which occur to the viral protein as a result of mutation have been intensively studied because this gene product can be obtained readily in purified form. One property of the viral protein, by which it is characterized and which sometimes changes upon mutation, is its amino acid sequence. It is a remarkable accomplishment that more than 200 mutants of TMV have been examined for sequence changes. This has been accomplished by workers in two laboratories, H. G. Wittmann at the Max-Plank Institute for Biology in Tübingen, and Tsugita and Fraenkel-Conrat at the University of California Virus Laboratory in Berkeley. This feat has been made possible by the initial determination of the amino acid sequence of the common strain protein. The determination was performed by two groups who independently arrived at the same sequence for 158 amino acids (Tsugita *et al.*, 1960; Tsugita and Fraenkel-Conrat, 1963; Anderer *et al.*, 1960; Anderer and Handschuh, 1962). The proteins of the mutants were compared with that of the common strain by two variations of the same method. Tsugita and Fraenkel-Conrat (1962a) determined directly the amino acid composition of the proteins by the technique of Spackman *et al.* (1958) with the aid of an automatic amino acid analyzer. Wittmann (1960) and Wittmann and Braunitzer (1959), on the other hand, first separated the tryptic peptides of the proteins by ion-exchange chromatography and determined the amino acid composition of each peptide. Peptide analyses were also performed by Tsugita and Fraenkel-Conrat (1962a) and Funatsu and Fraenkel-Conrat (1964) in order to locate an amino acid change, but only after they had first established that a mutant protein differed from the parent protein.

The German workers selected for analysis mutants which differed from the parent strain in the type of symptom induced in a systemic host, whereas Tsugita and Fraenkel-Conrat (1962b) chose to analyze mostly mutants which differed from the parent strain in their capacity to induce local necrotic lesions on a differential host.

The results obtained by these massive investigations have been useful in confirming two aspects of the genetic code. One concerns the nonoverlapping nature of the code. The other aspect is whether codons which have been proposed on the basis of other types of experimental data are also compatible with the amino acid replacement data.

A. *Evidence for a Nonoverlapping Code*

The current evidence indicates a genetic code in which three nucleotides in sequence, designated a codon, specify an amino acid (Crick *et al.*, 1961). A problem that has been considered is whether an individual nucleotide may be a part of two or more codons. If the nucleotide sequence AUAGCA is read AUA, GCA, then each nucleotide is a part of only a single codon and the code is termed nonoverlapping. The code would be overlapping, however, if it were read AUA UAG, AGC, GCA, etc., or even AUA, AGC, CA(x).

Analysis of the amino acid replacement data is clear in indicating a nonoverlapping code. An alteration of one of the nucleotide residues would alter a sequence of two or three amino acids in the viral protein if the code were overlapping, whereas only a single amino acid would be altered with a nonoverlapping code. Of 64 analyzed mutants which have protein alterations, 48 of them have only a single amino acid changed, 13 have 2 amino acid exchanges, and 3 have 3 amino acids replaced. Even the 16 mutants which have multiple changes do not support the notion of an overlapping code because in no case are two amino acid changes adjacent to each other.

Omitted from this analysis are five mutants analyzed by Tsugita and Fraenkel-Conrat (1962b). One of these has 7 exchanges, two have 16 changes, and two have 17 changes. These five mutants are similar to naturally occurring variants which have been carried as experimental laboratory strains. As pointed out by Wittmann (1962), a strong suspicion exists that these variants are not chemically induced mutants but were either isolated as contaminants or, what seems equally likely, were already present in the starting material as naturally occurring variants. The amino acid replacement data obtained from the chemically induced TMV mutants strongly support the hypothesis of a nonoverlapping genetic code.

B. *Matching Coding Triplets to the Amino Acid Replacements*

Nirenberg and Matthaei (1961) were the first to demonstrate that an *in vitro* system, consisting of *Escherichia coli* ribosomes and other appropriate additives, could be made to incorporate amino acids into polypeptides upon the addition of a synthetic polyribonucleotide. The first polynucleotide used, polyuridylic acid, stimulated the synthesis of polyphenylalanine. After this initial discovery, Nirenberg and co-workers (1962) at the National Institutes of Health and investigators of the New York University Biochemistry Department observed which and how much of each of the amino acids were incorporated into polypeptides when different polynucleotides of known composition were supplied to *E. coli* ribosomes. It was possible to deduce from the data that many combinations of three nucleotides were specific for different amino acids when the assumption was made that a sequence of three nucleotides comprised a code word for an amino acid. The data are of such a nature that the three nucleotides contained in a triplet code word can be deduced, but the specific order of the three nucleotides within the triplet codon have not been determined except in a limited number of cases (Bernfield and Nirenberg, 1965). For instance, CAU (cytosine, adenine, uracil) is a codon for threonine, but it has not yet been determined whether the sequence is CAU, CUA, AUC, ACU, UCA, or UAC.

There are 64 possible triplet combinations of the four bases found in ribonucleic acid. Because there are only 20 amino acids to be coded for, the question has arisen whether there is only one codon for each amino acid, in which case 44 of the combinations would be meaningless, or whether the code is degenerate in the sense that there are several codons for each amino acid. This question has been resolved with the clear demonstration that the code is degenerate. At the present writing 48 of the possible 64 triplets have been shown to code for one or another of the 20 amino acids in the *in vitro E. coli* ribosome system (Wahba *et al.*, 1963a,b).

It is pertinent to inquire whether the amino acid replacements observed in TMV protein following nitrous acid treatment are compatible with the codons derived from the *in vitro* system. Wittmann (1962) has proposed that the deamination of both cytosine and adenine can result in mutation. In the case of cytosine the conversion is directly

TABLE I

SUMMARY OF AMINO ACID REPLACEMENT DATA AND POSSIBLE BASE EXCHANGES

Amino acid replacement	No. of occurrences	No. of codons for each member of exchange	Possible triplet exchanges	Possible base exchange
Threonine → alanine	2	4–3	CCG → CUG; CAA → CAG; CCA → CCG	C → U; A → G; C → U
Threonine → isoleucine	10	4–3	CAU → UAU; CCA → CUA; CAA → UAA	C → U
Threonine → methionine	3	4–1	—	—
Serine → phenylalanine	8	3–2	CUU → UUU	C → U
Serine → leucine	2	3–4	CCU → CUU	C → U
Asparagine → serine	6	3–3	CAA → CAG	A → G
Aspartic acid → glycine	2	3–3	GUA → GUG; GCA → GCG	A → G
Aspartic acid → alanine	4	2–3	—	—
Isoleucine → valine	5	3–1	UUA → UUG	A → G
Isoleucine → methionine	1	3–1	AAU → AGU	A → G
Proline → serine	3	3–3	CCU → CUU; CCC → CCU	C → U
Proline → leucine	6	3–4	CCU → CUU; CCC → CCU	C → U
Leucine → phenylalanine	1	4–2	CUU → UUU; CCU → CUU	C → U
Glutamine → valine	2	3–2	—	—
Glutamine → arginine	1	3–3	AUC → GUC	A → G
Glutamic acid → glycine	2	2–3	GUA → GUG; AAG → AGG	A → G
Arginine → glycine	3	3–3	GAA → GGA	A → G
Arginine → lysine	1	3–2	—	—
Valine → methionine	1	1–1	—	—

to uracil. In order for the deamination of adenine to result in mutation, however, it is necessary to postulate that the replication of TMV RNA involves a complementary double-stranded stage. Hypoxanthine, the deamination product of adenine, behaves like guanine in its base-pairing properties. Therefore, the deamination of an adenine residue would be equivalent to an adenine-to-guanine change in the coding sequence.

In order to decide whether all of the nitrous acid-induced amino acid replacements can be accounted for by C to U shifts or whether A to G changes must also be invoked, we may examine the 48 codons that have been established for the different amino acids. Table I lists all of the nitrous acid-induced amino acid replacements that have been published by Wittmann and Wittmann-Liebold (1963), by Tsugita and Fraenkel-Conrat (1963), and by Funatsu and Fraenkel-Conrat (1964). Table II lists the individual amino acids, their frequency of occurrence in TMV protein, and the number of times that each has been a partner in an amino acid replacement. The data in Table I illustrate that the deamination of cytosine to uracil can account for 7 of the 19 types of amino acid replacement, and 8 of the replacement types can be accounted for by the A → H → G conversion. Of the total number of 63 replacements which can be analyzed in this manner, 30 can be accounted for by the C → U deamination, 20 by the A → H → G conversion, 2 by either deamination, and 12 cannot be accounted for by either type of deamination.

The fact that the genetic code is highly degenerate has an important bearing on the consideration of the amino acid replacement data. Because there are 64 possible codons and 20 amino acids, the assumption may be made that 16 of the amino acids will prove to have 3 codons, and 4 amino acids will prove to have 4 codons. To date, 4 amino acids have been assigned 1 codon each, 6 amino acids have each been assigned 2 codons, 7 have been assigned 3 codons, and 3 have 4 codons. The 9 amino acid replacements in Table I, each of whose partners have been assigned either three or four codons, can be accounted for by a deamination of either cytosine or adenine. One replacement, threonine to alanine, can be accounted for by deamination of either cytosine or adenine. Four replacements can be accounted for by a deamination of cytosine to uracil: threonine to isoleucine, serine to leucine, proline to leucine, and proline to serine. Four replacements (asparagine to serine, arginine to glycine, aspartic acid to glycine, and glutamine to arginine) can be accounted for only on the basis of an adenine to guanine exchange. The importance of examining exchanges involving amino acids each of which have been assigned at least three codons lies in the unlikelihood that additional codons will be found for these

TABLE II
SUMMARY OF THE PARTICIPATION OF AMINO ACIDS IN REPLACEMENTS

Amino acids	No. of residues in TMV subunit	No. of times replaced	No. of times replacement	Known codons
Threonine	16	15	0	CCA; CAU; CAA; CCG
Serine	16	10	9	CUU; CCU; CAG
Alanine	14	0	6	CUG; CAG; CCG
Valine	14	1	7	UUG
Leucine	12	1	8	UAU; UUC; UGU; UCC
Glutamine[a]	12	3	0	UAC; AAG; AAC
Arginine	11	4	1	GUC; GAA; GCC
Isoleucine	9	6	10	UUA; UAA; UAC
Asparagine[a]	9	6	0	UAA; CUA; CAA
Aspartic acid[a]	9	6	0	GUA; GCA
Proline	8	9	0	CCC; CUC; CAC
Phenylalanine	8	0	9	UUU; UUC
Glycine	6	0	7	GUG; GAG; GCG
Glutamic acid[a]	4	2	0	AUG; AAG
Tyrosine	4	0	0	AUU; ACU
Tryptophan	3	0	0	UGG
Lysine	2	0	1	AAA; AUA
Cysteine	1	0	0	GUU
Methionine	0	—	5	UGA
Histidine	0	—	0	AUC; ACC

[a] In addition, there are a number of replacements where it is not yet known whether the free acid (glutamic, aspartic) or the amide (glutamine, asparagine) is involved.

amino acids. The evidence, therefore, strongly supports the contention that the deamination of adenine as well as the deamination of cytosine may lead to mutation. This conclusion suggests that TMV RNA has an *in vivo* replicative form which consists of a complementary, double-stranded structure, because a DNA (deoxyribonucleic acid) type of replication allows for the replacement of a deaminated adenine residue (hypoxanthine) by guanine. It is of interest in this regard that several authors have reported the presence of a double-stranded form of RNA in homogenates of virus-infected leaves (Mandel *et al.*, 1964; Shipp and Haselkorn, 1964; Burdon *et al.*, 1964).

Five of the types of amino acid exchanges which have been observed cannot as yet be accounted for by deamination of either cytosine or adenine. These are methionine for threonine, alanine for aspartic acid, valine for glutamine, lysine for arginine, and methionine for valine. In these five cases, one of the partners in the exchange has so far been assigned fewer than three codons. The possibility still exists, therefore, that these exchanges can be explained on the same basis as the others, if additional codons are found to apply. It is also possible that several of the mutants that have been isolated following nitrous acid treatment are actually spontaneous mutants which were present before treatment. Still other possibilities can be imagined to explain the five odd replacements.

The possible existence of an RNA strand which is complementary to the viral RNA strand raises the question of which strand acts as the messenger RNA for the synthesis of virus protein. In the case of ϕX-174, a bacterial virus containing a single-stranded DNA, it is the complementary strand and not the viral strand that supplies the template for viral protein synthesis (Hayashi *et al.*, 1963). Several features of the amino acid replacement data make it highly likely that in the case of TMV it is the viral RNA strand, and not its complement, that acts as the template for viral protein synthesis. The data presented in Table II show that proline has been replaced 9 times but has never been a replacement, whereas phenylalanine has been a replacement 9 times but has never been replaced. The codons assigned so far to proline are CCC, CCU, and CCA, none of which can be derived by a deamination from another codon (CCU can only be derived from CCC). On the other hand, the phenylalanine codons are UUU and UUC, neither of which can serve to replace phenylalanine upon deamination. The opposite result would be expected if a strand complementary to the virus RNA strand were to act as the messenger; that is, proline would be a frequent replacement and phenylalanine would be frequently replaced. This type of evidence, therefore, strongly supports the notion that it is the viral RNA strand which acts as messenger RNA in the synthesis of the virus protein. This conclusion is in agreement with that of Tsugita *et al.* (1962), who observed that the *E. coli* ribosome system would synthesize a viruslike protein when provided with TMV RNA as a messenger. However, more recent evidence by Aach *et al.* (1964) casts doubt on the resemblance to virus protein of the proteinaceous substances whose synthesis is stimulated by TMV RNA *in vitro*.

Each amino acid does not have an equal probability of being replaced or of acting as a replacement. This is clearly shown by the data in Table II. It would be expected on a random basis that the frequency with which an amino acid is replaced would be equal to the frequency with which the amino acid occurs in the parent-treated protein. This is not so; for instance, there are 16 residues each of threonine and serine in the parent protein and these two amino acids have been replaced 15 and 10 times, respectively. On the other hand, alanine and valine, which occur almost as frequently (14 residues each) have been found to be replaced only once. Again, on a random basis, it would be expected that each amino acid would have an equal probability of acting as a replacement. It is seen, however, that only 10 of the amino acids have acted as replacements, and 8 of these 10 have been replacements at least 5 times. The other 10 amino acids have not been found to act as replacements. The amino acids that have been replacements have codons that are relatively rich in guanine and uracil, as one might expect. It is puzzling, therefore, that certain amino acids have not yet been found as replacements. For instance, both cysteine, with its single assigned codon GUU, and tryptophan, with GGU, appear excellent candidates to act as replacements, but they have not yet been found to do so. A possible explanation for such anomalies may be that nonfunctional proteins result when certain amino acids act as replacements. Evidence in favor of such a notion is supplied by the type of defective nitrous acid-induced mutants reported by Siegel *et al.* (1962). Host plants inoculated with these mutants are capable of supporting infection but no complete virus particles are synthesized. A nonfunctional, viruslike protein which will not reconstitute with TMV RNA is found in plants infected with the PM2 type of defective mutant. This protein has peculiar properties of aggregation (Fig. 1), but otherwise it resembles TMV protein in serological properties and amino acid constitution (Zaitlin and Ferris, 1964). It is the first mutant protein, however, which has aspartic acid (or asparagine) as a replacement (Zaitlin and McCaughey, 1964). It is not known whether the presence of aspartate as a replacement is responsible for the protein being nonfunctional, because there is also an isoleucine substituted for a threonine. This later type of amino acid replacement has been observed in nondefective mutants. The defective mutants which induce the synthesis of nonfunctional protein promise to provide valuable information relative to perturbations that TMV protein may undergo and still remain functional.

Gierer (1960b) has arranged the 64 possible codons into eight octets according to changes the triplets undergo as a result of deamination. It is possible, in some cases, to place the amino acids in the different positions of the octets without violating the conclusions from either the *E. coli* ribosome data or the amino acid replacement data. This can be done most easily with Wittmann's (1962) octet #8.

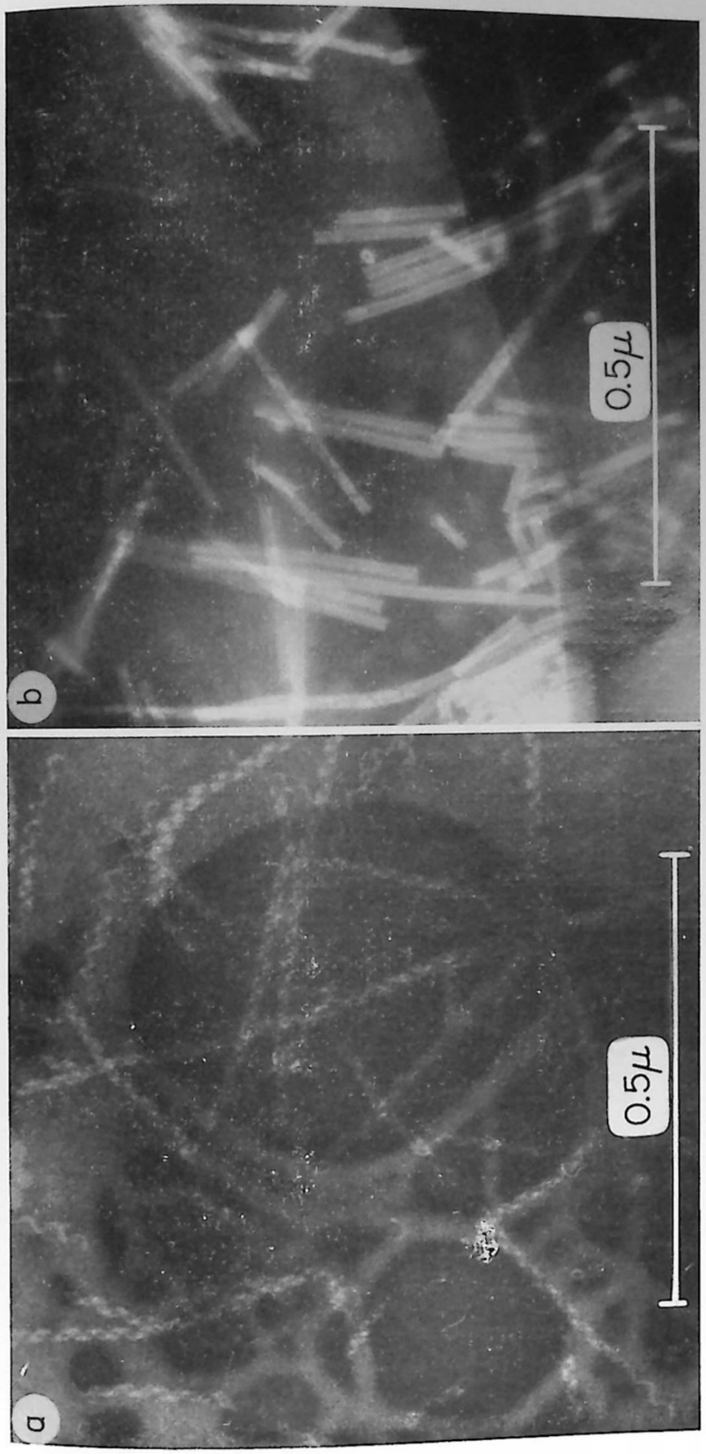
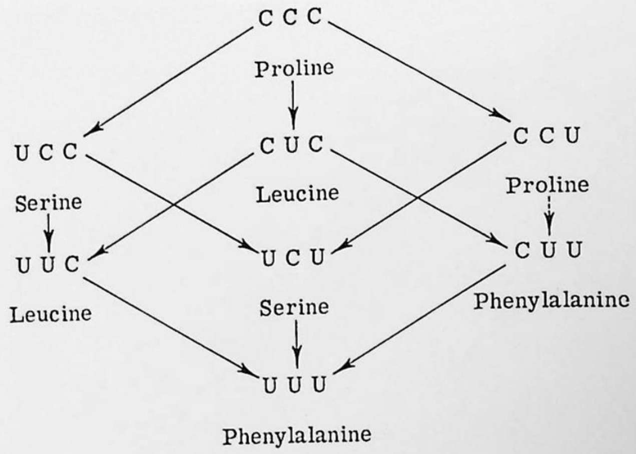


FIG. 1. Electron micrographs of (*a*) aggregated PM2 protein and (*b*) aggregated TMV A protein. Protein negatively stained with phosphotungstic acid was sprayed onto carbon-coated screens which contained holes. Magnification: × 120,000. (Zaitlin and Ferris, 1964.)



Scheme I

The amino acid positions in the octet are compatible with the available information, except that the proline → phenylalanine replacement, indicated by the dotted arrow, has not been observed. The base sequences shown for the different codons in Scheme I are those suggested by Bernfield and Nirenberg (1965).

An interesting feature of the replacement phenomenon that can be seen in octet #8 is that some deaminations will not result in amino acid replacement. For instance, when the CCC proline codon is deaminated, a CCU codon may result which also codes for proline. In the same manner, when CUU codon for phenylalanine is deaminated, the phenylalanine 3U codon is produced. In octet #8, 4 of the possible 12 deaminations result in no amino acid change. If the other 7 octets are similar in this regard, it may be concluded that about ⅓ of the nonkilling deaminations are unrecognized. This conclusion stands in seeming contrast to the notion that every deamination results in a detectable biological effect, either killing or mutation. However, the conclusion that every deamination can be recognized is based on imprecise data that can allow for a moderate level of ineffective deamination.

The above discussion is predicted on the assumption that the TMV host cell is capable of reading all codons assigned to an amino acid. This need not be so, for the host cell may not contain all transfer RNA's or enzymes that are necessary for this task. Deaminations that lead to codons that cannot be read might result in death or possibly

in the formation of defective mutants of a type which lacks viruslike protein. The type of codon which cannot be read has been termed nonsense by Benzer and Champe (1962).

The amino acid replacement data reveal that some positions of the protein are much more readily altered by nitrous acid treatment than are others. Of the 62 replacements that have so far been studied, the positions of 40 have been established in the amino acid sequence. One instance of exchange has been established for the seven positions 11, 21, 24, 55, 66, 73, and 91. Two replacements have been found at each of the nine positions 5, 20, 42, 59, 81, 97, 126, 129, and 148. Positions 25, 63, 107, 138, and 156 have been altered three times. It is probably too early to speculate on the meaning of the apparent nonrandomness of the replacement positions. Some factors which affect this type of data, however, can be mentioned. Part of the nonrandomness of the replacement data may be due to the technical problems of locating exchange positions. Another factor is that some of the positions in the amino acid sequence are occupied by amino acids whose codons consist largely of uracil and guanine, and, therefore, no amino acid replacement is to be expected upon nitrous acid treatment. It also is possible that some positions have codons which upon deamination become alternate codons for the same amino acid. Finally, the situation may exist that some portions of the nucleic acid, because of structural considerations, may be more readily deaminated than other portions.

VI. Discussion and Summary

There can be little doubt that mutagenic agents exist for TMV. The most conclusive evidence is supplied by the results of experiments in which the virus, or its nucleic acid, is treated with nitrous acid. The chemical reaction of nitrous acid with the viral nucleic acid is well known to result in the oxidative deamination of the nitrogenous bases. Adenine is converted to hypoxanthine and cytosine is converted to uracil when the intact virus is treated. In addition, guanine reacts with nitrous acid when infectious nucleic acid is treated; half of the guanine residues which react are converted to xanthine, and half are converted to an unknown product. The consequences of deaminations of the base residues are mostly inactivation and mutation, with the additional possibility that some base alterations may leave the genetic code unaltered. The deamination of any one of approximately half of the base residues results in loss of infectivity; deamination of most of the remaining residues results in detectable mutation.

The effects of mutation on one of the gene products of TMV RNA have been intensively studied. This gene product is the readily available virus protein. Two types of alterations may occur to this protein as a result of mutation. The protein may be altered in amino acid composition but still remain functional or, the protein may become nonfunctional as a result of a changed amino acid composition. The appearance of the latter class of mutants demonstrates that the viral protein is not essential to the maintenance of infection. It is questionable, therefore, whether a lethal deamination can occur in that part of the viral nucleic acid concerned with specifying the viral protein.

The type of mutation which results in an altered, but still functional, viral protein has been most intensively studied. All mutants of this class have their amino acid sequence altered in such a way that one amino acid replaces another at a particular position on the polypeptide chain. Most of the mutants of this class have a single amino acid replacement. A few mutants have two or three replacements, but these replacements have never been found to be close to each other. This information provides strong evidence for a nonoverlapping genetic code.

The nitrous acid-induced mutants do not have a random replacement of amino acids; some amino acids are much more frequently replaced than others. In like manner, not all of the amino acids act as replacements in a random fashion. The data accumulated up to the present are compatible with the notion that the amino acid replacements result from the deamination of a base so that a triplet codon for one amino acid is changed to the codon for another amino acid. The nonrandomness of the amino acids involved in replacements finds an explanation in the possibilities for deaminating bases of codons assigned to particular amino acids and to the probability that codons for other amino acids will result from a deamination. An example of the conformity of the data to the theoretical concept is illustrated by the behavior of the two amino acids proline and phenylalanine, both of which occur equally frequently in the TMV protein. Proline, whose assigned codons are rich in cytosine, has been frequently replaced and has never been a replacement. Phenylalanine, on the other hand, has codons rich in uracil, and has acted as a replacement a number of times but has never been replaced.

Forty-eight of the 64 possible triplet codons have been assigned to the 20 amino acids with the aid of the *in vitro E. coli* ribosome system. On the basis of this information, it is postulated that 6 of the 19 observed types of amino acid replacements result from a conversion of cytosine to uracil, 7 result from a conversion of adenine to guanine, one type may result from either conversion, and 5 types remain

unexplained on either basis. Several explanations have been advanced to account for these last 5 replacement types, but more information is needed to decide whether any of them are valid. The necessity of invoking the deamination of adenine to account for some of the replacements raises the possibility that a double-stranded stage exists during the replication of TMV. This is so because hypoxanthine, the deamination product of adenine, does not normally occur in nucleic acid. It does have, however, the base-pairing properties of guanine, and it is likely that hypoxanthine will be replaced by guanine if RNA replication involves a complementary strand.

Only those amino acid replacements which result from nitrous acid treatment have been subjected to intensive study. It is possible that the investigation of replacements which result from other mutagenic agents will also provide useful information. This is likely to be so especially for those mutagenic agents whose chemical reactions with TMV RNA are well known. Hydroxylamine appears to be an excellent candidate in this regard. It is mutagenic under particular reaction conditions, and its primary action is with cytosine, frequently converting it to uracil. This agent, therefore, should induce only those amino acid replacements which have been induced with nitrous acid and which have been attributed to a conversion of cytosine to uracil. It will be interesting to see whether this prediction turns out to be true.

Hydroxylamine reacts with TMV RNA only when the RNA is free, and not when it is part of an intact virus particle. Because of this feature, it might be possible to initiate mapping of the TMV genome. The suggested manner in which this may be accomplished is to react hydroxylamine with the ends of TMV RNA molecules which have been freed from the viral protein. Methods for stripping protein subunits from the ends of virus particles have been described (Hart, 1955, 1956). The results of such experimentation may reveal whether the viral protein cistron is closer to an end than to the middle of the RNA molecule (Schuster and Wittmann, 1963). Other mutagens may prove to be useful for describing the TMV genome as more becomes known about their chemistry of reaction with nucleic acid. Those that appear to be promising are dimethyl sulfate, which reacts with guanine residues, and *N*-bromosuccinamide.

The intensive work performed during the past few years on the induction and analysis of TMV mutants has yielded valuable knowledge about the relationship between viral nucleic acid and viral protein. The study of TMV mutation induction is proceeding apace, and it is to be expected that more biologically significant information will be yielded by this system in the next few years.

ACKNOWLEDGMENT

The author wishes to acknowledge assistance from the National Science Foundation, The American Cancer Society, and Atomic Energy Commission Contract AT(11-1)-873.

REFERENCES

Aach, H. G., Funatsu, G., Nirenberg, M. W., and Fraenkel-Conrat, H. (1964). *Biochemistry* **3**, 1362.
Anderer, F. A. (1963). *Advan. Protein Chem.* **18**, 1–35.
Anderer, F. A., and Handschuh, D. (1962). *Z. Naturforsch.* **17b**, 536.
Anderer, F. A., Uhlig, H., Weber, E., and Schramm, G. (1960). *Nature* **196**, 922.
Baltimore, D., and Franklin, R. M. (1963). *Biochem. Biophys. Res. Commun.* **9**, 388.
Bautz-Freese, E., and Freese, E. (1961). *Virology* **13**, 19.
Bawden, F. C. (1950). "Plant Viruses and Virus Diseases." *Chronica Botanica*, Waltham, Massachusetts.
Bawden, F. C. (1959). *Nature* **184**, B.A. 27–29.
Benzer, S., and Champe, S. P. (1962). *Proc. Natl. Acad. Sci. U.S.* **48**, 1114.
Bernfield, M. R., and Nirenberg, M. W. (1965). *Science* **147**, 479.
Burdon, R. H., Billeter, M. A., Weissmann, C., Warner, R. C., Ochoa, S., and Knight, C. A. (1964). *Proc. Natl. Acad. Sci. U.S.* **52**, 768.
Caspar, D. L. D. (1963). *Advan. Protein Chem.* **18**, 37–121.
Cohen, M., Siegel, A., Zaitlin, M., Hudson, W. R., and Wildman, S. G. (1957). *Phytopathology* **47**, 694.
Crick, F. H. C. (1963). *Progr. Nucleic Acid Res.* **1**, 163.
Crick, F. H. C., Barnett, L., Brenner, S., and Watts-Tobin, R. J. (1961). *Nature* **192**, 1227.
Dahl, D., and Knight, C. A. (1963). *Virology* **21**, 580.
Fraenkel-Conrat, H. (1961). *Biochim. Biophys. Acta* **49**, 169.
Fraenkel-Conrat, H., and Williams, R. C. (1955). *Proc. Natl. Acad. Sci. U.S.* **41**, 690.
Funatsu, G., and Fraenkel-Conrat, H. (1964). *Biochemistry* **3**, 1356.
Gierer, A. (1957). *Nature* **179**, 1297.
Gierer, A. (1960a). *Progr. Biophys. Biophys. Chem.* **10**, 229.
Gierer, A. (1960b). Cited by Wittmann, H. G. (1963). *Proc. Intern. Congr. Biochem., 5th, Moscow*, 1961, Vol. 1, p. 240. Macmillan (Pergamon), New York.
Gierer, A., and Mundry, K. W. (1958). *Nature* **182**, 1457.
Gierer, A., and Schramm, G. (1956). *Z. Naturforsch.* **11b**, 138.
Ginoza, W. (1958). *Nature* **181**, 958.
Gordon, M. P., and Staehelin, M. (1958). *J. Am. Chem. Soc.* **80**, 2340.
Gowen, J. W. (1941). *Cold Spring Harbor Symp. Quant. Biol.* **9**, 187.
Hart, R. G. (1955). *Virology* **1**, 402.
Hart, R. G. (1956). *Biochim. Biophys. Acta* **20**, 388.
Haruna, I., Nozu, K., Ohtaka, Y., and Spiegelman, S. (1963). *Proc. Natl. Acad. Sci. U.S.* **50**, 905.
Hayashi, M., Hayashi, M. N., and Spiegelman, S. (1963). *Proc. Natl. Acad. Sci. U.S.* **50**, 664.
Holmes, F. O. (1934). *Phytopathology* **24**, 845.

Holoubek, V. (1963). *J. Mol. Biol.* **6**, 164.
Jensen, J. H. (1933). *Phytopathology* **23**, 964.
Johnson, J. (1947). *Phytopathology* **37**, 822.
Kassanis, B. (1957). *Virology* **4**, 187.
Kausche, G. A., and Stubbe, H. (1939). *Naturwissenschaften* **29**, 501.
Kausche, G. A., and Stubbe, H. (1940). *Naturwissenschaften* **28**, 824.
Kleczkowski, A. (1963). *Biol. Rev. Cambridge Phil. Soc.* **38**, 364.
Knight, C. A., (1959). *In* "The Viruses" (F. M. Burnet and W. M. Stanley, eds.), Vol. 2, p. 127. Academic Press, New York.
Kramer, G., Wittmann, H. G., and Schuster, H. (1964). *Z. Naturforsch.* **19b**, 46.
Kunkel, L. O. (1940). *Publ. Am. Assoc. Advan. Sci.* **12**, 22.
Kunkel, L. O. (1947). *Ann. Rev. Microbiol.* **1**, 85.
McKinney, H. H. (1926). *Phytopathology* **16**, 893.
Mandel, H. G., Matthews, R. E. F., Matus, A., and Ralph, R. K. (1964). *Biochem. Biophys. Res. Commun.* **16**, 604.
Miller, P. M. (1953). *Phytopathology* **43**, 80.
Mundry, K. W. (1957a). *Z. Induktive Abstammungs-Vererbungslehre* **88**, 407.
Mundry, K. W. (1957b). *Z. Induktive Abstammungs-Vererbungslehre* **88**, 115.
Mundry, K. W. (1959). *Virology* **9**, 722.
Mundry, K. W. (1963). *Ann. Rev. Phytopathol* **1**, 173.
Mundry, K. W., and Gierer, A. (1958). *Z. Vererbungslehre* **89**, 614.
Nirenberg, M. W., and Matthaei, J. H. (1961). *Proc. Natl. Acad. Sci. U.S.* **47**, 1588.
Nirenberg, M. W., Matthaei, J. H., and Jones, O. W. (1962). *Proc. Natl. Acad. Sci. U. S.* **48**, 104.
Pfankuch, E., Kausche, G. A., and Stubbe, H. (1940). *Biochem. Z.* **304**, 238.
Rappaport, I., and Wildman, S. G. (1957). *Virology* **4**, 265.
Rappaport, I., and Wildman, S. G. (1962). *Nature* **195**, 1029.
Rappaport, I., and Wu, J.-H. (1962). *Virology* **17**, 411.
Sadasivan, T. S. (1940). *Ann. Appl. Biol.* **27**, 359.
Schuster, H. (1961). *J. Mol. Biol.* **3**, 447.
Schuster, H., and Schramm, G. (1958). *Z. Naturforsch.* **13b**, 697.
Schuster, H., and Wilhelm, R. C. (1963). *Biochim. Biophys. Acta* **68**, 554.
Schuster, H., and Wittmann, H. G. (1963). *Virology* **19**, 421.
Shipp, W., and Haselkorn, R. (1964). *Proc. Natl. Acad. Sci. U.S.* **52**, 401.
Siegel, A. (1959). *Virology* **8**, 470.
Siegel, A. (1960). *Virology* **11**, 156.
Siegel, A. (1961). *Virology* **15**, 212.
Siegel, A., and Wildman, S. G. (1960). *Ann. Rev. Plant Physiol.* **11**, 277.
Siegel, A., Zaitlin, M., and Sehgal, O. P. (1962). *Proc. Natl. Acad. Sci. U.S.* **48**, 1845.
Spackman, B. H., Stein, W. H., and Moore, S. (1958). *Anal. Chem.* **30**, 1190.
Sugiyama, T., and Fraenkel-Conrat, H. (1963). *Biochim. Biophys. Acta* **72**, 524.
Sukhov, K. S. (1956). Cited by Kassanis (1957).
Takahashi, W. N. (1956). *Phytopathology* **46**, 654.
Thiry, L. (1963). *Virology* **19**, 225.
Tsugita, A., and Fraenkel-Conrat, H. (1960). *Proc. Natl. Acad. Sci. U.S.* **46**, 636.
Tsugita, A., and Fraenkel-Conrat, H. (1962a). *J. Mol. Biol.* **5**, 284.
Tsugita, A., and Fraenkel-Conrat, H. (1962b). *J. Mol. Biol.* **4**, 73.

Tsugita, A., and Fraenkel-Conrat, H. (1963). *In* "Molecular Genetics" (J. H. Taylor, ed.), p. 477. Academic Press, New York.

Tsugita, A., Gish, D. T., Young, J., Fraenkel-Conrat, H., Knight, C. A., and Stanley, W. M. (1960). *Proc. Natl. Acad. Sci. U. S.* **46**, 1463.

Tsugita, A., Fraenkel-Conrat, H., Nirenberg, M. W., and Matthaei, J. H. (1962). *Proc. Natl. Acad. Sci. U. S.* **48**, 846.

Veldee, S., and Fraenkel-Conrat, H. (1962). *Virology* **18**, 56.

Wahba, A. J., Gardner, R. S., Basilio, C., Miller, R. S., Speyer, J. F., and Lengyel, P. (1963a). *Proc. Natl. Acad. Sci. U.S.* **49**, 116.

Wahba, A. J., Miller, R. S., Basilio, C., Gardner, R. S., Lengyel, P., and Speyer, J. F. (1963b). *Proc. Natl. Acad. Sci. U.S.* **49**, 880.

Weissmann, C., Simon, L., and Ochoa, S. (1963). *Proc. Natl. Acad. Sci. U.S.* **49**, 407.

Wildman, S. (1959). *In* "The Viruses" (F. M. Burnet and W. M. Stanley, eds.), Vol. 2, p. 1. Academic Press, New York.

Wittmann, H. G. (1960). *Virology* **12**, 609.

Wittmann, H. G. (1962). *Z. Vererbungslehre* **93**, 491.

Wittmann, H. G., and Braunitzer, G. (1959). *Virology* **9**, 726.

Wittmann, H. G., and Wittmann-Liebold, G. (1963). *Cold Spring Harbor Symp. Quant. Biol.* **28**, 589.

Wu, J.-H., Hildebrandt, A. C., and Riker, A. J. (1960). *Phytopathology* **50**, 587.

Zaitlin, M., and Ferris, W. (1964). *Science* **143**, 1451.

Zaitlin, M., and McCaughey, W. F. (1964). Personal communication.

PLANT VIRUS-VECTOR RELATIONSHIPS

Kenneth M. Smith*

Department of Biophysics, University of Pittsburgh, Pittsburgh, Pennsylvania†

I. Introduction 61
II. Mechanical Vectors 62
III. The Vector Relationships of Tobacco Mosaic Virus 64
IV. The Problem of Aphid-Virus Relationships 67
  A. Stylet-Borne Viruses 68
  B. Circulative Viruses 71
  C. Propagative Viruses 72
  D. Variation in Transmission of a Single Virus by a Single Aphid Species. 74
  E. Variation in Transmission of a Single Virus by Different Aphid Species. 75
  F. "Helper" Viruses and Aphid Transmission 76
  G. Artificial Feeding and Virus Injection of Aphids 78
V. Biological Transmission 79
VI. Vectors Outside the Insecta 87
  A. Mites (Eryiophyidae) 87
  B. Eelworms (Nematoda) 88
  C. Fungi [*Olpidium brassicae* (Wor.) Dang.] 90
VII. Discussion 91
  References 93

I. Introduction

Not many years ago the title of this chapter would, perforce, have been "Plant Virus-Insect Vector Relationships" chiefly because of an apparent unwillingness on the part of plant virologists to recognize any other type of organism as a potential vector of plant viruses. No doubt this attitude of mind was responsible for the slow start of investigations into possible kinds of vectors other than insects, just as the axiom that plant viruses were not soil-transmitted delayed research into that aspect of virus spread.

Actually there was precedent for the search for noninsect vectors in an early paper which suggested a phytophagous mite as the vector of "reversion" in the black currant (Amos *et al.*, 1927); it was not till many years later that this was shown to be the case (Massee, 1952). The discovery by Hewitt *et al.* (1958) that an eelworm trans-

* Work was supported by U.S. Public Health Service Grant No. G.M. 10403 and by the National Science Foundation G-24046.

† Present address: Cell Research Institute, Department of Botany, University of Texas, Austin, Texas.

mitted the fanleaf disease of the grapevine probably instigated the present-day intensive research into the long-neglected field of soil transmission. Similarly the incrimination of another mite as the vector of wheat streak mosaic virus (Slykhuis, 1953, 1955) suggested that mites might be concerned in the spread of other plant viruses.

Now the search has widened and vectors have been discovered outside the animal kingdom altogether, and certain root-infecting fungi, long suspected, have been confirmed as vectors of some plant viruses. In addition, then, to biting and sucking insects, the list of vectors includes mites, eelworms, and fungi.

It will not be possible in a single review to deal adequately with all the minutiae of the relationships between plant viruses and their vectors, but it is hoped to give a fairly comprehensive account of the main factors in these relationships.

II. Mechanical Vectors

Mechanical transmission of plant viruses in this context refers to a mode of transfer in which there is no evidence of any biological relationship between virus and vector. The question as to whether some aphid-borne viruses are mechanically transmitted is discussed in the section dealing with aphid-virus relationships and, with one exception, this section is concerned with virus transmission by insects with biting mouth-parts. It is, of course, fairly obvious that any virus which is transmitted mechanically by an insect is also easily sap-transmissible; the four viruses mentioned here all fall into that category.

Perhaps the best example of a purely passive transfer of a plant virus is given by that of tobacco mosaic (TMV); Walters (1952) showed that the large grasshopper, *Melanoplus existientialis,* would carry the virus on its jaws and infect a healthy tobacco plant after first feeding on a mosaic-infected plant. The writer has also observed a certain amount of virus transfer on the feet of grasshoppers which have been allowed to wander, without necessarily feeding, on a mosaic-diseased tobacco plant and then transferred to plants of *Nicotiana glutinosa.* This plant reacts to infection by TMV with the production of localized foci of infection, and a few of these local lesions developed where the insects had placed their feet. Walters also demonstrated that the grasshopper would transmit potato virus X (PVX) by contamination of its jaws. Some experiments with the same virus were carried out by the writer using the potato flea beetle (*Psylliodes* sp.) as a possible vector; the results of these experiments were all negative. Schmutterer (1961) showed that TMV can be carried for a short period only on the mouth parts, but not the legs, of two beetles, *Tettigonia viridissima* and *T. cantans,* and of a caterpillar, *Barathra brassicae.* The virus appeared to be inactivated in less than an hour by the saliva, and cleaning the mouth parts of *T. viridissima* with KCN increased the infection rate. Heating to 65°C. reversed the inactivation of TMV by salivary gland or intestine homogenate of *T. viridissima.* Exposure to higher temperatures increased the strong virus inactivation by the blood which, however, still contained traces of active TMV after long feeding periods. Schmutterer considers that virus inactivation is probably due to enzymes such as amylase. It is probable that the most important vector, in the field, of TMV and potato virus X is man himself by means of the contamination of his hands and implements.

Two good examples of the purely passive transfer of animal viruses by insects are the spread of fowl-pox virus on the proboscis of a blood-sucking fly and of the rabbit myxoma virus on the mouth parts of the mosquito, called by Fenner the "flying pin." We can find a parallel to this in the plant viruses; Costa *et al.* (1958) demonstrated the transmission of the virus of sowbane mosaic (SbMV) on the ovipositor of a fly (*Liriomyza langei* Frick) during the process of depositing its eggs in the epidermis of leaves in which the larvae subsequently live as "leaf miners."

The methods of mechanical transmission dealt with, so far, have been concerned only with external contamination; there are, however, other examples of mechanical transmission in which more than this is involved. These are viruses of which the natural vector is a beetle, an insect with biting mouth parts; a good example is the virus of turnip yellow mosaic (TYM), which is spread in the field by one or more species of flea beetle (*Phyllotreta* sp.). In the discussion on the transmission of plant viruses by aphids and other hemipterous insects it will be shown that the saliva plays an important part in that process; it is interesting therefore to find that the absence of saliva in biting insects is the essential fact in virus transmission. Although the turnip flea beetle is the natural vector of TYM virus it can be transmitted by any type of beetle which will feed on the necessary susceptible plants. The explanation seems to be as follows, since beetles have no salivary glands it is necessary for them to regurgitate part of the contents of the foregut while eating; this apparently helps in the process of digestion. Regurgitation brings into contact with the leaf any infective tissue previously eaten and this, during the process of mastication, is inoculated to a healthy susceptible plant. Transmission of this type is not confined to beetles, so far as TYM virus is concerned, and it can be spread by grasshoppers and earwigs (Forficulidae) which feed in a similar manner. The virus, however, is not spread by caterpillars, which are also biting insects, but which do not regurgitate whilst feeding. Transmitting beetles or their larvae can retain infective power for several days without further access to a source of virus; this is thought to be due merely to the length of time infective tissue remains in the fore-gut. When this is digested the insect must feed once more on an infected plant before it can again transmit the virus.

III. The Vector-Relationships of Tobacco Mosaic Virus

It has long been a puzzle to plant virologists that tobacco mosaic virus (TMV), the most infectious plant virus known, has no efficient vector other than man himself. We have already considered in Section I the purely mechanical transmission of TMV by biting insects, such as grasshoppers and beetles (Walters, 1952), so that aspect of the subject will not be referred to again, except incidentally.

The literature contains many references to apparently successful transmission of TMV by different species of insects, mainly belonging to the Hemiptera, but attempts to repeat these transmissions have invariably failed.

The earliest record seems to be the report of Allard (1914, 1917) that a virus, which he believed to be TMV, was transmitted by an aphid. It is possible, in such early days, that Allard mistook for TMV a strain of cucumber mosaic virus (CMV) which, of course, is easily transmitted by aphids; there are several strains of CMV which, from the symptoms produced on the tobacco plant, might be confused with TMV.

It is more difficult, however, to explain the successful results of Hoggan (1931, 1934), who carried out an extensive investigation on the transmission of TMV by aphids. According to this work, four species of aphids transmitted the virus from tomato to tobacco and various other solanaceous hosts, but not, apparently, from tobacco to tobacco. The aphids used were, *Aulacorthum solani* (Kalt.) (= *Myzus pseudosolani* Theob.), *Macrosiphum euphorbiae* (Thomas [= *M solanifolii* (Ashmead)], *A. circumflexus* (Buckt) [= *Myzus circumflexus* (Buckt)] and *Myzus persicae* (Sulz). Of these, *M. persicae* was the least efficient vector. Hoggan used large numbers of aphids, about 300 per plant; they were allowed to feed on the infected plants for 2–5 days and were then transferred on detached leaves, placed on pieces of paper near the plants to be colonized. In another experiment, Hoggan (1934) transferred the aphids from the TMV-infected plants to hosts which react only with local lesions. One lesion was produced by *each* 129 *A. solani*, 140 *M. euphorbiae*, and 812 *M. persicae*.

There are several other recorded instances of apparently successful

transmission of TMV by aphids and other sap-sucking insects. Cornuet and Morand (1960a,b) describe a strain of this virus which affects *Fragaria vesca* L. In subsequent experiments Cornuet and Morand claim to have transmitted this strain of TMV, by means of the aphid *Pentatrichopus fraegaefolii* Cock, not only to strawberry plants but also from tomato and tobacco to tobacco.

Early claims to have transmitted TMV by mealybugs were made by Olitsky (1925) and Elmer (1925), using *Pseudococcus citri* (Risso) and *P. maritimus* Ehr., respectively. Newton (1953), using *P. citri*, obtained 100% transmission with 4 insects on each of 5 tobacco plants, and on a local lesion host 1–3 lesions developed after the feeding of 10 mealybugs.

Orlob (1963), who has made a reappraisal of the transmission of TMV by insects, repeated all these apparently successful experiments with uniformly negative results. In Table I is reproduced Orlob's table in which the results of previous authors' work are compared with those of his experiments.

TABLE I

Results of Transmission of Tobacco Mosaic Virus by Sucking Insects Obtained by Various Authors, Compared with Results of Present Work[a]

Experiment no.	Author	Virus source	Test plant	Vector	No. of insects/test plant or leaf	Results of author	Results of present work
1	Hoggan	*Lycopersicon esculentum*	*Nicotiana tabacum*	*Macrosiphum euphorbiae*	300	30/50[b]	0/12[b]
				Aulacorthum solani	300	39/50[b]	0/12[b]
2	Hoggan	*L. pimpinellifolium*	*N. glutinosa*	*M. euphorbiae*	?	27/3775[c]	0/785[c]
3	Cornuet & Morand	*Fragaria vesca*	*F. vesca*	*Pentatrichopus fragaefolii*	15	+[d](4/4[b]?)	0/20[b]
4	Cornuet & Morand	*N. tabacum*	*N. tabacum*	*P. fragaefolii*	20	+(1/2[b]?)	0/20[b]
5	Olitsky	*N. tabacum*	*N. tabacum*	*Pseudococcus citri*	5	3/5[b]	0/8[b]
6	Olitsky	*N. tabacum*	*N. glutinosa*	*P. citri*	10	1–3/10[c]	0/20[c]
7	Newton	*N. tabacum*	*N. tabacum*	*P. citri*	4	–5/5[b]	0/8[b]

[a] After Orlob (1963).
[b] Number of test plants infected/number colonized.
[c] Number of local lesions/total number of insects used.
[d] + = Positive result.

Brčák (1959) also was unable to transmit TMV with 300 *P. maritimus* transferred to 71 test plants or with 500 whiteflies (Aleyrodidae).

It has been suggested that aphids cannot transmit TMV because they do not imbibe the virus. Sukhov (1944) thought that the salivary sheath surrounding the aphid puncture in the tissue acted as a filter and prevented the uptake of virus. Soest and de Meesters-Manger Cats (1956) were unable to detect the presence of TMV in the droplets exuding through stylets, cut off but left *in situ*, by means of the electron microscope or by serological means, and they also concluded that the aphid *Myzus persicae* did not take up TMV from an infected plant. However, it has now been proved that both aphids and leafhoppers do in fact imbibe this virus. Kikumoto and Matsui (1962) demonstrated the presence of TMV rods in the midguts of aphids which had fed on infected plants (see also Ossiannilsson, 1958).

Several workers have shown that TMV can pass through the gut of biting insects and can be recovered from the feces Brčák, 1957; Walters, 1952. The writer (Smith, 1941) was unable to transmit TMV by means of several species of caterpillars but viable virus was easily recoverable from the feces. Orlob (1963) was also able to recover virus from the feces of the potato flea beetle, *Epitrix cucumeris* Harr.; in addition he found this beetle could act as an inefficient mechanical vector, the virus only being transmitted if the insect scraped the epidermis of the leaf without ingesting the tissue.

What then can be the reason for the nontransmission of TMV by hemipterous insects, especially aphids? In the next section we shall discuss a type of mechanical transmission by aphids in which the virus adheres to the distal end of the stylets. Why is the very infectious TMV not transmitted in a similar manner? It cannot be a question of the shape of the virus particle; *Hyoscyamus* mosaic virus and potato virus Y (PVY), both rod shaped, are easily aphid-transmitted but TMV and potato virus X (PVX) also with rod-shaped particles, are not so transmitted.

Orlob (1963) has carried out a number of experiments to investigate the relationships between the aphid's stylets and TMV. Wetting the stylets of aphids or of other sap-sucking insects and the mouth parts of the potato flea beetle with TMV or with TMV nucleic acid failed to make any insects viruliferous with the exception of the last-named. Virus could be recovered from the bared stylets but not after the labium had extended down over them.

It has now been proved that TMV is imbibed by aphids and other sucking insects and it can be demonstrated inside the mid-gut of aphids (Kikumoto and Matsui 1962). What then prevents the virus from passing out of the gut and reaching the saliva and thus being returned to the plant? Although the presence of the two rod-shaped viruses of *Hyoscyamus* mosaic and PVY, which are aphid-transmitted, has not been demonstrated in the mid-gut of aphids, they can be legitimately assumed to be there. However, this is not necessary for the transmission of these two viruses, since they adhere to the stylets and are transmitted as soon as the stylets enter a susceptible cell. We have, then, apparently comparable aphid relationships of TMV, *Hyoscyamus* mosaic virus, and PVY, namely, presence of the virus in the gut and adherence to the stylets, but there is still some factor preventing the transmission of TMV. Can the explanation be a purely quantitative one? Orlob has shown that there is very little virus left on the stylets once the labium has extended over them. Yet this must apply in an even greater degree to the other two viruses which are aphid-transmitted. In the present state of our knowledge of the subject we can only assume that aphids do not transmit TMV because not enough virus adheres to the stylets to make an infective dose. There is some evidence in support of this in the fact that some biting insects easily transmit the virus provided there is gross contamination of their jaws.

IV. The Problem of Aphid-Virus Relationships

In this section we can visualize the gradual development from the purely passive transfer of virus to more intimate relationships between insect vector and plant virus.

The problem of aphid-virus relationships is no simple one, and, in spite of many ingenious experiments and much theory, there is still considerable confusion of thought. Perhaps the best way to present the problem will be to state the many facts which have been discovered and then to discuss the various explanations which have been offered.

The pioneers in this field were Watson (1936, 1938, 1946) and Watson and Roberts (1939). Using the virus of *Hyoscyamus* mosaic, they confirmed earlier observations which had been made on the aphid transmission of cucumber mosaic virus and potato virus Y, namely, that some viruses were rapidly lost by the aphid vector. Aphid-transmitted viruses were divided into two categories, the *nonpersistent* (or external) which were usually lost by the aphid after feeding on one or two plants, and the *persistent* (or internal) which were retained by the aphid for long periods.

It was recognized, however, that there was no hard and fast dividing line and that these two categories tended to merge into each other.

Here we may digress for a moment to discuss the nomenclature of these different types of aphid-virus relationships. A third category was added, the *semipersistent* viruses, to include those cases in which retention of virus by the aphid is relatively brief but is, nevertheless considerably longer than in the case of the nonpersistent viruses.

Just recently Kennedy *et al.* (1962) have introduced terms which are less empirical and which give some indication of the location of, and route followed by, the virus in the insect. For nonpersistent viruses the term *stylet-borne* is suggested; persistent viruses become *circulative* and those viruses which have a definite biological relationship with their vectors are known as *propagative*. This type of virus is more usually associated with leafhopper vectors, but, as we shall see later, there is evidence that they are also associated with aphids. That leaves the semipersistent viruses unaccounted for, and it seems a matter of choice as to which category they should be assigned; thus Maramorosch (1963) designates them as stylet-borne, and Sylvester (1962), for convenience, and for lack of more precise knowledge, includes them in the circulative viruses.

These terms suggested by Kennedy *et al.* seem likely to be adopted by plant virologists and they will be used throughout this review.

We shall now return to the discussion of these three types of aphid-virus relationships, giving the facts known about each and the various explanations put forward concerning them.

A. *Stylet-Borne Viruses*

The following facts about the stylet-borne viruses have been elucidated by Watson and her co-workers.

1. A preliminary fasting period by the aphid greatly increases the capacity to transmit virus.
2. A short feed (acquisition feed) is more effective than a long period of feeding, and aphids making many penetrations retained infectivity longer than those making prolonged single penetrations.
3. Prolonged feeding reduces the efficiency of virus transmission.

In discussing the various theories put forward in explanation of these facts, it must be borne in mind that there is another phenomenon in connection with the stylet-borne viruses: that is the question of aphid specificity. In other words, one species of aphid can transmit a particular virus while another apparently similar species cannot do so.

Some rather complicated, but not very precise, theories have been put forward by various workers to account for these phenomena, and to explain the question of aphid specificity. Watson and Roberts (1939) were the first to suggest an "inactivator-behavior theory" and this was followed by Day and Irzykiewicz (1954) who introduced a *mechanical-inactivator-behavior* hypothesis, which suggested contamination of the stylets together with selective inactivation of virus on the stylets by fasting labile components of the saliva. Differences in behavior of the different aphid species might help to account for selective transmission. As we shall see later, the behavior of the aphids themselves plays an important role in virus transmission.

Van der Want (1954) suggested a *mechanical-surface adherence* hypothesis; here emphasis is laid on surface differences of the stylets of various aphid species, so that specificity is tied to differential adsorption to, and elution of the virus from, the stylets. In this connection it should be mentioned that van Hoof (1957) found differences in structure of the stylets of various aphids. Low power electron micrographs revealed the presence of different excrescences, pockets, or ridges to which virus might be expected to adhere.

Sylvester (1954) proposed a *mechanical-inactivator-compatibility* hypothesis in which specificity depended upon the compatibility of the combination of the virus, saliva, and host cell inoculated; the actual transmission being effected by virus carried in or on the stylets.

The idea of a purely mechanical process was reintroduced by Bradley (1952), and later (1959) he emphasized the role of behavior in at least some of the transmission phenomena. This was an important observation, and the relevance of aphid behavior and the light it throws on some of the rather peculiar facts stated above, will now be discussed. It was noticed that after a fasting period the aphid tended to make a number of very short penetrations of the leaf, mainly in the epidermis where many of the stylet-borne viruses are located. Furthermore, saliva was not secreted and virus contamination occurred during these short penetrations.

Bradley also observed that many aphids when removed from a feeding position have the stylets protruding beyond the tip of the rostrum. It is not possible for these thin and delicate stylets to pierce the leaf epidermis unsupported; the aphid must therefore wait until the rostrum is again extended down over them. The behavior of the aphid then offers two reasons why a preliminary starving period enhances virus transmission. First, it allows time for the extended stylets to be restored to their proper position for leaf penetration; and second, it encourages numerous short stabs into the epidermis which appear to offer optimum conditions for transmission.

Another of the observations made by Watson was that the longer the aphid fed on the diseased plant the less efficient it became as a vector. This may be partly explained by the fact that the stylets penetrate, during prolonged feeding, into deeper tissue where the stylet-borne viruses are not located. Sylvester (1962), who has made a careful study of the feeding habits of aphids, has suggested what may be called an *infective salivary* plug concept of transmission, as an additional reason for this phenomenon. When a fasted aphid lowers its rostrum to the leaf epidermis, saliva begins to flow. The stylets begin to penetrate the cuticle and the stylet movements mold the salivary liquid

FIG. 1. Section through potato leaf showing stylet track of the aphid *Myzus persicae* Sulz. Note that the stylets have penetrated intercellularly. Magnification: × 1200.

into a gelatinous tube which eventually congeals to surround and support the flexible stylets (Fig. 1). Sylvester noticed that during brief penetrations of 15 to 20 seconds the salivary sheath was incomplete, and examination of the stylets, after such a brief penetration, revealed a plug of saliva clinging to the tips of the stylets which is brought

into contact with the tip of the labium when the latter ensheathes the stylets. Thus virus-contaminated material will be smeared over the tip of the rostrum and the stylets and inoculated into the cell at the next penetration. On the other hand, prolonged feeding tends to eliminate transmission as the stylets would have to be drawn through the long close-fitting tube shown in Fig. 1 and this would tend to remove any virus-contaminated material adhering to them. It is relevant here to draw attention to some experiments by Bradley (1956), who showed that penetration through a thin membrane, stretched over the leaf surface, reduced the efficiency of both acquisition and inoculation probes.

To sum up, then, we can partially explain the transmission of the stylet-borne viruses by mechanical adherence of the virus to about 15 μ of the distal end of the stylets. Most of the other phenomena associated with this type of virus transmission are explainable in terms of aphid behavior. The problem of aphid specificity, however, is still not satisfactorily solved.

B. Circulative Viruses

As we have mentioned earlier, there is lack of precise knowledge concerning the semipersistent type of viruses. Some workers consider they are stylet borne while others put them in the category of circulative viruses, in which they are included here for convenience. One factor in deciding whether a semipersistent virus belonged in the stylet-borne or circulative groups would be its retention through a molt. When molting occurs, the interior linings of the fore- and hind-gut are shed along with the stylets and skin of the insect. Hence, any virus transmitted after a molt, provided the aphid had no access to a further source of virus, must have been present in the mid-gut. However, so far as the semipersistent virus of sugarbeet yellows is concerned, repeated attempts to test for retention of this virus after molting have been unsuccessful (Day, 1955; Sylvester, 1962). Some recent work by Nault *et al.* (1964) shows that the circulative virus of pea enation mosaic (PEMV) is retained by the aphid vector after molting. Virus was retained by 16 aphids through at least one molt; 11 of 16 aphids transmitted virus after 3 molts. Some aphids were injected with hemolymph from other aphids which had been reared on infected pea plants, and in 3 out of 4 experiments the injected aphids transmitted PEMV to healthy plants. Nymphs were more efficient than adults in transmitting the virus during short probes, and the fact that the virus can be spread by short probes suggests that, unlike most circulative aphid-borne viruses, it may not be necessary for PEMV to be inoculated into the phloem of the susceptible host. Although it cannot definitely be said that PEMV multiplies in the aphid vector, this work demonstrates a biological relationship of some kind between virus and vector.

The chief differences between the true stylet-borne (nonpersistent) and the semipersistent viruses are the longer periods of retention by the aphid of the semipersistent virus, and the facts that preliminary starving does not influence subsequent transmission, while prolonged initial feeding increases rather than decreases the likelihood of virus transmission.

With most of the circulative viruses (persistent) there is a delay in the development of the infective power within the insect. In other words, the aphid does not become capable of transmitting the virus until a certain period has elapsed after acquisition of the virus. This latent period varies considerably from a few hours to several days; those which have the longest latent period are strawberry virus 3 (Prentice and Woollcombe, 1951) and sow thistle yellow vein virus (Duffus, 1963). In the first case this varies from 10–19 days, and in the second 8–46 days according to the temperature.

Other circulative viruses are those of potato leaf roll (Smith, 1931a; Day, 1955), pea enation mosaic (Osborn, 1935, Chaudhuri, 1950; Simons, 1954), barley yellow dwarf (Toko and Bruehl, 1959) and radish yellows (Duffus, 1960). The two components causing the rosette complex disease of tobacco, the vein-distorting and mottle viruses, are also both circulative; the aphid vector *Myzus persicae* retains these for a period of 30 days and probably for the remainder of its life (Smith, 1946).

C. *Propagative Viruses*

In the discussion on the relationships of plant viruses with leafhopper vectors convincing evidence that certain viruses do multiply in these insects will be presented. The situation as regards propagative viruses in aphids is less clear-cut, but there are two instances where the evidence for virus multiplication in the aphid is fairly convincing.

The first of these concerns the virus of potato leaf roll and the aphid vector *Myzus persicae*. It was with this virus that the serial inoculation technique, long used with leafhoppers, was first applied to aphids.

It was first shown by Smith (1931a) that the aphid *M. persicae* retained infectivity with the leaf roll virus, even after feeding on an immune plant, such as cabbage, for as long as 7 days. In 1955 Day also presented evidence which suggested the possibility that the potato leaf roll virus might multiply in the aphid, *M. persicae*. In the same year Heinze (1955) carried out experiments on the inoculation of aphids

with plant extracts which opened the way to the subsequent work carried out by Stegwee and Ponsen (1958). Aphids were injected with aphid hemolymph and not with plant extracts which tended to be toxic. It was found that after injection with hemolymph from virus-bearing (viruliferous) aphids, about 50% of the injected aphids became infective after an average incubation period of 20 hours. When the hemolymph was diluted with saline the incubation period was extended to 7 or 10 days. Once infected with the potato leaf roll virus by injection the aphids retained their infectivity. Serial transmissions were then carried out as follows: Viruliferous aphids were colonized on a virus-immune plant such as Chinese cabbage for 7 days, hemolymph from these aphids was then injected into a series of known virus-free aphids. After 7 days the process was repeated, and this was carried out 15 times. At each passage the presence of the leaf roll virus was demonstrated by feeding the aphids on susceptible plants. It is calculated that if no multiplication had taken place the dilution would have reached 10^{-21}, while the actual dilution end point of the virus in hemolymph is 10^{-4}.

A method of isolating the potato leaf roll virus from the hemolymph of viruliferous aphids by means of gradient centrifugation has recently been described (Stegwee and Peters, 1961).

As we shall see later in discussing the biological relationship of some plant viruses with their leafhopper vectors, in some cases there is an adverse effect of the virus upon the insect vector. The question then arises: Is there any histological or metabolic disturbance caused by the leaf roll virus in the aphid? Opinion is divided on this question, although Schmidt (1959, 1960) found differences in the shape and size of the nuclei in the alimentary canal; in the viruliferous aphids the nuclei were smaller and of abnormal shape.

Ehrhardt (1960) studied the metabolic changes in the aphid *Myzus persicae* and observed a 30% decrease in oxygen consumption in viruliferous, as compared with virus-free, aphids.

A second case of the apparent multiplication of a plant virus in an aphid has been described by Duffus (1963). The virus in question, causing the yellow vein disease of sow thistle, *Sonchus oleraceus*, is transmitted by the aphid *Amphorophora lactucae* (L.). The latent period is very long and transmission is independent of the quantity of virus ingested. The shortest insect-latent period recorded was 8 days at 25°C.; the longest latent period was 46 days at 5°C. From experiments carried out on the incubation and latent periods in both the plant and the insect, it seems that a certain virus concentration must be built up in the aphid vector before transmission occurs. Aphids retain infectivity throughout their lives. The sow thistle yellow vein virus (SYVY) is transmitted in essentially the same manner as those plant viruses which multiply in their leafhopper vectors (see Section IV,D).

The only other plant virus known at present, with a similar long latent period in the aphid vector, i.e., 10–19 days, is that of strawberry crinkle (Prentice and Woollcombe, 1951).

D. Variation in Transmission of a Single Virus by a Single Aphid Species

As Rochow (1963) has pointed out in his review of aphid variation in transmission, there are three kinds of variation in an aphid species, among different *clones* or *strains*, among various *developmental* stages, and among *different forms* of one species. Stubbs (1955) working with the yellows virus of spinach, a virus of the circulatory type, found considerable variation in the ability of different cultures of *Myzus persicae* to transmit the virus.

Björling and Ossiannilsson (1958) studied the transmission by *Myzus persicae*, among other species, of two viruses, those of beet yellows and potato leaf roll, a circulative and a propagative virus, respectively.

They found that they could arrange the 85 strains of *M. persicae* into groups in which the percentage of transmission ranged from 10 to 80%, the majority falling into the 40–50% group. These differences were about the same for both viruses and the ability to transmit was not related to the host plant from which the aphids originally came.

Björling and Ossiannilsson made crosses among the different clones of the aphid and obtained some evidence of the genetic basis for the variation.

Rochow (1960a) has found considerable differences in the ability of another aphid (*Toxoptera graminum* Rondani) to transmit the virus of barley yellow dwarf, another circulative virus. He made collections of the aphid from Florida, Wisconsin, and Illinois, and found that those from Wisconsin and Illinois were fairly efficient vectors but aphids from Florida were virtually unable to transmit the virus. Rochow points out a slight anatomical difference in the beak tip of the inactive clones of aphid.

Somewhat similar clonal variation occurs in the transmission of the stylet-borne viruses. Simons (1954) showed that the cotton aphid (*Aphis gossypii* Glover) differed in its ability to transmit the virus of southern cucumber mosaic. He also found that these differences are not paralleled in the transmission by the same aphid of a different stylet-borne virus such as potato virus Y.

There are two records in the literature of differences in transmitting power according to the *developmental* stage of the aphid; both are concerned with circulative viruses. In one of these the young nymphs of *Macrosiphum geranicola* (Hille Ris Lambers) acquired the virus of filaree red leaf virus more readily than the adults and the minimum latent period was shorter in the nymphs (Anderson, 1951). The second case concerns the virus of pea enation mosaic of which the first instar nymphs of the pea aphid (*Macrosiphum pisi*, Kalt) are more efficient vectors and have a shorter latent period than the adults (Simons, 1954).

Variation in the ability of different *forms* of the same aphid species has been observed on several occasions, but there seems to be little correlation. For example, Paine and Legg (1953) were able to transmit the virus of hop mosaic by means of the spring winged form of the aphid *Phorodon humuli* (Schrank) but not by the wingless form. On the other hand, Orlob and Arny (1960) found the wingless oviparous forms and the fundatrigeniae of *Rhopalosiphum fitchii* (Sanderson) to be capable of transmitting barley yellow dwarf virus, but not the other types including the winged forms.

Again, Orlob, (1962) found that the winged migratory form of *Aphis nasturtii* (Kalt) could transmit potato virus Y, but not the oviparae and fundatrices which spend their entire life cycle on the winter host.

E. Variation in Transmission of a Single Virus by Different Aphid Species

The question of why one aphid species can transmit a particular virus while a second aphid species is not able to do so has already been discussed in the section dealing with stylet-borne viruses. In this section another aspect of the situation is briefly considered in which the inability to transmit a particular virus by a particular aphid species appears to be due to a change in the virus itself. As regards stylet-borne viruses a good example is that of cucumber mosaic virus and the aphid vector *Myzus persicae*. Badami (1958), working with a strain of this virus, isolated from spinach, which was easily transmitted by *M. persicae*, found that the aphid lost its transmitting ability in so far as this strain of the virus was concerned. The aphid, however, was still able to transmit other strains of cucumber mosaic virus and the original strain was still aphid-transmissible by other species such as *Aphis gossypii* and *Myzus ascalonicus*.

The loss of their insect vector relationships by viruses is no new thing. It seems to occur when a virus has been propagated mechanically or by vegetative reproduction of the plant host over periods of years. This appears to have happened with the virus of potato paracrinkle after years of virus propagation by the tubers. It has also been recorded with the virus of tomato spotted wilt and the thrips vector and with some leafhopper-borne viruses where there has been no contact between virus and vector for periods of years. In the case of cucumber mosaic virus and *Myzus persicae*, the case is slightly different because the loss of vector relationship is confined to one virus strain only and to only one out of several vectors.

Another example of variation in transmission is that of barley yellow dwarf virus, of the circulative type. There is a remarkable specialization among isolates of the virus and some of the aphids that transmit it. Five aphid species were shown to be vectors and others have been described. The original studies were made by Oswald and Houston (1953) and since then many papers have been published by other workers, notably Toko and Bruehl (1956, 1957, 1959), Slykhuis *et al.* (1959), Watson and Mulligan (1960) and Rochow (1959, 1960b, 1961). The whole subject has recently been reviewed by Rochow (1963) and much of this information is taken from his paper.

Direct comparisons of three aphid species and different isolates of barley yellow dwarf virus have shown the existence of at least three vector-specific strains of the virus. One strain (MGV) is transmitted efficiently by *Macrosiphum granarium* (Kirby) but not transmitted regularly by *Rhopalosiphum padi* (L.) or *R. maidis* (Fitch). A second strain (RPV) is transmitted efficiently by *R. padi* but not transmitted regularly by *M. granarium* or *R. maidis*. The third strain (RMV) is transmitted fairly efficiently by *R. maidis* but not transmitted regularly by *M. granarium* or *R. padi*.

In discussing the basis for this specificity Rochow suggests that the major basis for the variation lies in the virus rather than in the aphids. As to the mechanism controlling this specificity it might be centered in any one, or in a combination, of three main areas: acquisition of virus from the plant, physiology of virus in the aphid after acquisition, or inoculation of virus to plants by the aphid vector. The evidence suggests that failure to acquire virus from a source plant is not the major basis for the specificity. First the specificity persisted whether acquisition of the virus was by feeding or by injection (Mueller and Rochow, 1961). Second, it was shown by direct tests that the "nonvectors" actually acquired the virus (Rochow and Pang, 1961). Rochow concludes that the results of the various studies on vector specificity of barley yellow dwarf virus are perhaps best explained by assuming that the virus is extremely variable and that existing strains differ in the efficiency with which each aphid species can transmit them.

F. "Helper" Viruses and Aphid Transmission

The manner in which plant viruses react upon each other in the same host is a large and fascinating subject which has recently been reviewed in this series by Kassanis (1963). One aspect of this behavior relates to the transmission of virus complexes by aphids. Selective transmission by the aphid is of various kinds; for example, when fed upon a potato plant containing the X and Y viruses the vector selects out virus Y because virus X is not aphid-transmitted (Smith, 1931b). Again from a complex of cabbage black ringspot and cauliflower mosaic viruses in the same host, the aphid *M. persicae* will transmit both viruses, whereas another aphid species will select one virus only (Kvíčala, 1945). There is, however, another related phenomenon which is little understood; that is the dependence of one virus upon another for aphid transmission. In other words, it is necessary for a second virus to be present in the plant with the first virus for the latter to be picked up, or at least transmitted by, the aphid.

The term "helper virus" is suggested for the second virus since the first is dependent upon it for its spread to other hosts.

The first example of this phenomenon was pointed out by Clinch *et al.* (1936), who stated that potato aucuba mosaic virus was only transmitted by aphids in the presence of potato virus A. They did not, however, present any experimental data, and the first study of this phenomenon was made by Smith (1946) on the virus complex in tobacco, known as "rosette." Two viruses are present in this disease, the vein-distorting and the mottle viruses; of these two only the mottle virus is sap-transmissible. Both viruses are of the circulative type and are retained by the aphid vector, *Myzus persicae*, for long periods, generally for the remainder of its life. From the rosette disease the aphid transmits both viruses together with great regularity. When the two viruses are separated, however, and exist alone in individual tobacco plants, only the vein-distorting virus is aphid-borne.

In experiments performed at intervals over a period of years the mottle virus has never been aphid-transmitted. Various explanations of this phenomenon have been put forward but there is little supporting evidence for any of them. It was thought that possibly the presence of the vein-distorting virus increased the concentration of the mottle virus to a point where it could be picked up by the insect, but dilution tests do not bear this out. Another possibility is that the vein-distorting virus adsorbs the mottle virus and so may be said literally to carry it. Examination of the leaf tissues of rosette plants, however, by ultrathin sections and electron microscopy has so far failed to reveal the viruses.

Kassanis (1961) has investigated further the problem of the aphid-transmission of potato aucuba mosaic virus and potato viruses A and the closely allied virus Y; these are all stylet-borne viruses compared to the circulative-type viruses of the rosette complex. Kassanis found that not all the strains of potato aucuba mosaic virus behaved similarly;

the ease with which they become aphid-transmissible varied and some strains did not become aphid-transmissible at all. Also two strains were transmitted in the presence of potato virus Y but not in the presence of potato virus A.

What appears to be a similar case of the "helper" virus has recently been reported by Watson (1962). She has worked on the yellow net virus of sugarbeet in England, and she found that the yellow net virus (YNV) was invariably accompanied in the sugarbeet by a second virus, yellow net mild yellows (YNMY) without which YNV could not be aphid-transmitted. Watson refers to YNMY virus as a "carrier" virus and suggests that it may help YN to invade sugarbeet or the two may combine to form an aphid-transmissible combination, one virus presumably being adsorbed by the other. However, Kassanis (1963) thinks there is not yet sufficient experimental evidence that this is another case of the "helper" virus. He points out that the interaction was suggested because YNV is rarely transmitted from *Nicotiana clevelandii*, which is immune to YNMY, but can easily be transmitted from sugarbeet plants in which it is always found associated with YNMY. It is rather surprising that the yellow net virus should always have been transmitted together with the mild virus. Kassanis suggests that a simpler explanation for the results is that the aphids very rarely transmit yellow net virus from *N. clevelandii*. He quotes an apparently similar situation in which dandelion yellow mosaic virus is transmitted by aphids with difficulty from dandelion to lettuce plants but readily from lettuce to lettuce plants (Kassanis, 1947). Furthermore the existence of YNMY has not been reported in the United States where YNY is aphid-transmitted (Sylvester, 1949).

G. *Artificial Feeding and Virus Injection of Aphids*

Much of our knowledge on the relationships of plant viruses and leafhoppers, which are discussed in the next section, has arisen from the artificial feeding and injection methods that have been developed. Similar techniques applicable to aphids have been slower in development because of the more fragile nature of these insects and the presence in the body of large numbers of developing young. However, as we have previously pointed out, these two techniques are now being used successfully, largely because of the pioneer work of Heinze (1955), Stegwee and Ponsen (1958), and more recently of Rochow (1963).

The artificial feeding method is carried out by placing aphids that have been starved for several hours into a small feeding chamber. Each feeding chamber is topped with an animal-capping skin membrane previously fastened over the end of a glass tube. Recently, however, the animal membrane has been replaced by stretched Parafilm [Rochow (1964), personal communication]. The liquid to be tested, partially purified virus preparations, is placed in the glass tube so that the aphids are able to feed through the membrane top of the feeding chamber into the test solution above it in the tube. Following an acquisition feeding period of about 18 hours at 15°C, the glass tubes are removed from the feeding cage and the aphids are transferred to test plants.

A method for the artificial feeding of individual aphids has been described by Hutchinson and Matthews (1963). The stylets of the aphid are placed in the bore of a small capillary tube, and the aphid is effectively immobilized in this position by securing its legs to the outside of the tube with wax. Using purified solutions of turnip yellow mosaic virus (TYM), heavily labeled with P^{32} and S^{35}, it has been shown that the virus enters the gut of the nonvector aphid *Hyadaphis brassicae* and that substantial quantities of apparently intact virus persist in the gut for periods of days.

Using the needle-injection method, Mueller and Rochow (1961) have regularly transmitted the barley yellow dwarf virus from infected plants to aphids.

The glass needles used in this technique are prepared from 2 mm. tubing. Each needle is drawn three times over successively smaller flames and a final point is produced by chipping with a razor blade. The completed needles, which have a point diameter of 25–40 μ, are fastened to rubber tubing connected to a rubber syringe bulb. To fill a needle, the operator places its tip in the test solution, presses the syringe bulb with his foot, and allows the solution to rise in the needle. Injection is usually made in the back of the aphid between abdomen and thorax. Liquid is forced into the aphid by pressing with the foot on the syringe bulb. Use of the foot control allows both hands to be used for manipulation of the needle and holding the aphid by means of a brush on the stage of a binocular microscope. If necessary the aphids can be anesthetized with CO_2. Although injected aphids often transmit the virus of barley yellow-dwarf within 1 day, an inoculation test-feeding period of 5 days is usually allowed (Rochow, 1963).

V. Biological Transmission

As used in this context, biological transmission indicates a definite relationship between plant virus and insect vector. We have seen something of this in the discussion of the viruses of potato leaf roll and *Sonchus* yellow vein and their aphid vectors, but it is in regard to some of the leafhopper-borne viruses that our knowledge of this phenomenon is greatest.

All the viruses that are transmitted by leafhoppers and related insects (Cicadellidae) appear to be of the circulative or propagative type; none of the stylet-borne type are known,* and, with the exception of potato yellow-dwarf virus, they are not sap-transmissible.

Although the connection of certain plant diseases with leafhoppers has been known in Japan since 1895 and in the United States since 1909, it was not until 1926 that the first evidence was submitted of a biological relationship between the leafhopper and the virus it transmitted. In that year Kunkel, who was a pioneer in this type of work, published an extensive study of aster yellows and pointed out that there was a delay in the development of infective power of at least 10 days, after feeding on an aster plant with "yellows," before the aster leafhopper (*Macrosteles fascifrons* Stal.) became infective to a healthy susceptible plant. Kunkel interpreted this delay as an "incubation period" while the virus developed in the insect to a sufficient concentration to form an infective dose. At the time this interpretation was not accepted by many workers. Then in 1933 a Japanese worker, Fukushi, demonstrated that the virus of dwarf disease of rice was transmitted to the progeny of the leafhopper vector (*Nephotettix apicalis* var. *cincticeps*) but only if the female insect was viruliferous (virus-bearing). This means, of course, that the virus passed by way of the egg, but not by the sperm, a fact underlined, as we shall see later, by the occurrence of the virus in the cell cytoplasm, and not the nucleus, of the insect vector. Over the years Kunkel carried out a number of experiments designed to show that the virus of aster yellows did in fact multiply in the transmitting leafhopper. In one of these experiments (1937) a number of viruliferous leafhoppers with the aster yellows virus were subjected to a temperature of 32°C. for varying periods, and the effect of this on the subsequent ability of the insects to transmit the virus was studied. Kunkel found that the high temperature deprived the leafhoppers of their infectivity for a period, and that the length of this period was dependent on the length of time of their exposure to the high temperature. In other words, leafhoppers kept at 32°C. for 1 day regained their infectivity in a few hours; if they were exposed for several days it required 2 days for them to regain infectivity, and exposure for 12 days resulted in a permanent loss of infectivity. Kunkel interpreted these results as indicating that the amount of virus in the insect vector was reduced by the high temperature to a point below infectivity level, and a period of time was therefore necessary for the virus to multiply up again to reach the necessary concentration for infection. Long exposure to heat apparently inactivated all the virus in the insect, which therefore lost infectivity until fed once more on a source of virus. This interpretation was received in 1937, when the results were published, with a good deal of skepticism, but in view of the more direct evidence on plant virus multiplication in leafhoppers it is now generally accepted.

* K. C. Ling (*in litt.* 1965) reports the tungro virus of rice nonpersistent in the leafhopper (*Nephotettix impicticeps*).

One more experiment of Kunkel's (1955) is of interest in this connection. It is well-known that there is a type of acquired immunity among some plant viruses whereby a plant infected with a given virus is resistant to infection by another strain of the same virus, a phenomenon known as "cross-protection." The current explanation of this is that since all available multiplication sites are occupied by the first virus, there is no place for the second virus to gain access. There are two strains of the aster yellows virus known respectively as the New York and California strains, and the first step was to find a differential host plant which would distinguish between the two strains. Having found such a plant Kunkel demonstrated that infection with one strain precluded the entrance of the other. The insect transmission experiment was carried out as follows: leafhoppers were fed first on a plant infected with New York aster yellows, then transferred to a plant infected with California aster yellows, and finally to the healthy test plant. Next, the experiment was repeated except that this time the order of feeding on the virus sources was reversed. The results were interesting: in every case when the insect was fed successively on the two strains, it transmitted only that on which it fed first. These results clearly show the existence of some biological relationship between insect and virus; it may be that certain cells, possibly of the fat body, were occupied by the strain of virus first imbibed, and this precluded, not necessarily the entrance, but probably the multiplication, of the second virus strain.

These early experiments of Kunkel and others suggested the possibility of plant virus multiplication in insect vectors and laid the foundations for future work.

There are now two main methods of approach to this problem, and a third which is not yet universally applicable. These are the study of transovarial virus transmission, serial transmission of virus from insect to insect by injection, and the visualization of the virus in the insect vector by electron microscopy.

The results of all these methods of study now leave no doubt that some, but not all, leafhopper-transmitted plant viruses do multiply inside the body of the insect vector.

We have seen that in 1933, Fukushi demonstrated that the virus of dwarf disease of rice was transmitted transovarially to the progeny of the vector. Then, in 1940, Fukushi showed that the virus could be passed through six generations involving 82 infective leafhoppers, all derived from a single virus-bearing female without access to a further source of virus. The virus, however, was only passed through the egg and not through the sperm.

Next, Black (1944) discovered the virus of clover club-leaf, not in a plant, but in a leafhopper, *Agalliopsis novella* (Say); he showed (1950) that this virus was transmitted transovarially for more than 5 years through 21 generations from the original viruliferous parents with no further access to a source of virus.

These experiments of both Fukushi and Black prove the multiplication of the virus in the insect, since otherwise the dilution involved would be far too great.

Since these original experiments other examples of leafhopper-borne viruses that are transmitted transovarially have been discovered. For instance, the virus of rice stripe disease has been transmitted in this way through 40 generations of *Delphacodes striatella* (Fallen), a planthopper belonging to the Delphacidae (Shinkai, 1955). The same worker (Shinkai, 1960) demonstrated the transovarial transmission of rice dwarf virus through *Deltocephalus* (*Inazuma*) *dorsalis* Motschulski, as well as through *Nephotettix cincticeps*, as originally shown by Fukushi. For further examples of this phenomenon the reader is referred to a recent review by Maramorosch (1963).

The second method of studying the question of virus multiplication in leafhoppers is by serial injection. The pioneers in this approach were Merrill and TenBroeck (1934) who showed that the virus of equine encephalomyelitis multiplied in the mosquito vector, *Aedes aegypti;* they made injection from one mosquito to the next until the dilution was high enough to make multiplication of the virus the only explanation for continued infectivity in the mosquito.

This injection technique was first applied to leafhoppers and plant viruses by Maramorosch (1952), who obtained by this means direct evidence for the multiplication of aster yellows virus in its vector *Macrosteles fascifrons*. Black and Brakke (1952) also made serial transmission of the wound tumor virus in *Agallia constricta* Van Duzee, and proved that this virus, too, multiplied in the leafhopper.

The third method of approach, i.e., the examination of ultrathin sections if viruliferous insects in the electron microscope, has so far only been applied with success in one or two cases. Fukushi *et al.* (1962, 1963) showed the virus of rice dwarf disease *in situ* in the abdomen of the vector *Nephotettix apicalis* var. *cincticeps*. The virus occurred in microcrystals in the cytoplasm of abdominal cells; it was not observed in the cell nuclei or in mitochondria. It is interesting that the microcrystals of virus are somewhat reminiscent of an insect

virus, the *Tipula* iridescent virus (TIV) (Smith, 1962), which also occurs in microcrystals in the cell cytoplasm of infected insects; it is an icosahedron as apparently is the virus of rice dwarf disease. Shikata *et al.* (1964) have demonstrated the presence, by means of electron microscopy, of the wound tumor virus in both the insect vector and the plant tumor (Figs. 2 and 3). Virus particles, obtained from both insect and plant, appeared to be of similar size and morphology. In Fig. 3 can be seen the crystalline arrangement of the virus particles in the fat body of the vector *Agallia constricta*. This arrangement appears similar to that of the virus of rice dwarf disease in the abdomen of the insect vector.

Whitcomb and Black (1961) have made an important contribution in this field by assaying wound tumor-soluble antigen in the insect vector. They measured the rate of increase of wound tumor-soluble antigen at 27.5°C. in the vector *Agallia constricta* following injection of massive doses of wound tumor virus. After injection, soluble antigen was first detected at 4 days, and the soluble antigen titer developed rapidly to a plateau level within 8 to 10 days and was maintained at that level for weeks. A method was developed for determining the presence or absence of soluble antigen in a single insect vector by means of the precipition ring test. Almost all insects injected with massive doses of wound tumor virus supported the synthesis of soluble antigen; no injected insects negative for soluble antigen were found to transmit wound tumor virus to plants.

Since it has now been proved that some plant viruses do multiply in the leafhoppers that transmit them, we are now faced with the question: Are these agents primarily plant or insect viruses? From this follows another question: Are the leafhoppers which support these viruses themselves adversely affected? Some evidence that this is so is available. Littau and Maramorosch (1956, 1960) have described cytological changes which occur in aster leafhoppers carrying the virus of aster yellows. It has been established that these changes occur in the nuclei of fat body cells of male leafhoppers 18 to 28 days after acquiring the virus by feeding or by injection. In viruliferous insects the nuclei tended to be stellate rather than rounded and the cytoplasm was reticulate instead of homogeneous with many vacuoles. The first plant virus shown to be definitely injurious to the insect vector was that causing Western X disease of stone fruits and a yellows disease in celery. This virus has been shown to cause a significant reduction in the life span of the vector *Colladonus montanus* (Van Duzee) (Jensen, 1958, 1959a,b). Viruliferous individuals also produce fewer eggs than do noninfective controls (Jensen, 1962). Incidentally, we might mention that not all individuals of a given species

FIG. 2. Clusters of virus particles in fat body cells from a leafhopper (*Agallia constricta*) infected with the wound-tumor virus. Magnification: × 24,000. (Courtesy E. Shikata *et al.*, 1964.)

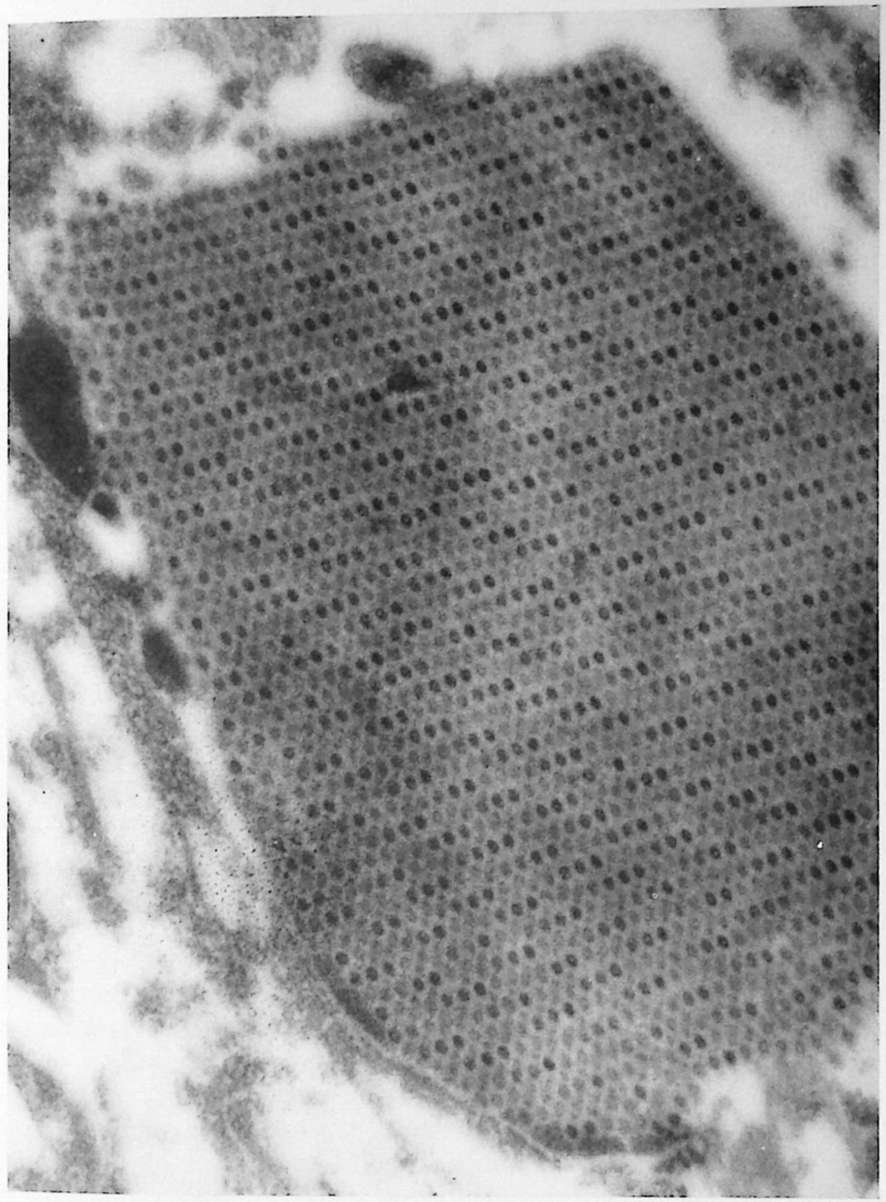


FIG. 3. Crystalline arrangement of wound-tumor virus particles in fat body cells of *Agallia constricta* (♂). Magnification: × 52,800. (Courtesy E. Shikata.)

of leafhopper vector are necessarily able to transmit the virus in question. Lamey *et al.* (1964) have made a study of the transmission of the Hoja blanca virus to rice plants, and they find that a normal population of the vector *Sogata orizicola* Muir contains only 10% of potential transmitters.

The effect generally of plant viruses on insects has been reviewed at some length by Jensen (1963). See also a review article on the harmful and beneficial effects of plant viruses in insects (Maramorosch and Jensen, 1963).

Before concluding the discussion on biological transmission of plant viruses mention must be made of a virus disease of sugarbeet known as "beet savoy" in the United States, and of a very similar disease known as "beet leaf curl" in Germany. The vector in both cases is somewhat unusual, it is not a leafhopper but a "lace bug," so called, belonging to the *Piesmidae; Piesma cinerea* in the United States, and *P. quadrata* in Germany. The American disease has been studied by Coons *et al.* (1958) and by Volk and Krczal (1957) in Germany. There is a good deal of evidence of a biological relationship between virus and vector, particularly in the length of time the virus is retained by the insect, apparently right through the hibernation period of the adult.

A recent paper (Proeseler, 1964) affords further evidence on this biological relationship, the author carried out four serial passages of the virus from insect to insect by means of carefully prepared micropipettes, but lost all adult insects in the fifth passage. From a personal communication (1964) from the author to Karl Maramorosch, it appears that a mysterious mortality several times prevented continuous serial passages and often the second or third group suddenly died. Maramorosch suggests [also in a personal communication (1964)] that this could be due to an increased virulence of the virus toward the insect vector, and further states that he has had a similar experience in serial transmissions with aster yellow virus through the leafhopper vector and could only overcome this by using large numbers of insects.

Proeseler's conclusion is that the beet virus does multiply in its vector *Piesma quadrata*, since the calculated dilution of the original inoculum was 10^8, while the incubation period in insects indicated no reduction in titer. The dilution in larvae, also used for serial passages, was 10^{11} in the last group.

A new type of insect vector has recently been recorded, and may conveniently be mentioned here although a biological relationship between virus and vector has not yet been established. The virus in question causes "pear decline" and the vector is the pear psyllid *Psylla pyricola* Foerster; this is apparently the first species of the family

Psyllidae that has been shown to transmit a plant virus (Jensen *et al.* 1964).

VI. Vectors Outside the Insecta

A. *Mites* (Eriophyidae)

As we have already mentioned, the first evidence of the transmission of plant viruses by noninsect vectors is an early paper by Amos *et al.* (1927), who suggested that the big bud mite *Phytoptus* (= *Eriophyes*) *ribis* (Westw.) was the vector of the disease of black currant known as "reversion"; this was confirmed by Massee (1952). In addition to reversion there are five other mite-transmitted viruses known and others are suspected. Three of these infect only grasses, i.e., wheat streak mosaic, wheat spot mosaic, and ryegrass mosaic, the other three affect only woody perennials and cause peach mosaic, fig mosaic, and as we have seen, black currant reversion.

All the mite vectors so far identified belong to the same group, the Eriophyidae, which seems to be distinct from other phytophagous mites (Keifer, 1952). The next mite-transmitted virus disease to be discovered, after black currant reversion, was wheat streak mosaic, this was described by Slykhuis (1953, 1955), who has done much work on mite transmission of the viruses affecting grasses. The vector of wheat streak mosaic virus (WSMV) is *Aceria tulipae* Keifer; this virus is easily sap-transmissible. A second virus disease of wheat called wheat spot mosaic (WSpMV) is transmitted by the same species of mite, with the difference that this virus is not sap-transmissible and is retained by the mite for 12 days without further access to a source of virus (Slykhuis, 1956). A single mite can carry both WSMV and WSpMV at the same time. The third virus of this group causes a mosaic disease of ryegrass and has been studied by Mulligan (1960). The mite vector of this virus is *Abacarus hystrix* (Nalepa), and although it occurs commonly on wheat it is unable to transmit WSMV or WSpMV. Ryegrass mosaic virus (RMV) is easily sap-transmissible.

The first tree virus shown to be transmitted by a mite was that of fig mosaic (Flock and Wallace, 1955, 1956). The vector is the fig mite *Aceria ficus* (Cotte). As Slykhuis points out, it is not surprising that fig mosaic is so widely spread, since the host plant is a perennial and the mite is an efficient and abundant vector. It was shown in tests that a single viruliferous mite per test plant infected 7 out of 10 plants.

The successful transmission of peach mosaic virus was first reported by Wilson *et al.* in 1955, the mite vector being *Eriophyes insidiosus*. The method of transmission of peach mosaic had been sought for many years and various sucking insects such as aphids and leafhoppers had been tested without results. Other species of mites commonly found on diseased peach trees such as *Vasates cornutus* failed to transmit the virus.

None of the mite-transmitted viruses affecting woody plants appear to be sap-transmissible. Owing to their small size and the difficulty of adequately handling individual mites there is not much information on the relationships between virus and mite. However, Slykhuis (1962) considers there is a close biological relationship; he points out that all are specifically transmitted by only one mite species even though other species may be present on the same host. Of the six mite-transmitted viruses known, only two, WSMV and RMV, are sap-transmissible. RMV appears to be similar to the stylet-borne (nonpersistent), aphid-transmitted viruses in that infectivity is lost by the mite within 24 hours after removal from the source of virus. On the other hand, WSMV has been found to persist in its vector for 9 days, WSpMV for 12 days, and peach mosaic virus for 2 days, after removal from their diseased hosts. None of the mite-transmitted viruses appears to be transmitted transovarially, but WSMV and WSpMV persist in the mite *A. tulipae* through the molts. Slykhuis (1962) points out the interesting fact that when different stages of the mite *A. tulipae* are colonized on diseased wheat, the nymphs acquire WSMV but the adults do not. This is a parallel case to the transmission of tomato spotted wilt virus by the thrips (Samuel *et al.*, 1930), where the adult thrips are unable to pick up the virus *de novo* but can do so if the virus is acquired in the nymphal state. There seems to be no satisfactory explanation of this phenomenon at present. According to Thresh (1964), infection of black currant bushes with the virus disease known as reversion induces at the same time an increased susceptibility on the part of the plant toward infestation by the mite vector of the virus, *Phytoptus ribis* Nel.

The subject of mite-transmission of plant viruses is reviewed by Slykhuis in this series (1965).

B. Eelworms (Nematoda)

Some years ago a new virus appeared somewhat mysteriously, or so it was thought at that time, in one of the insect-proof glasshouses at Cambridge, England, affecting a plant of Rock Cress, *Arabis hirsuta*, in which it caused a mosaic disease (Smith and Markham, 1944).

It is interesting now to find that this virus is the same as, or closely related to, a number of soil-borne viruses which have since been described affecting the vine, the cherry, the strawberry, and the raspberry; these viruses are all transmitted by the dagger nematodes, various species of the one genus *Xiphinema*.

The first demonstration that eelworms were capable of transmitting plant viruses was made by Hewitt *et al.* (1958). They showed that the fanleaf disease of grapevines was spread by the eelworm *Xiphinema index*. The distribution of fanleaf disease is very wide, it occurs commonly in California, it is common also in Europe where it is known by a variety of names: roncet, court noué, reisigkrankheit, etc.

Hewitt and his co-workers have investigated the relationship between the grape fanleaf virus and the vector *X. index*.

Preliminary experiments suggest that the time necessary for the eelworm to acquire the virus (acquisition feed) is comparatively short, but on the other hand the period during which the eelworm remains infective (viruliferous) without access to a further source of virus may be as long as 30 days. Other preliminary experiments suggest that the fanleaf virus can be transmitted by any of the stages of nematode development and that transovarial passage of the virus does not take place.

There is also some information on the relationships between tobacco ringspot virus (TRSV) and the eelworm vector *Xiphinema americanum*. The eelworm became viruliferous 8–24 hours after having access to an infected plant. Viruliferous individuals of *X. americanum*, when stored in soil at 10°C. for 49 weeks, were still able to transmit TRSV to 60% of the indicator plants (Bergeson and Athow, 1963). McGuire (1964) has studied the efficiency of the eelworm *Xiphinema americanum* as a vector of tobacco ringspot virus to young cucumber plants. He found that TRSV could be transmitted by a single eelworm, but percentages of infected plants increased with an increase in the number of nematodes. There seemed to be no difference in transmitting power between larvae and adults since four developmental stages tested transmitted the virus. Single nematodes acquired TRSV within 24 hours, the shortest acquisition feeding time tested. Symptoms of virus infection usually appeared in 13–25 days in the leaves of plants to which transmission had been effected. McGuire concludes that *X. americanum* is an efficient vector of TRSV.

On the other hand, Bergeson *et al.* (1964) studied the transmission, movement, and vector relationships of tobacco ringspot virus in soybeans. They did not seem satisfied that nematode transmission alone could account for the natural spread of TRSV in soybeans and suggested an additional vector, possibly thrips.

There seems to be, therefore, as with the mite-transmitted viruses, some kind of biological relationship between virus and eelworm. Thus, not every species of plant-feeding nematode can transmit virus, and the fact that virus can be retained by the eelworm for long periods seems to rule out a purely mechanical mode of transfer. As further proof of this, Harrison (1964) has demonstrated that there are specific nematode vectors for serologically distinctive forms of raspberry ringspot and tomato black ring viruses. The list of eelworm-transmitted viruses is now very long and is likely to be increased; thus the group of ringspot viruses, so-called from a common symptom on certain herbaceous hosts, includes tomato black ring, tomato ringspot, raspberry ringspot, arabis mosaic, cherry rosette, and peach yellow bud mosaic (the last-named has recently been shown by Cadman to be serologically related to tomato ringspot virus).

Strains of tomato black ring virus have been described under different names in different countries. The original, or type strain, was described in England by Smith (1946), the potato bouquet strain from German potato crops (Köhler, 1952), and the beet ringspot strain from sugarbeet, potato, turnip, oat, raspberry, strawberry, and weed plants in Scotland (Harrison, 1957, 1958). The biology of soil-borne viruses has been reviewed in this series (Harrison 1960).

C. *Fungi* [*Olpidium brassicae* (Wor.) Dang.]

The tobacco necrosis viruses form an interesting group of soil-borne agents, and there has been much speculation on their mode of transmission. These viruses infect the roots of many plant species without becoming systemic in their host. In the past it had been suggested that the vector might be a root fungus, but the first circumstantial evidence of this was pointed out by Teakle (1960) and by Teakle and Yarwood (1962), who showed that the fungus *Olpidium brassicae* was closely associated with infection of plants by one of the tobacco necrosis virus group (TNV).

Teakle (1962) has carried out a number of experiments which seem to prove that *Olpidium* does in fact transmit TNV. For example, TNV infection of roots was induced only when actively swimming *Olpidium* zoospores were able to encyst on the roots of mung bean (*Phaseolus mungo* L.) seedlings in the presence of TNV. Mild treatments, such as aging for half an hour, heating to 35°C. for 10 minutes, and addition of a very weak solution of copper sulfate inactivated the zoospores and prevented TNV infection of plants subsequently placed in the suspensions. The amount of TNV infection was approximately proportional to the number of zoospores.

In addition to the Californian strain of TNV used in the above

experiments, it was shown that a strain of TNV from England was transmitted in a similar manner to cowpea (*Vigna sinensis*), mungbean, tomato, and tobacco roots.

It is interesting to find evidence suggesting that certain strains of *Olpidium* transmit TNV with great regularity, whereas others fail to do so. Teakle reports that several crucifer strains of *Olpidium* have not transmitted TNV but lettuce strains have consistently done so.

In a more recent paper Teakle and Gold (1963) carried out experiments designed to show that the *Olpidium* spore acquires and harbors TNV and that transmission of the virus is not a mere surface contamination. Thus it was found that when dilutions of TNV and its antiserum were mixed and added to zoospores released into water, no virus transmission to roots resulted from the mixture, but transmission resulted when zoospores were released from roots into the virus suspension and the antiserum was added later. Again, the addition of TNV antiserum to roots bearing zoospores that had encysted on the roots in the presence of TNV did not prevent TNV infection, although the antiserum inactivated the virus in suspension.

It was not found possible to induce *Olpidium* to transmit certain other soil-borne viruses, such as those of tobacco ringspot and tobacco rattle. However, there is another virus causing "big vein" of lettuce which is also apparently transmitted by *Olpidium brassicae* (Campbell and Grogan, 1963). For a long time it was thought that big vein of lettuce was caused by tobacco necrosis virus but it is now known that a separate independent virus is the cause. Campbell and Grogan (1964) postulate that *Olpidium brassicae* carries the virus of lettuce big vein internally in the zoospores, as well as within the resting sporangia, and that exchange of the virus from fungus to host or from host to fungus occurs during the early stages of infection.

VII. Discussion

When the writer first commenced studies on plant viruses, only a comparatively few were known and the same was true of the insect vectors. It was considered that the power to transmit was the property of only a few insects whose feeding methods specifically fitted them to achieve this. At the present time, however, one is compelled to change this view. We know now that vectors occur among almost every kind of organism that feeds upon plants and that the relationships consist of all grades from a purely mechanical contamination to a close biological relationship between virus and vector. To recapitulate these stages, there is the purely mechanical contamination of the jaws of a biting insect; then there is the next stage where mere contamination is not enough and regurgitation of virus-containing fluid from the fore-gut is necessary for infection. With sap-sucking insects, as we have seen, there is again a gradation of relationship between virus and vector in which three stages are recognized. These are the stylet-borne, the circulative, and the propagative; it might be permissible to consider the stylet-borne viruses as mechanically transmissible were it not for the question of specificity of transmission among similar aphid species. Some work carried out by Gamez and Watson (1964) is relevant here; they attempted to transmit the stylet-borne virus of henbane (*Hyoscyamus*) mosaic by the artificial insertion of the stylets of anesthetized aphids into the leaves of infected and healthy tobacco plants. They conclude that although micromanipulated penetration probably only superficially resembles natural probing by aphids, it is still difficult to understand why henbane mosaic virus was never transmitted in these experiments if it is carried merely as a contaminant of the stylet tips.

Then, as regards the circulative type of transmission, why is circulation of the insect's body by the virus necessary? Presumably in one sense all aphid-transmitted viruses are circulative since all must be swallowed with the plant sap. Those viruses which are only transmitted after passage of the insect's body are mostly, but not entirely, phloem viruses not mechanically transmitted. Such viruses, then, obviously could not be transmitted by short epidermal stabs but only by prolonged penetration of the phloem with a plentiful supply of virus from the gut of the vector.

The propagative type brings us a stage further in the relationship between virus and vector. Now we have arrived at the point, in some cases at all events, where the virus can infect both plant and insect vector and can be considered as a plant virus or an insect virus.

How do vectors arise? Can it be only an extension of the host range by frequent contact, with transmission of the virus merely incidental and arising from this contact. We know that plant viruses lose their vector relationships by long separation of virus and vector, so why should not the reverse be true and vectors arise by long association between the virus and some common plant-feeding organism. Examples of this might be the long association of fig mosaic virus with the fig mite, or the root-infecting viruses with such common root organisms as eelworms and fungi. On the other hand, it is not always true that the most common organism which feeds upon a particular plant species is necessarily a vector of viruses affecting that plant. This is the case with the "phony" disease of the peach tree and the spike disease of sandal.

It could be that in considering the vector transmission of plant viruses, we are merely observing the gradual extension of the host range of the viruses by long-continued association of insect and plant, with transmission an incidental factor arising out of this long-continued association.

References

Allard, H. A. (1914). *U.S. Dept. Agr. Bull.* **40**, 33 pp.

Allard, H. A. (1917). *J. Agr. Res.* **10**, 615–632.

Amos, J., Hatton, R. G., Knight, R. C., and Massee, A. M. (1927). *Ann. Rept. East Malling Res. Sta. Kent* **13**, 126–150.

Anderson, C. R. (1951). *Phytopathology* **41**, 499.

Badami, R. S. (1958). *Ann. Appl. Biol.* **46**, 554–562.

Bergeson, G. B., and Athow, K. L. (1963). *Phytopathology* **53**, 871. Abstr.

Bergeson, G. B., Athow, K. L. Laviolette, F. A., and Thomasine, M. (1964). *Phytopathology* **54**, 723.

Björling, K., and Ossiannilsson, F. (1958). *Socker Handl. II* **14**, 1–13.

Black, L. M. (1944). *Proc. Am. Phil. Soc.* **88**, 132–144.

Black, L. M. (1950). *Nature* **166**, 852–853.

Black, L. M., and Brakke, M. K. (1952). *Phytopathology* **42**, 269–273.

Bradley, R. H. E. (1952). *Ann. Appl. Biol.* **39**, 78.

Bradley, R. H. E. (1956). *Can. J. Microbiol.* **2**, 539.

Bradley, R. H. E. (1959). *Proc. Intern. Botan. Congr. 7th, Montreal, 1960.*

Brčák, J. (1957). *Phytopathol. Z.* **30**, 414–428.

Brčák, J. (1959). *Virusol.* **8**, 171–176.

Campbell, R. N., and Grogan, R. G. (1963). *Phytopathology* **53**, 252–259.

Campbell, R. N., and Grogan, R. G. (1964). *Phytopathology* **54**, 681–690.

Chaudhuri, R. P. (1950). *Ann. Appl. Biol.* **37**, 342.

Clinch, P., Loughnane, J. B., and Murphy, P. (1936). *Sci. Proc. Roy. Dublin Soc.* **21**, 431.

Coons, G. H., Dewey, S. Bockstahler, H. W., and Schneider, C. L. (1958). *Plant Disease Reptr.* **42**, 502–511.

Costa, A. S., de Silva, D. M., and Duffus, J. E. (1958). *Virology* **5**, 145–149.

Cornuet, P., and Morand, J. D. (1960a). *Compt. Rend. Acad. Sci.* **250**, 1583–1584.

Cornuet, P., and Morand, J. D. (1960b). *Compt. Rend. Acad. Sci.* **250**, 1750–1752.

Day, M. F. (1955). *Exptl. Parasitol.* **4**, 387.

Day, M. F., and Irzykiewicz, H. (1954). *Australian J. Biol. Sci.* **7**, 251.

Duffus, J. E. (1960). *Phytopathology* **50**, 289.

Duffus, J. E. (1963). *Virology* **21**, 194–202.

Ehrhardt, P. (1960). *Entomol. Exptl. Appl.* **3**, 114–117.

Elmer, O. H. (1925). *Iowa Res. Bull.* **82**, 91.

Flock, R. A., and Wallace, J. M. (1955). *Phytopathology* **45**, 52–54.

Flock, R. A., and Wallace, J. M. (1956). *Calif. Agr.* **11**, 12.

Fukushi, T. (1933). *Proc. Imp. Acad. (Tokyo)* **9**, 451.

Fukushi, T. (1940). *J. Fac. Agr. Hokkaido Univ.* **45**, 83–154.

Fukushi, T., and Shikata, E. (1963). *Virology* **21**, 503.

Fukushi, T., Shikata, E., and Kimura, I. (1962). *Virology* **18**, 192–205.

Gamez, R., and Watson, M. (1964). *Virology* **22**, 292–295.

Harrison, B. D. (1957). *Ann. Appl. Biol.* **45**, 462.

Harrison, B. D. (1958). *J. Gen. Microbiol.* **18**, 450.

Harrison, B. D. (1960). *Advan. Virus Res.* **7**, 131.
Harrison, B. D. (1964). *Virology* **22**, 544.
Heinze, K. (1955). *Phytopathol. Z.* 25, 103.
Hewitt, W. B., Raski, D. J., and Goheen, A. C. (1958). *Phytopathology* **48**, 586.
Hoggan, I. A. (1931). *Phytopathology* **21**, 199–212.
Hoggan, I. A. (1934). *J. Agr. Res.* **49**, 1135–1142.
Hutchinson, P. B., and Matthews, R. E. F. (1963). *Virology* **20**, 169–175.
Jensen, D. D. (1958). *Phytopathology* **48**, 394. Abstr.
Jensen, D. D. (1959a). *Virology* **8**, 164–175.
Jensen, D. D. (1959b). *Pan-Pacific Entomologist* **35**, 65–82.
Jensen, D. D. (1962). *Proc. Internt. Congr. Entomol. 11th, Vienna, 1960* pp. 790–791.
Jensen, D. D. (1963). *Ann. N.Y. Acad. Sci.* **105**, 685–712.
Jensen, D. D. Griggs, W. H., Gonzales, C. G., and Schneider, H. (1964). *Phytopathology* **54**, 1346, 1351.
Kassanis, B. (1947). *Ann. Appl. Biol.* **34**, 312.
Kassanis, B. (1961). *Virology.* **13**, 93.
Kassanis, B. (1963). *Advan. Virus Res.* **10**, 219–255.
Keifer, H. H. (1952). *Bull. Calif. Insect Survey* **2**, No. 1.
Kennedy, J. S., Day, M. F. and Eastop, V. F. (1962). *"A Conspectus of Aphids as Vectors of Plant Viruses,* 114 pp. Commonwealth Inst. Entomol., London.
Kikumoto, T., and Matsui, C. (1962). *Virology* **16**, 509–510.
Köhler, E. (1952). *Phytopathol. Z.* **19**, 284.
Kunkel, L. O. (1926). *Am. J. Botany* **13**, 646.
Kunkel, L. O. (1937). *Am. J. Botany* **24**, 316.
Kunkel, L. O. (1955). *Advan. Virus Res.* **3**, 251.
Kvíčala, B. (1945). *Nature* **155**, 174.
Lamey, H. A., Hendrick, R. D., Everett, T. R., and Showers, W. B. (1964). *Phytopathology* **54**, 624. Abstr.
Littau, V. C., and Maramorosch, K. (1956). *Virology* **2**, 128–130.
Littau, V. C., and Maramorosch, K. (1960). *Virology* **10**, 483–500.
McGuire, J. M. (1964). *Phytopathology* **54**, 799–801.
Maramorosch, K. (1952). *Phytopathology* **42**, 59–64.
Maramorosch, K. (1963). *Ann. Rev. Entomol.* **8**, 369–414.
Maramorosch, K., and Jensen, D. D. (1963). *Ann. Rev. Microbiol.* **17**, 495–530.
Massee, A. M. (1952). *Ann. Rept. East Malling Res. Sta. Kent 1951*, pp. 162–165.
Merrill, H. M., and TenBroeck, C. (1934). *Proc. Soc. Exptl. Biol. Med.* **32**, 421.
Mueller, W. C., and Rochow, W. F. (1961). *Virology* **14**, 253–258.
Mulligan, T. (1960). *Ann. Appl. Biol.* **48**, 575–579.
Nault, L. R., Gyrisco, G. C., and Rochow, W. F. (1964). *Phytopathology* **54**, 1269–1272.
Newton, W. (1953). FAO Plant Protect. Bull. **2**, 40.
Olitsky, P. K. (1925). *Science* **62**, 442.
Orlob, G. B. (1962). *Virology* **16**, 301–304.
Orlob, G. B. (1963). *Phytopathology* **53**, 822–830.
Orlob, G. B., and Arny, D. C. (1960). *Virology* **10**, 273–274.
Osborn, H. T. (1935). *Phytopathology* **25**, 160.
Ossiannilsson, F. (1958). *Kgl. Lantbruks-Hogskol. Ann.* **24**, 369–374.
Oswald, J. W., and Houston, B. R. (1953). *Phytopathology* **43**, 128–136.
Paine, J., and Legg, J. T. (1953). *Nature* **171**, 263–264.

Prentice, I. W., and Woollcombe, T. M. (1951). *Ann. Appl. Biol.* **33**, 50.
Proeseler, G. (1964). *Z. Angew. Entomol.* **54**, 325–333.
Rochow, W. F. (1959). *Plant Disease Reptr. Suppl.* **262**, 356–359.
Rochow, W. F. (1960a). *Phytopathology* **50**, 881–884.
Rochow, W. F. (1960b). *Plant Disease Reptr.* **44**, 940–942.
Rochow, W. F. (1961). *Phytopathology* **51**, 809–810.
Rochow, W. F. (1963). *Ann. N. Y. Acad. Sci.* **105**, 713–729.
Rochow, W. F., and Pang, E-Wa. (1961). *Virology* **15**, 382–384.
Samuel, G., Bald, J. G., and Pittman, H. A. (1930). *Australia Commonwealth Council Sci. Ind. Res. Bull.* **44**.
Schmidt, H. B. (1959). *Biol. Zentr.* **78**, 889–936.
Schmidt, H. B. (1960). *Monatsber. Deut. Akad. Wiss. Berlin* **2**, 214–223.
Schmutterer, H. (1961). *Z. Angew. Entomol.* **47**, 277–301, 416–439.
Shikata, E., Orenski, S. W., Hirumi, H., Mitsuhashi, J., and Maramorosch, K. (1964). *Virology* **23**, 441–444.
Shinkai, A. (1955). *Ann. Rept. Kanto Tosan Phytopathol. Entomol. Soc.* pp. 5–6. In Japanese.
Shinkai, A. (1960). *Plant Protect.* **14**, 146–150. In Japanese.
Simons, J. N. (1954). *Phytopathology* **44**, 282.
Slykhuis, J. T. (1953). *Can. J. Agr. Sci.* **33**, 195–197.
Slykhuis, J. T. (1955). *Phytopathology* **45**, 116–128.
Slykhuis, J. T. (1956). *Phytopathology* **46**, 682–687.
Slykhuis, J. T. (1962). *In* "Biological Transmission of Disease Agents" (K. Maramorosch, ed.), pp. 41–61. Academic Press, New York.
Slykhuis, J. T. (1965). *Advan. Virus Res.* **11**, 97.
Slykhuis, J. T., Zillinsky, R. J., Hannah, A. E., and Richards, W. R. (1959). *Plant Disease Reptr.* **43**, 849–854.
Smith, K. M. (1931a). *Ann. Appl. Biol.* **18**, 141.
Smith, K. M. (1931b). *Proc. Roy. Soc.* **B109**, 251–267.
Smith, K. M. (1941). *Parasitology* **33**, 110–116.
Smith, K. M. (1946). *Parasitology* **37**, 126.
Smith, K. M. (1962). *Advan. Virus Res.* **9**, 195–240.
Smith, K. M, and Markham, R. (1944). *Phytopathology* **34**, 324.
Stegwee, D., and Peters, D. (1961). *Virology* **15**, 202–203.
Stegwee, D., and Ponsen, M. B. (1958). *Entomol. Exptl. Appl.* **1**, 291.
Stubbs, L. L. (1955). *Australian J. Biol. Sci.* **8**, 68–74.
Sukhov, K. S. (1944). *Compt. Rend. Acad. Sci. U.R.S.S.* **42**, 226–228.
Sylvester, E. S. (1949). *Phytopathology* **39**, 117.
Sylvester, E. S. (1954). *Hilgardia* **23**, 53.
Sylvester, E. S. (1962). *In* "Biological Transmission of Disease Agents" (K. Maramorosch, ed.), pp. 11–31. Academic Press, New York.
Teakle, D. S. (1960). *Nature* **188**, 431–432.
Teakle, D. S. (1962). *Virology* **18**, 224–231.
Teakle, D. S., and Gold, A. H. (1963). *Virology* **19**, 310–315.
Teakle, D. S., and Yarwood, C. E. (1962). *Abstr. Phytopathology* **52**, 366.
Thresh, J. M. (1964). *Nature* **202**, 1028.
Toko, H. V., and Bruehl, G. W. (1956). *Plant Disease Reptr.* **40**, 284–288.
Toko, H. V., and Bruehl, G. W. (1957). *Phytopathology* **47**, 536. Abstr.
Toko, H. V., and Bruehl, G. W. (1959). *Phytopathology* **49**, 343–347.
van der Want, J. P. H. (1954). "Onderzoekingen over Virusziekten van der Boon

(*Phaseolus vulgaris* L.)" 6 pp. Veenman and Zonen, Wageningen.
van Hoof, H. A. (1957). Koninkl. Ned. Akad. Wetenschap. Proc. C60, 314.
van Soest, W., and de Meesters-Manger Cats, V. (1956). *Virology* **2**, 411–414.
Von Volk, J., and Krczal, H. (1957). *Nachrbl. Deut. Pflanzenschutz dienst Berlin* **9**, 17–22.
Walters, H. J. (1952). *Phytopathology* **42**, 355.
Watson, M. A. (1936). *Phil. Trans. Roy. Soc. London*, **B226**, 457.
Watson, M. A. (1938). *Proc. Roy. Soc.* **B125**, 144.
Watson, M. A. (1946). *Proc. Roy. Soc.* **B133**, 200.
Watson, M. A. (1962). *Ann. Appl. Biol.* **50**, 451.
Watson, M. A., and Mulligan, T. (1960). *Ann. Appl. Biol.* **48**, 711–720.
Watson, M. A., and Roberts, F. M. (1939). *Proc. Roy. Soc.* **B127**, 543.
Whitcomb, R. F., and Black, L. M. (1961). *Virology* **15**, 136–145.
Wilson, N. S., Jones, L. S., and Cochran, L. C. (1955). *Plant Disease Reptr.* **39**, 889–892.

MITE TRANSMISSION OF PLANT VIRUSES

J. T. Slykhuis

Plant Research Institute, Canada Department of Agriculture, Ottawa, Canada

I. Introduction 97
II. Characteristics of Mites That Transmit Plant Viruses 98
A. Eriophyidae 98
B. Tetranychidae, the Spider Mites 100
III. Virus Transmission by Eriophyidae 102
A. Currant Reversion 102
B. Wheat Streak Mosaic 109
C. Wheat Spot Mosaic 117
D. Fig Mosaic 119
E. Peach Mosaic 121
F. Ryegrass Mosaic 124
G. Suspected Virus Transmission by Eriophyidae 125
IV. Virus Transmission by Tetranychidae 129
A. Potato Virus Y 129
B. Suspected Virus Transmission by Tetranychidae 130
V. Conclusions 131
References 134

I. Introduction

The author feels almost apologetic writing a review on this subject when there have been so few pertinent additions to the literature since previous reviews were written. Nevertheless, a recent report that the two-spotted spider mite can transmit a strain of potato virus Y is significant, especially since it is the first report with substantial experimental evidence to show that a mite other than an eriophyid can transmit a plant virus. Also, there have been several additions to the literature on currant reversion and wheat streak mosaic. Otherwise, there have been few new reports of vector experiments with known mite-transmitted viruses, and no other new examples of mite transmission appear to have been proved. There are a number of reports of suspected mite transmission that remain in doubt because they are still inadequately supported by experimental proof. These cannot be either accepted or ignored until proved one way or the other.

As we review the investigations that have been done on mites and the viruses they transmit, we note the peculiarities of each relationship and become impressed by the opportunities, difficulties, and hence the challenges that remain in this relatively little explored field of virology.

II. Characteristics of Mites That Transmit Plant Viruses

Only mites in the families Eriophyidae and Tetranychidae have been implicated with plant virus transmission. The mites in both families are specialized for piercing plant cells and sucking their contents, but they differ in many physical and behavioral characteristics.

A. *Eriophyidae*

1. *General Characteristics*

Most mites reported to transmit plant viruses belong to the Eriophyidae, a taxonomically distinct family that does not appear to be closely related to any other group of mites (Keifer, 1952). They are tiny creatures that average about 0.2 mm. in length. Their main distinction is the possession of only four legs. The genitalia are just behind the legs, and the abdomen is elongate with the surface characterized by narrow transverse rings typically bearing beadlike microtubercles.

The developmental history of eriophyids is simple and may be completed in 6 days (Staples and Allington, 1956) but usually takes 10–14 days at summer temperatures. There are two nymphal instars, the second terminating in a resting period or pseudopupa, during which the genitalia form and protrude through the body wall. Males are usually smaller than females and in some species are rarely observed. Some species have two types of females, one being specialized for hibernation.

2. *Host Specificity*

Eriophyids have intimate and usually highly specific host relations. Some species parasitize only certain species of one plant genus. Others have hosts in several genera, but it is rare for one mite species to have hosts in more than one plant family. All are essentially parasites of perennial plants because they have no resistant forms that can survive long periods in the absence of a living host plant. Annuals do not ordinarily afford the necessary stable basis for colony founding and perpetuation, but there are exceptions in which annuals become infested by mites from perennials. Sometimes an annual favorable to the mites grows in an overlapping sequence, providing suitable immature plants to sustain mites throughout the year. Thus eriophyid mites are perpetuated on wheat in areas where wheat streak mosaic virus is a serious problem (Slykhuis, 1955).

3. *Feeding Mechanism and Habits*

Eriophyid mites feed by sucking plant juices in a manner that appears to be well suited for the transmission of plant viruses. Their slender stylets, which rest in a groove in the rostrum, puncture plant tissue but cause little apparent damage to the cells. The rostrum is a jointed structure, the apex of which is a pair of pads that apparently conduct saliva to the stylets and suck up plant juices.

Eriophyids usually feed on young tissues and so are frequently found in buds and on young leaves. Most species cause no noticeable injury to their hosts, and since they are so minute, their presence is usually overlooked. A minority of species cause visible injuries that may range from leaf discolorations to varied malformations, galls, and bud blasting. It is sometimes very difficult to distinguish virus symptoms from mite injury.

4. *Movement*

The range of independent movement of eriophyids is limited by their small size and their dependence on specific hosts for food and protection from desiccation. Massee (1928) reported that black currant gall mites were transported by aphids, bees, and coccinellid beetles. Although *Aceria tulipae* may also be carried to a limited extent by insects (Gibson and Painter, 1957), wind is of major importance in dispersing these mites in wheat fields (Slykhuis, 1955), and the spread of wheat streak mosaic is strongly influenced by wind. The black currant gall mite is stimulated by certain wind speeds to stand erect, then leap; thus it accommodates itself to be carried away by the wind (Smith, 1959).

5. *Special Techniques for Experimentation with Eriophyid Vectors*

The extremely small size and the fragility of the eriophyids necessitates special considerations not needed for experimentation with larger vectors. A lens (10 ×) is required to see them, a dissecting microscope (20–40 ×) is needed when manipulating them individually, and a compound microscope with oil immersion lens is essential when making taxonomic determinations. It is indeed an advantage that they cannot hop, run about quickly, or fly like leafhoppers and thrips. Instead, they crawl about and will cling to many objects that contact them. A single fine hair from a camel's-hair brush cemented to a handle about the size of a pencil is a convenient tool for transferring either mites or eggs because they cling to the hair but can be wiped off without injury.

Caging is simple for some types of experimentation but difficult for others. In the greenhouse it is easy enough to prevent spread from one potted plant to another by covering the plants with cages constructed of clear sheet plastic, using a close-weave, white Dacron taffeta cloth over ventilator openings. Sometimes it is convenient to grow seedlings of a host plant such as wheat in sterilized soil in large test tubes (2.5 × 25 cm.) tightly plugged with cotton. The test tubes thus confine the plants and any mites placed on them, and exclude unwanted mites or predators. However, the small size and mobility of the mites on the plant cause difficulties in experiments that require repeated handling of individual mites. A mite placed on a plant will usually wander about before settling down to feed. It is very difficult to find again, without damaging the plant during the search, especially if it enters a leaf whorl or bud. We have developed a cage to confine mites on a leaf on a wheat plant and prevent their escape to the whorl, but efficient transmission of virus has been achieved only when mites were allowed freedom to move to the younger tissues at the base of the leaf. Certain eriophyids feed in buds, galls, or other sheltered locations. This creates serious difficulties if you wish to observe feeding or ascertain when the plant is free from all mites. Techniques for working with mite transmission of viruses on different hosts vary, but the problems related to manipulating such tiny vectors are difficult for all.

B. *Tetranychidae, the Spider Mites*

1. *General Characteristics*

The Tetranychidae are all plant feeders, and receive the name "spider mite" from the ability of most species to spin a fine web over the leaves of the plants on which they feed. At times the entire plant may be covered by the webbing. These mites vary in color from yellowish, greenish, and orange to red. They are 8-legged, medium-sized mites (up to 0.80 mm. long), oval or pear-shaped with the narrow portion toward the rear, and possess more or less marked shoulders. A pair of eyes is located on each side of the propodosoma (Baker and Wharton, 1952).

Some species overwinter as eggs, but *Tetranychus telarius* L. (the two-spotted spider mite) overwinters as adult females. After mating, the females lay eggs at the rate of 2 to 6 a day, each depositing a total of 70 or more during her lifetime. The spherical, shiny eggs are usually attached to the web on the underside of the leaf. In warm conditions they hatch in 4 or 5 days, emerging as 6-legged larvae, but after molting they emerge as 8-legged protonymphs. The molted skins remain adhering to the leaf, and, if numerous enough, give the leaf a silvery appearance.

The females molt three times, the males only twice (Metcalf *et al.*, 1962). The females become larger and more globose than the males. In the summer the females predominate, but as cold weather approaches the numbers of males and females are more nearly equal. The females live about 17 days in midsummer to several months in winter, and are not very active. The males are shorter lived than the females and move about rapidly when not mating. They are smaller and narrower than the females, and the abdomen can telescope considerably during mating. Parthenogenesis has been reported, but only males are produced from the unfertilized eggs (Baker and Wharton, 1952).

The developmental period of spider mites varies widely with temperature. One female can give rise in 1 month, through succeeding generations, to a progeny of 20 mites at 60°, about 13,000 at 70°, and well over 13 million at 80°F. constant temperature (Smith, 1952).

2. *Host Specificity*

The Tetranychidae are not usually host specific. When one food plant becomes crowded they will migrate to other plants. Practically all species are or appear to be potential pests, and frequently several species may be found on one plant. The Tetranychidae are probably the most destructive family of plant-feeding mites and may occur on a wide range of economic plants (Metcalf *et al.*, 1962).

3. *Feeding Mechanism*

They feed by piercing the leaves with styletlike chelicerae and sucking cellular material. The leaves become blotched with pale yellow and reddish-brown spots, ranging from small specks to large areas on both leaf surfaces. When mite populations are large the leaves develop a pale, sickly appearance, eventually die, and drop from the plant.

4. *Movements*

Spider mites can migrate from plant to plant. They can be dispersed with infected plant material and probably by wind. They are common pests both in the field and in greenhouses. Their mobility and wide host range favor their widespread distribution.

5. *Techniques for Experimenting with Tetranychidae*

Since the Tetranychidae may feed and breed on a wide range of plants, there is considerable choice of hosts for rearing and virus transmission experiments. Schulz (1963) reared *Tetranychus telarius* on bush bean, then used potato and *Nicotiana glutinosa* as virus sources and potato as test plants. *Tetranychus telarius* is not as small or fragile as the eriophyids and apparently can be manipulated and observed more readily during transmission experiments.

III. Virus Transmission by Eriophyidae

The first evidence that mites transmitted a plant virus was presented by Amos *et al.* (1927) when they reported a correlation between infestation with the black currant gall mite, and the development of reversion disease of black currants. Substantiating evidence was not published until 1952 (Massee, 1952). Late that year there was evidence that another eriophyid mite, was a vector of a very different virus, wheat streak mosaic virus (Slykhuis, 1953b, 1955). Eriophyid mites are now known to transmit six different viruses (Table I). Although there are indications that other viruses may also be transmitted by eriophyids, substantiation seems difficult to achieve.

Three of the mite-transmitted viruses—currant reversion virus (CRV), fig mosaic virus (FMV), and peach mosaic virus (PMV)—appear to infect only specific woody plants, and can be transmitted artificially only by grafting and perhaps by dodder. The other three—wheat streak mosaic virus (WSMV), wheat spot mosaic virus (WSpMV) and ryegrass mosaic virus (RMV)—infect only grasses. Only WSMV and RMV have been transmitted manually by the leaf-rubbing method.

In the following, the viruses transmitted by eriophyids are discussed in the order in which their transmissibility by mites was discovered.

A. *Currant Reversion*

Reversion of black currants (*Ribes nigrum* L.) was the first plant disease suspected to be caused by a mite-transmitted virus (Amos *et al.*, 1927; Massee, 1952).

1. *The Disease and Its Distribution*

The disease was called "reversion" because the character of the leaves changed and the affected bushes were thought to have reverted to the wild type. Reversion was recorded in Holland and Germany in 1904; by 1912 it was becoming well-known in black currants in southern England (Anonymous, 1927), where it became widespread even in plantations of the most up-to-date growers. It incited much concern because most reverted bushes soon ceased to bear fruit and appeared to be sources of further infection. Reversion has been reported widespread and destructive in coastal districts of Norway, (Jøstad, 1928), in Denmark (Anonymous, 1929), Sweden (Lihnell, 1949), and the U.S.S.R. (Dounin, 1939). It has been reported in only one field in Canada (McLarty, 1942), and was recorded for the first time in Austria in 1960

(Glaeser, 1961). In Denmark, black currants appeared to be the principal host, but red currants (*R. vulgare*) and flowering currants (*R. sanguinea*) showed symptoms of reversion under conditions indicating that the disease had spread from black currants.

2. *The Black Currant Gall Mite*

The black currant gall mite, *Phytoptus* (= *Eriophyes*) *ribis* (Westw.) Nalepa, which usually accompanies reversion, was recognized as a serious pest of black currants long before the reversion disease was recognized (Massee, 1928). It was known in a number of localities in Scotland as far back as 1850, and was described by Westwood in 1869. A condition called "big bud" develops as a result of mites infesting buds and causing them to swell (Plate I). Although black currants are the host of major concern, *P. ribis* may also infest red and white currants, the mountain currant (*Ribes alpinum* L.) and gooseberry.

P. ribis is dependent on *Ribes* spp. throughout its life history. The mites emerge from infested buds from early March to mid-June, reaching the height of their migration during the latter part of April or in May. Temperature seems to be one of the main controlling factors. During the migration period they live and feed without protection on the outer bud scales, shoots, and leaves. They disperse by crawling down bud stems. They sometimes make a sudden leap and light on leaves near the buds, or attach themselves to insects, or are carried by the wind to other bushes. Some make their way to the petiole and enter between its upper surface and the main stem, where they feed, reproduce, and eventually enter the almost invisible buds. As the lower leaves of the new shoots harden, the mites migrate toward the newly developing leaves near the apex, where most of the infestation finally develops. They have been observed to copulate on leaves, then they may enter new buds from the end of May to late July. They lay eggs and multiply rapidly in the buds. Big buds may appear as early as July and become abundant by September to November but sometimes continue to develop until March. The buds become globular and twice normal size. Slightly swollen buds may produce small, crumpled leaves, but most dry up and die in early summer. Sometimes mite-infested buds do not enlarge but dry out the following spring or summer (Plate I, Figs. 1–5).

3. *Differentiation of Reversion Symptoms*

Since the "big bud" mites had been associated with serious damage to currants for many years and were usually quite abundant on diseased

Plate I

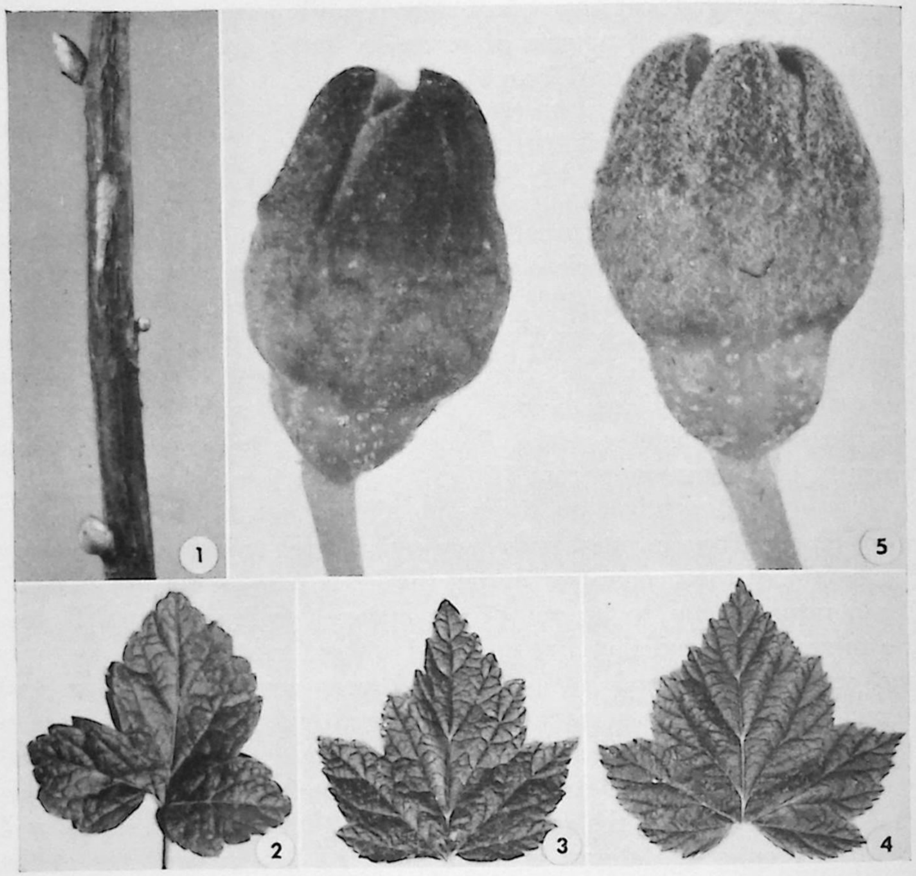


Fig. 1. A dormant shoot of black currant bearing a normal elongate bud (top), a swollen mite-infested bud (bottom), and a small infested bud (center).

Fig. 2. Black currant leaf severely malformed by effects of mites (*Phytoptus ribis*).

Fig. 3. Reverted black currant leaf.

Fig. 4. Normal black currant leaf.

Fig. 5. An almost hairless flower bud of a reverted bush (left) and a normal flower bud (right). (Photographs supplied by J. M. Thresh.)

bushes, Lees at first suspected that the abnormal leaf symptoms called "reversion" were caused by mites feeding in the buds, the numbers of mites determining the severity of damage (Thresh, 1964b). Later he differentiated "false reversion," a temporary condition, which the plants outgrew and which was not associated with crop loss. Amos and Hatton (1927) stressed the fact that although a number of abnormalities such as "nettlehead" (a crowded, woody growth from lateral buds), abnormal

flowers, and sterility were often associated with reversion, these conditions were not always evident on diseased bushes. They could result from different causes, so they were not in themselves good diagnostic symptoms. True reversion could be detected earliest from leaf characteristics of shoots from buds near the top or base of the previous year's wood. The reverted leaf tended to be narrower and smaller than normal, and flattened at the base by a reduction in depth of the basal sinus. The leaf was deficient in subsidiary veins, the margin was more coarsely toothed with fewer teeth that do not each receive a subvein, and the leaf surface was more coarsely rugose. However, the similar-appearing "false reversion" condition sometimes developed from damaged or divided tips, by the forcing into growth of dormant buds, or by growth impeded by drought. As such shoots grew, the new leaves tended to be normal. In true reversion the basal leaves of shoots could have five or more submain veins, and only the later-formed leaves reveal any shortage.

Eventually it was shown that reversion symptoms developed on all previously normal plants grafted to or inarched with reverted plants when there was strong evidence that mites were not present (Amos *et al.*, 1927). This suggested that reversion was caused by a virus and was not just a manifestation of mite injury; however, it appears that there was no absolute certainty that mites were not present. Thresh (1963) described leaf malformations "false" reversion, which could be confused with reversion symptoms (Plate I, Figs. 2–4). These were attributed to *P. ribis* alone because the plants recovered after the mites were eliminated, but true reversion symptoms persisted. Transmission of reversion with assurance that mites were not transferred on the scion material appears to have been accomplished by patch bark grafting (Cropley *et al.*, 1964).

4. *Relation of Mites to Reversion Disease*

Although true reversion symptoms sometimes appeared on shoots or even whole bushes on which mites were not observed, and some mite-infested bushes did not develop reversion symptoms, a correlation was shown between infestation by the big bud mite and the development of reversion (Amos *et al.*, 1927). In experiments at East Malling, 90% of normal bushes developed reversion after mites from reverted bushes were established on them, but only 8% of the controls in which big bud did not appear showed symptoms. Since the cause of reversion appeared to be graft-transmissible, these and results reported by Lees (1925) suggested that reversion was caused by a mite-transmitted virus.

The foregoing circumstantial evidence was later confirmed in experiments by Massee (1952). He selected 30 healthy Baldwin black currant bushes and planted them in 8-inch pots in November 1942; 24 plants to be inoculated were placed in an insectary compartment along with 6 reverted bushes. The other 6 healthy plants were placed in a separate insectary to serve as untreated controls. All test bushes received similar pruning, fertilizer, and tar oil treatment during the 3 years of the test. The 6 control plants were kept as free from mites as possible by spraying with 3% lime sulfur each year at the end of March and by dusting with flowers of sulfur and nicotine from time to time each summer. The 24 test plants were inoculated by placing 40 mites on each of 12 buds on each plant, by cutting up big buds from reverted plants and then placing portions in the axils of 12 buds of each plant, and by placing other portions of buds on the soil by each plant. Such multiple inoculations were repeated monthly from December 1942 to April 1943, started again in November 1943 and continued to April 1944, and repeated during the winter of 1944–1945. Although Massee indicated that at other times it was often very difficult, if not impossible, to transfer mites successfully from reverted to healthy plants, in this experiment mites were established on all 24 inoculated plants during the first year. The numbers of big buds per plant varied considerably after one year but did not increase materially during the second and third years. Reversion symptoms were evident on 10 bushes during the first year, 9 in the second, and on each of the 24 bushes by the third year, whereas none of the 6 control plants showed reversion.

Although the above results indeed confirmed the previous circumstantial evidence, actually they too provided only circumstantial evidence that the mites transmitted a virus causing reversion. The large numbers of mites which include 2400 transferred to each bush each year, plus the undetermined numbers that would emerge from the pieces of buds placed on each plant, is a tremendous number of mites for virus transmission. Since the mites that were transferred and the numbers that developed on the bushes were sufficient to cause big buds, it seems possible they could have caused reversionlike leaf symptoms, even if a virus were not present. The controls were plants kept mite-free so we still do not know the effects of virus-free mites. Another factor in Massee's experiments is that the bushes were kept in close association with reverted bushes. It therefore remains possible that vectors other than mites could have come from the reverted bushes.

The latter criticism appears to have been avoided by Smith (1961), who achieved more facility in establishing mites on new bushes by using mites from galled buds collected from reverted bushes during the normal mite migration period in spring and early summer. The mites were manipulated with a single hair, and placed, individually or in groups, near or in the axils of new shoots of Baldwin black currant seedlings, or on 1-year-old plants transplanted from the nursery. He reported that although the mites he transferred penetrated to the centers of many buds, they survived and reproduced in fewer than 1% of the buds. However, even a single mite was found capable of starting an infestation leading to bud galling and the transmission of reversion.

This experiment again confirms the association between the mites and the development of reversion symptoms, but again sufficient mites were present to cause big buds before reversion symptoms developed. It does not prove that mites without a virus could not cause the symptoms.

Thresh (1964b) evidently avoided direct damage by mites. He infested small currant seedlings with mites from diseased bushes, and after 4 days he dipped the plants in 0.05% endrin solution to destroy the mites. Although it is not evident what assurance there was that this treatment destroyed all mites that may have been sheltered within buds, he reported that the seedlings developed no recognized mite injury or bud galls, yet some developed reversion symptoms. The presence of reversion virus was confirmed by patch-grafting to healthy bushes.

Smith (1962) was unable to demonstrate a correlation between the distribution of reversion and the spread of gall mites from reverted bushes to healthy plants in a currant plantation, but the time of reversion spread correlated with the time of mite dispersal between April 1 and June 28, 1960. Earlier observations by Lees (Thresh, 1964b) and Swarbrick and Berry (1937) indicated that healthy currant bushes were not as readily infested with mites as bushes with reversion symptoms. This was confirmed by Cropley *et al.* (1964) on patch bark graft inoculated bushes, and by Thresh (1964a), who suggested that the decreased hairiness on flowers (Plate I, Fig. 5) and vegetative parts facilitated the movement of mites and their entry into susceptible buds, thus decreasing the period of exposure during which they may desiccate or starve. Thresh interprets the phenomenon as a striking evolutionary adaptation between mites and reversion of mutual advantage. The virus increases the susceptibility of currant bushes to the mites without decreasing the vegetative vigor or numbers of buds available for colonization by mites. The heavy populations of mites that develop on reverted bushes increase the rate of spread of mites to other bushes. Although the mites may not readily colonize healthy bushes they may inoculate them with reversion, which will eventually render the bushes more favorable for mite colonization. He suggests that the damage caused by mites as vectors of reversion, which causes sterility, is much more important than their effects on buds.

5. *Mites in Relation to Disease Control*

Early recommendations for the control of reversion included removal of reverted bushes to eliminate sources of disease, and the application of lime sulfur sprays shortly after leaves appeared in spring to kill mites emerging from buds (Amos *et al.*, 1927). A voluntary scheme for inspection and certification of black currant planting stock was implemented in England and proved effective in reducing crop losses. A similar scheme initiated in Denmark in 1949 is credited with largely eliminating reversion in most nurseries under official supervision, but the disease is still widespread in gardens (Kristensen and Christensen, 1957).

Although the disease is still widespread in England, the control measures, designed primarily to control the mites, are considered effective and practical (Thresh, 1964c). No systemic acaricides suitable for killing mites protected within the buds have been found, but lime sulfur is still an effective spray if used judiciously (Smith, 1960). Wettable sulfur, endosulfan, and endrin have also proved effective. Time of spray applications is important. Ten weekly applications of endrin during the migration period of the mites completely prevented spread of mites and reversion, but fewer applications of any of the recommended miticides were less effective (Thresh, 1964d). Recent confirmation that reverted bushes are more susceptible and thus become more heavily colonized by mites than healthy bushes emphasizes the importance of removing reverted bushes to reduce mite populations and spread (Thresh, 1964a).

6. *Problems of Investigating Mite-Transmission of Currant Reversion Virus*

Although successive experiments and practical aspects of control confirm the hypothesis that currant reversion is caused by a mite-transmitted virus, uncomplicated proof has been difficult to achieve. As assessed by Thresh (1964b), serious technical problems hamper critical experiments. Indeed, the tiny, fragile mites are difficult enough to manipulate, but they are even more difficult to culture on healthy black currants under conditions sufficiently controlled to assure exclusion of contaminants. It is difficult to establish virus-free colonies of mites. Their freedom from infectivity cannot be determined quickly because reversion symptoms do not develop until the bushes have been infected for 1 to 3 years. The earliest symptoms are often missed and chronic symptoms may be masked by mite, insect damage, or confused with effects of other factors. On the other hand, it can be difficult to maintain mite-free currants without repeated spraying. Even after miticide treatments it is not always possible to be certain, without dissecting the buds, that plants are absolutely mite-free.

Progress in studying virus-vector relationships has been understandably slow, and little is known about physical properties of reversion virus. The absence of a reliable rapid means of detecting reversion virus has remained a handicap to virus and vector research. Perhaps the difficulties have discouraged confirmatory experiments on the transmission of currant reversion in the many areas of northern Europe where reversion and the currant gall mites have been reported, but it seems highly desirable that the relation of mites to virus spread should be studied in at least a few of these other areas.

B. *Wheat Streak Mosaic*

Wheat streak mosaic virus (WSMV) was the second plant virus shown to be transmitted by an eriophyid mite. In many respects a comparison of wheat streak mosaic and currant reversion is a study in contrasts. Despite the similar type of vector, the hosts, viruses, and diseases differ greatly.

1. *The Disease and Its Distribution*

The disease identified as wheat streak mosaic was first collected in Kansas in 1932, but a mosaic found on wheat in Nebraska in 1922 appears to have been caused by a juice-transmissible virus and was probably wheat streak mosaic (McKinney, 1937). Since then it has caused heaviest losses of wheat yield in western Kansas in 1949 and 1959 (Fellows, 1949; King and Sill, 1959), and from time to time has caused serious losses in other states over the great plains including Nebraska (Staples and Allington, 1956), South Dakota (Slykhuis, 1952), Colorado (Atkinson, 1949), Wyoming (Walters, 1954), Montana (McNeal and Dubbs, 1956), and as far north as southern Alberta, Canada (Slykhuis, 1953b). Although less serious, we have found it also in southwestern Saskatchewan. It has been reported in western North Dakota (Timian and Bissonnette, 1964), Idaho (Finley, 1957), Washington (Bruehl and Keifer, 1958), and California (Houston and Oswald, 1952). The most southerly limit has been northern Oklahoma (Wadsworth, 1949) and the Texas panhandle (Ashworth and Futrell, 1961). It has not been reported further east in the United States than the western edge of Iowa (Wallin, 1950), but in 1964 a few mosaic-diseased plants collected near London, Ontario, Canada, were found to be infected with WSMV (Slykhuis, 1964). The only countries in which WSMV has been identified outside North America are Rumania (Pop, 1962) and Jordan (Slykhuis and Bell, 1961). A disease of wheat in the Krasnodar region in the U.S.S.R. may be wheat streak mosaic (Razvyazkina *et al.*, 1963).

In all areas where WSMV has been reported, winter wheat culture is practiced. Although the virus may attack spring wheat, corn, and other spring-sown crops, its occurrence is still associated with winter wheat. When wheat is heavily infected while very young the plants can be severely stunted and may die without producing seed; thus the entire yield of heavily infected fields may be lost. In 1959 approximately 20% of the wheat crop of Kansas was lost because of severe infection with WSMV (King and Sill, 1959).

2. *Differentiation of Wheat Streak Mosaic Virus*

Although chlorotic dashes and streaks, stunting, and necrosis of wheat plants may be caused by different factors, the presence of WSMV can be checked readily by simple, manual transmission procedures. Fresh diseased leaves may be ground with mortar and pestle, or the juice extracted by other means, diluted 1/4 to 1/40 with water to increase the volume of inoculum, and then, either with or without an abrasive such as fine carborundum powder or celite, rubbed on leaves of healthy wheat plants, preferably in the 2- to 3-leaf stage. If the plants are then kept in a greenhouse at 20°–25°C., faint chlorotic dashes and streaks will be visible in 6 to 8 days and should show clearly in 2 to 3 weeks. By such tests, wheat streak mosaic was identified as a virus disease long before its natural means of transmission was surmised.

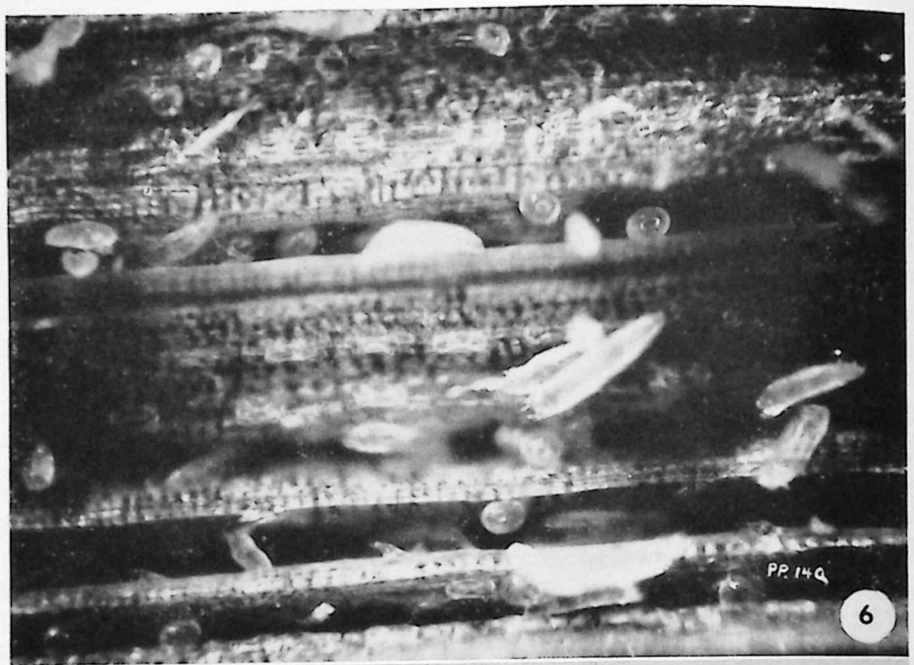
Strains of WSMV and other viruses were initially characterized by the symptoms each produced on wheat under standard conditions, but differences in reactions of other hosts were important characteristics (McKinney, 1937, 1944; Slykhuis, 1952, 1961a). A number of alternative methods can now be used to distinguish WSMV from other viruses causing mosaic symptoms on wheat. Electron microscopy reveals the particles of WSMV to be flexuous rods about 700 mμ long, in contrast to the much shorter rods of barley stripe mosaic virus and the soil-borne wheat mosaic virus (Brandes and Wetter, 1959), and the polyhedral particles of bromegrass mosaic virus (Chiu and Sill, 1963). The particles of WSMV are indistinguishable from those of *Agropyron* mosaic virus (Staples and Brakke, 1963), but we have found these viruses are distinguishable by their synergistic reaction in combined infections in wheat, and by specific serological reactions as well as by differential hosts. Thus WSMV, which can be transmitted so readily artificially and which has such a short incubation period in a host that is easily grown from seed in the greenhouse, provides many conveniences for investigation not available to investigators of currant reversion virus.

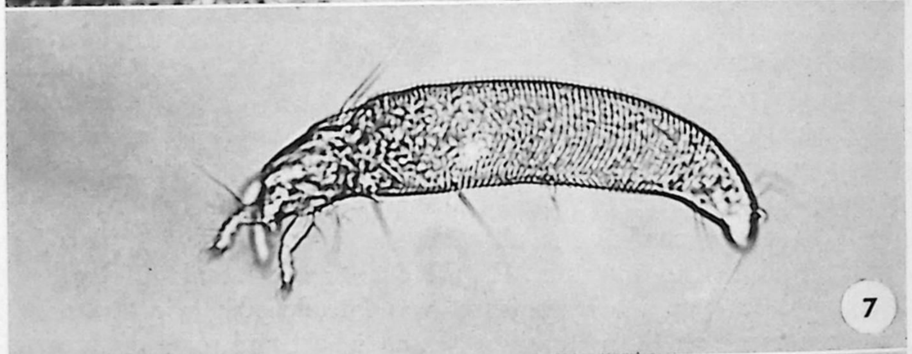
3. *The Vector Hunt*

Early tests had evidently indicated that WSMV was not soil-borne (McKinney, 1944), but there is little record of attempts to find a vector until the serious outbreaks of the disease occurred in Colorado in 1947, and in Kansas and Nebraska in 1949. Although Atkinson (1949, 1952) reported that the aphids *Toxoptera graminum* (Rond.) and *Rhopalosiphum prunifoliae* (Fitch.) transmitted western wheat mosaic virus (a strain of WSMV) in Colorado, such results were not confirmed by other investigators. It now appears probable that his aphids were transmitting barley yellow dwarf virus, which was not yet recognized but which is now known to be transmitted by the species named, or perhaps the real vector of WSMV was present with the aphids he tested. At least 23 species of insects were tested in Kansas (Connin, 1952). The leafhopper *Endria inimica* (Say) was associated with virus transmission in some of these tests, but it failed to transmit the virus in later tests. Field experiments in South Dakota showed that WSMV was being spread naturally from early June until September; most spread occurred during July and August (Slykhuis, 1952). There was circumstantial evidence that insects were associated with transmission, but no specific insect was shown to transmit WSMV. *Endria inimica* induced mosaic symptoms, but these were symptoms of a hitherto unrecognized virus disease later named wheat striate mosaic (Slykhuis, 1953a). In some tests naturally diseased wheat was kept in cages with openings joined to cages confining healthy plants. Insects were introduced to test their ability to transmit WSMV. Sometimes test plants developed streak mosaic even when no insect was introduced. Since thrips were usually present on the naturally diseased wheat and some of them migrated to the test plants, they were suspected to be vectors. However, tests with thrips manually transferred from diseased wheat failed to show that they could transmit.

When wheat streak mosaic was found in southern Alberta in 1952, tests with thrips were continued. Since cages with screen fine enough to exclude thrips did not always prevent the spread of WSMV from naturally diseased to healthy wheat, something smaller than thrips must have been the vector. Attention therefore became focused on tiny eriophyid mites, *Aceria tulipae* (K.) (Plate II, Figs. 6–8), noticed on diseased wheat (Plate III, Fig. 9) (Slykhuis, 1953b, 1955). The first test showed that mites from diseased wheat induced streak mosaic symptoms (Plate III, Fig. 10) on test plants, and the presence of WSMV was confirmed by artificial transmission. Mites transferred at any stage, including first nymph, second nymph, or adult, or while molting, could

PLATE II





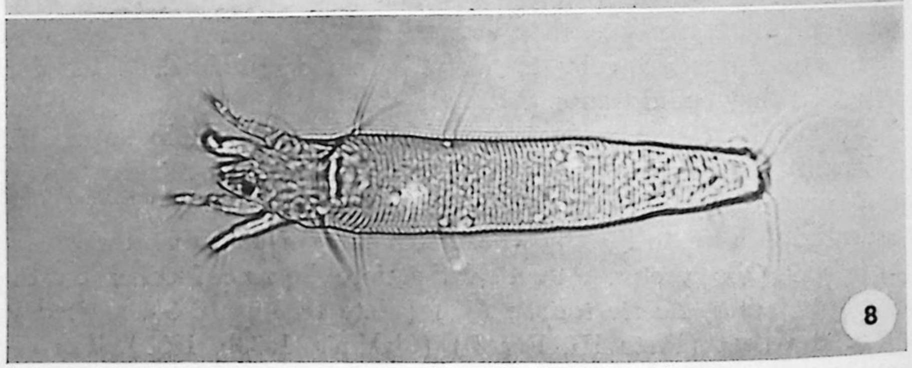


FIG. 6. Eggs and various stages of *Aceria tulipae* on the upper surface of a wheat leaf.

FIG. 7. Lateral view of an adult *Aceria tulipae* about 250 μ long.

FIG. 8. Ventral view of *Aceria tulipae* female.

infect wheat seedlings with WSMV. About 1/3 of the mites tested singly on wheat seedlings transmitted. The virus was not carried by eggs; hence eggs hatched on healthy plants were the origin of noninfective colonies of mites that did not induce mosaic symptoms until after they were fed on diseased plants. Nymphs acquired the virus during access periods of 30 minutes or longer, but older adults did not appear able to acquire virus. Mites reared on diseased wheat remained infective until the ninth day after removal to *Lolium perenne* L. plants which were immune to WSMV.

Mite transmission of WSMV was soon confirmed in Kansas (Connin, 1953) and Nebraska (Staples and Allington, 1956). Since then *A. tulipae* has been found to be a vector of WSMV everywhere it has been tested in the United States. Furthermore, the same mite species is the only known vector of a virus identified as WSMV in Rumania in 1960 (Pop, 1962). Also, WSMV collected in Jordan in 1961 was transmitted by our Canadian cultures of *A. tulipae* (Slykhuis and Bell, 1963).

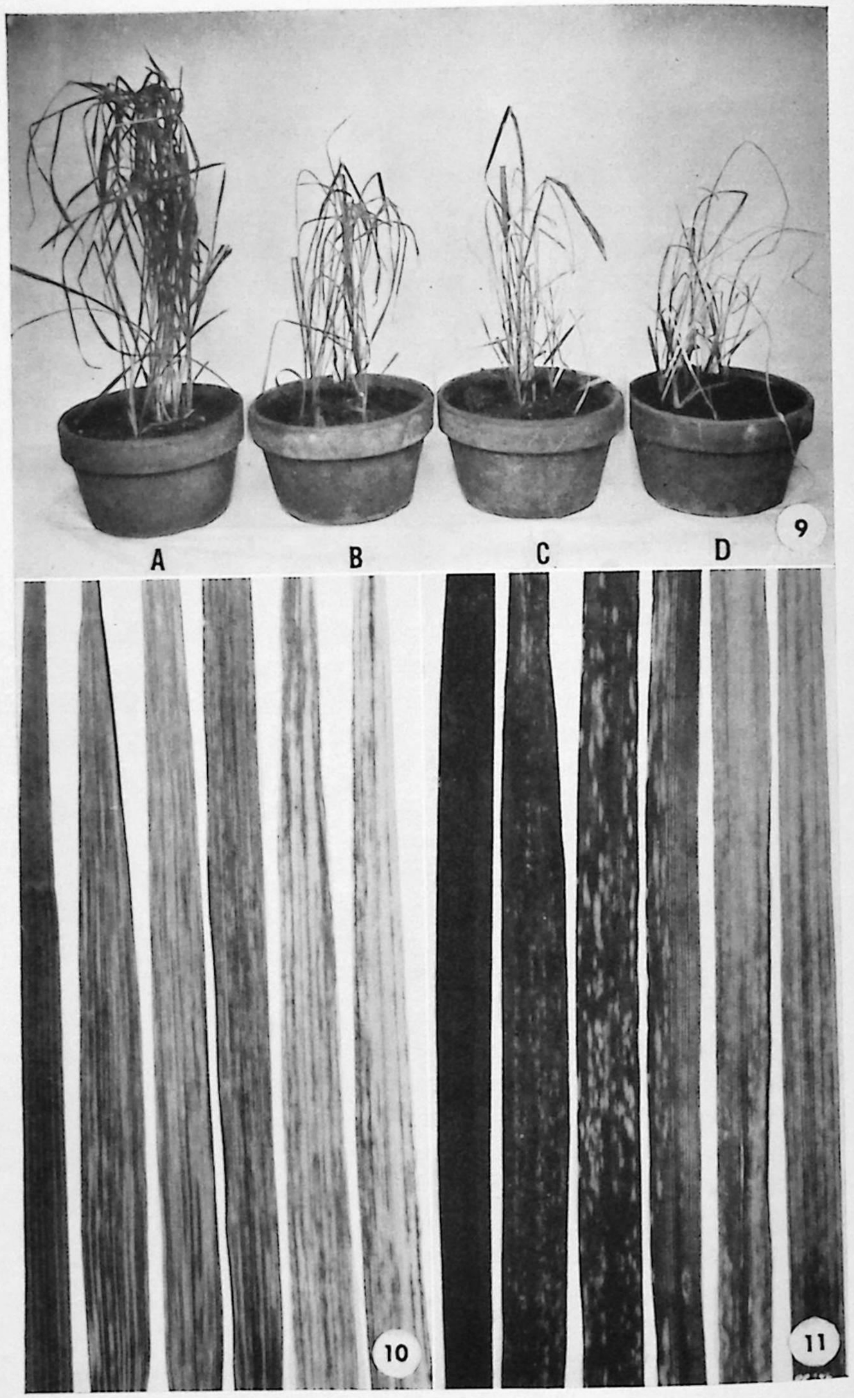
4. *Aceria tulipae in Relation to the Epidemiology of Wheat Streak Mosaic*

In contrast to the concern about mites on currants long before currant reversion was recognized, no attention was paid to eriophyid mites on wheat until *A. tulipae* was found to be the vector of WSMV. Since then this mite has received considerable attention and mite infestations on wheat have been correlated with the spread of the disease.

Usually all stages of *A. tulipae* can be found on immature diseased wheat. Although the mites may be extremely abundant they are usually overlooked unless a deliberate search is made with a $10\times$ lens. The sizes of eggs vary from $35 \times 42\,\mu$ to $46 \times 65\,\mu$; first nymphs, $33 \times 80\,\mu$ to $47 \times 150\,\mu$; second nymphs, $38 \times 140\,\mu$ to $57 \times 206\,\mu$; and adults, $38 \times 173\,\mu$ to $63 \times 285\,\mu$. Reproduction appears to be entirely parthenogenic on wheat and each individual can produce at least 12 to 16 eggs. Since the life cycle from egg to egg can be completed in 7 to 10 days at 24° to 27°C., tremendous populations can develop quickly on immature wheat during summer. The mites cause wheat leaves to roll inward, forming a tube within which the mites are protected. The tip of the new leaf emerging as the plant grows is often caught in the rolled leaf immediately below it; thus the new leaf is usually looped as well as rolled (Plate III, Fig. 9). Nonviruliferous mites do not normally cause chlorotic symptoms that could be confused with mosaic symptoms on wheat.

Wheat streak mosaic can be transmitted by the mites to barley, oats, corn, rye, and a number of wild annual grasses as well as to

Plate III



wheat. *Aceria tulipae* from wheat can colonize some common perennials including *Poa pratensis* L., *P. nervosa* (Hook.) Vasey, *Agropyron smithii* Rydb., and *Hordeum jubatum* L., which are apparently immune to WSMV. It has also colonized *Agropyron trichophorum* (Link) Richt., *Elymus canadensis* L., *E. virginicus* L., *Oryzopsis hymenoides* (Roem. & Schult.) Ricker, and *Poa compressa* L., a few plants of each of which have been infected with WSMV (Connin, 1956; McKinney and Fellows, 1951; Sill and Connin, 1953; Slykhuis, 1955; Staples and Allington, 1956). Some of these or other perennial grasses may be the natural reservoirs of WSMV and mites that attack wheat, but as yet no perennial that harbors both mites and WSMV has been shown to be a source of infection for wheat in the field.

Not all mites identified as A. *tulipae* on perennial plants will colonize wheat readily. Although Connin (1956) transferred *A. tulipae* successfully from naturally infested *Elymus canadensis* and *Agropyron smithii* to wheat, Slykhuis (1955) failed. Similarly, *A. tulipae* from *Poa compressa* in Alberta (Holmes *et al.*, 1961) did not survive when transferred to wheat. However, Del Rosario (1959) reported a low infestation of wheat with A. *tulipae* from *Agropyron smithii* (1.9%) and from onions (9.23%), and mites from both sources were able to transmit WSMV from wheat to wheat.

Although perennial plants have been considered the primary host of eriophyids because they provide a continuity of living tissue to sustain the mites around the year (Keifer, 1952), the annual plant, wheat, is the major host involved in epidemics of *A. tulipae* and WSMV. Immature wheat is such a favorable host that large populations of viruliferous mites can develop quickly. As the wheat matures or dies it cannot sustain the mites, but mites spread by wind or insects to other immature wheat multiply rapidly and perpetuate the population. Thus, winter wheat infected with mites and the virus in the fall harbors them over the winter. The next spring and summer the mites multiply on the overwintered wheat, which then may be a source of serious infection in spring wheat. Lightly infected spring wheat can carry mites and

---

Fig. 9. Effects on winter wheat of *Aceria tulipae* and viruses it carries. Kharhov 22 MC wheat was infested with (A) noninfective mites, (B) mites that acquired wheat streak mosaic virus from manually inoculated wheat, (C) mites carrying wheat spot mosaic virus only, and (D) mites carrying both wheat streak and wheat spot mosaic viruses.

Fig. 10. Mosaic symptoms caused on wheat by wheat streak mosaic virus transmitted by *Aceria tulipae*.

Fig. 11. Symptoms caused on wheat by wheat spot mosaic virus transmitted by *Aceria tulipae*.

virus without obvious damage. Volunteer wheat or shoots of hailed crops can perpetuate the species and the virus through late summer after the winter wheat crops have matured. Such infested immature wheat surviving through the summer until a new winter wheat crop emerges near it in the late summer or early fall can complete an annual cycle of immature wheat most favorable to mite and virus perpetuation. A break in the continuity of immature wheat almost eliminates the disease; therefore, this disease is not known in spring wheat in Alberta, Saskatchewan, or South Dakota outside the areas where winter wheat is grown, or in other wheat-growing areas where, for one reason or other, immature wheat is absent for long periods before a new crop is planted (Slykhuis, 1952, 1955).

Although wheat is the main plant to consider in the epidemiology of WSMV and *A. tulipae*, other less susceptible annuals such as barley may sometimes contribute to oversummering the disease. Fall rye is not readily infected with WSMV and is a poor host for mites; however, there was circumstantial evidence in 1964 that overwintered rye was a source of streak mosaic infection of some spring wheat fields in southern Alberta (Atkinson and Grant, 1964). This situation may have developed because conditions were exceptionally favorable for the spread of wheat streak mosaic to all susceptible hosts in September and October 1963 (T. G. Atkinson and Slykhuis, 1963).

5. *Mites in Relation to Disease Control*

Even before *A. tulipae* was found to be a vector of WSMV, it was realized that the early seeding of winter wheat near diseased immature wheat exposed the winter wheat to infection, and control recommendations were devised accordingly (Slykhuis, 1952). The discovery of the role of *A. tulipae* in virus spread accentuated the importance of most aspects of these measures and helped clarify the relative importance of virus hosts other than wheat.

Although certain native perennial grasses may be natural reservoirs perpetuating both the virus and the mites in areas where wheat streak mosaic develops frequently, the present evidence does not indicate that either annual or perennial wild grasses need be considered for effective control of the disease (Slykhuis, 1955; Staples and Allington, 1956). Control has therefore been centered principally around wheat. Although WSMV can spread surprisingly rapidly from diseased immature wheat into new crops nearby and can cause complete loss of crop yield, the preventive measures, which consist simply of an interruption of the overlapping sequence of immature wheat in the vicinity, can be spectacularly effective. In southern Alberta this is normally achieved

by refraining from planting winter wheat before early to mid-September, after all spring wheat in the area has matured, and by eliminating all immature wheat in the vicinity a week or more before seeding (Slykhuis *et al.*, 1957).

As was drastically illustrated in the fall of 1963, the date-of-seeding recommendation alone may not always be effective. In 1963 much of the spring wheat crop in some areas of southern Alberta emerged very late because of extreme drought from seeding time to late June. After heavy rains, a heavy growth of late spring wheat developed and did not mature until late September or early October. The weather remained very mild through September and until late October, and winter wheat seeded in the area even during the normally recommended seeding time, while there was still an abundance of spring wheat, became heavily infected. Thus, thousands of acres of winter wheat were destroyed or severely damaged by disease. Nevertheless, a few fields of winter wheat that were not seeded until spring wheat in the district was ripe and volunteer wheat eliminated suffered little or no damage (Atkinson and Slykhuis, 1963).

Late seeding and elimination of volunteer wheat are also effective in controlling streak mosaic in Nebraska (Staples and Allington, 1956), but the conditions necessary for control are sometimes difficult to achieve in Kansas, where the fall season is often long, warm, and favorable for continued growth of volunteer wheat, for the multiplication and spread of mites (King and Sill, 1959). Mosaic-tolerant varieties of wheat have been credited with reducing yield losses from the disease in Kansas.

Miticides to eliminate or reduce mite populations have been tested but none has proved sufficiently effective for practical control of the disease (Kantack and Knutson, 1958).

C. *Wheat Spot Mosaic*

1. *The Disease and Its Distribution*

Wheat spot mosaic virus (WSpMV) was detected when *A. tulipae* was proved a vector of WSMV in Alberta (Slykhuis, 1953b, 1955, 1956). It was frequently found associated with WSMV between 1952 and 1955, and was readily reisolated in 1958. Although cultures of the virus were lost in 1960, WSpMV was assumed to be readily available in association with WSMV until failures were encountered in attempts to reisolate it from diseased plants collected in Alberta in 1962 and 1963. However, it was reisolated from diseased wheat collected near Lethbridge in May 1964 (Slykhuis, 1964).

Very mild chlorosis symptoms induced by *A. tulipae* collected from wheat in Ontario (Slykhuis, 1961b) are believed to be caused by a mild strain of virus resembling WSpMV. Other than this, no one has reported a virus similar to WSpMV even from other areas where WSMV is a constant menace to wheat. The extent of its distribution even in southern Alberta is not known because it is difficult to recognize in the field, where symptoms are confused with wheat streak mosaic and the chlorotic effects of rust and other leaf diseases. It has been necessary to perform time-consuming transmission tests with mites to determine its presence in diseased wheat; hence a general survey of spot mosaic has not been undertaken.

2. *Detection and Differentiation of Wheat Spot Mosaic*

When mites from some of the wheat plants naturally diseased with wheat streak mosaic in southern Alberta were tested singly on wheat seedlings, some of the test plants, instead of developing streak mosaic symptoms, developed chlorotic spots in 3–10 days (Plate III, Fig. 11). Some leaves became severely chlorotic. Necrotic areas developed, often resulting in death of the entire plant. Since no manually transmitted virus was detected, the symptoms were at first attributed to a toxic effect of the mites, but the symptoms continued to develop even on plants freed of mites. Furthermore, mites reared from eggs hatched on healthy wheat did not produce such symptoms unless they were first colonized on diseased wheat. The symptoms were therefore attributed to a virus. The severity of symptoms caused by different isolates on young wheat plants varied from mild spotting and chlorosis to such severe effects that it was difficult to maintain cultures on wheat because of rapid death of plants.

Wheat spot mosaic has not been detected with assurance in the field. Chlorotic spots caused by WSpMV are usually masked by other leaf diseases. Since it has usually been found associated with WSMV, and a combination of the two viruses is synergistic, its presence has sometimes been detected because the plants it infected were more severely chlorotic, necrotic, and stunted than plants infected with WSMV alone (Plate III, Fig. 9).

Proof of the presence of WSpMV in diseased wheat has required the transfer of single mites from the naturally diseased wheat to single wheat test plants growing in sterilized soil in large, tightly plugged test tubes. Each plant that developed chlorotic spots or chlorosis had then to be tested by manual juice transmission tests to wheat to ascertain that the symptoms were not caused by WSMV.

3. *Relation of Aceria tulipae to WSpMV*

Like WSMV, WSpMV is carried by all stages of *A. tulipae* except the eggs. In one series of comparative tests with individual mites, 65% transmitted WSpMV and 34% transmitted WSMV. Mites reared on diseased wheat remained infective until the thirteenth day after transfer to *Lolium perenne* L. plants which were immune to the virus. Single mites simultaneously carried both WSMV and WSpMV.

Since WSpMV is so efficiently transmitted by the mite vector of WSMV, the general factors affecting epidemiology could be assumed to be similar if both viruses infect the same hosts. The two viruses appear to have a number of annual hosts in common, including barley, corn, rye, and several wild grasses, in addition to wheat. Not enough is known about the perennial hosts of either virus to indicate which are important natural reservoirs. It is expected that the reservoirs of the virus must also harbor *A. tulipae*.

4. *Control*

Since wheat spot mosaic has been found only associated with wheat streak mosaic, for control purposes it can be considered part of a wheat streak mosaic complex, and no separate measures are recommended for its control.

D. *Fig Mosaic*

Fig mosaic is the first tree disease demonstrated to be caused by a mite-transmitted virus.

1. *The Disease and Its Distribution*

In 1933 Condit and Horne (1933) described a mosaic disease of figs (*Ficus carica* L.) in California. It caused mottling, spotting, and distortion of leaves and was sometimes associated with leaf and fruit dropping. It has since been reported widespread in a number of countries, including Australia (Noble, 1934), the British Isles (Ainsworth, 1935), Bulgaria (Atanasoff, 1935), China (Ho and Li, 1936), New Zealand (Li and Procter, 1944), Italy (Graniti, 1954), and India (Nagaich and Vashisth, 1962).

Condit and Horne (1933) reported that of the five leading commercial varieties in California at that time, Kadota and Calimyrna were little affected. White Adriatic and Brown Turkey were sufficiently resistant under good cultural conditions, while Mission was severely affected. Of over 100 varieties in an experimental plot at Riverside, only one, an entire-leaf caprifig form of *F. palmata,* appeared to be immune. Other varieties appeared highly resistant, but most were susceptible. More recent inoculation tests in Florida showed fig trees in seven species to be susceptible, whereas *Cudronia tricuspidata* (Artocarpaceae) is the first susceptible host found outside the genus *Ficus* (Burnett, 1961a,b).

2. The Fig Mite and Its Effects

The fig mite, *Aceria ficus* (Cotte), is usually found in buds and on young leaves, but many become abundant on old leaves and inside fruit. It may cause russeting or browning on the plant surface, blasting of buds, chlorosis of leaves, and defoliation of branches or entire trees. Mosaic and leaf distortion are commonly associated with an abundance of mites, but it is difficult to differentiate these effects of the mites and effects of the mosaic virus.

3. Differentiation of Disease Symptoms

Condit and Horne (1933) concluded that mosaic symptoms of fig were caused by a virus because the symptoms appeared on new growth from symptomless seedlings grafted to diseased orchard trees. They also observed the fig mite and suggested that certain injuries, including leaf spotting, might result from the effects of mite feeding. As indicated by Flock and Wallace (1955), the graft transmission experiments of Condit and Horne were made on mite-infested trees; thus the mosaic could have resulted from toxicogenic effects of the mites rather than a graft-transmitted virus. They indicated similar difficulties in interpreting the cause of mosaic symptoms that developed on fig seedlings on which had been placed mite-infested bud scales and young leaves from field fig trees. Since the seedlings were not treated to eliminate mites, the symptoms could have resulted from the continuous presence of mites. However, they differentiated mosaic symptoms from the effects of mites by eliminating mites with sulfur. Then they demonstrated graft transmission in the continued absence of mites.

Transmission of fig mosaic virus by the mite *A. ficus* was proved by transferring mites from diseased trees to each healthy test seedling, and, after 3–5 days, killing the mites with sulfur. In the first tests, in which large numbers of mites were used on each plant, some mosaiclike symptoms appeared in less than 10 days. The plants partially recovered from these symptoms but later developed more severe symptoms. Mites from eggs that hatched on disease-free seedlings caused leaf distortion and slight chlorosis but no mosaic symptoms. This indicated that the earlier symptoms induced by mites from diseased trees

could be attributable to mite feeding, but the symptoms developing after the tenth day were attributed to fig mosaic virus.

4. Relation of Mites to Disease Spread

In tests with one viruliferous mite per test plant, 7 out of 10 plants developed mosaic symptoms, and with larger numbers nearly all test plants were infected. With such an efficient vector it is not surprising that all field-grown fig trees are infected in California, where the fig mite is abundant. Although this disease has not been considered of major importance, it is so universal that there is no standard of comparison to indicate the effect of fig mosaic and the fig mite on yield of any variety.

5. Control

Flock and Wallace suggested that it would be necessary to start mosaic-free fig trees in isolated plantings to determine the possible benefits of field control, but they stated there were no virus-free fig varieties available in California. However, satisfactory production continues, apparently because the varieties in commercial production are not severely affected by the disease.

E. Peach Mosaic

Peach mosaic was the second disease of trees experimentally transmitted by eriophyid mites.

1. The Disease and Its Distribution

Peach mosaic was described as a new virus disease of peaches in Texas in 1932 (Hutchins, 1932). Most of 56 nursery peach trees grafted or budded in July 1931 with Texas material showing symptoms suggestive of mosaic developed similar symptoms in 1932. These included shortening of the internodes, profuse growth in leaf axil buds, and mosaic patterns on leaves, which were often small, narrow, crinkled, and irregular in outline. The symptoms also developed on new aerial growth and suckers and was communicated by grafting either roots or shoots. There appeared no doubt that this disease was caused by a graft-transmissible virus.

According to the first report, the disease was found only in two orchards in Texas; hence further spread from these sources by grafting could easily be avoided. However, in 1934 a severe outbreak of mosaic was reported in Colorado, where in some orchards all peach trees were infected (Bodine, 1934). Eradication was begun in attempts to curtail spread. By 1937 it was also recognized in California, Utah, New Mexico, and Arizona (Hutchins *et al.*, 1937). It appeared to be so highly contagious and destructive that the commercial value of an entire planting could be destroyed in 3 to 6 years. Vigorous eradication measures as initiated in Colorado (Bodine, 1936) were adopted in other states.

Control by eradication required recognition of the disease in other hosts. The virus was transmitted by grafting from almond, apricot, plum, and prune, but some of these hosts were symptomless carriers (Cochran and Hutchins, 1938). However, the effectiveness of such hosts as sources of infection was not easily assessed when the natural means of transmission was not known. Experiments in Colorado gave what appeared to be proof that the green peach aphid [*Myzus persicae* (Sultz.)] transmitted the virus (Daniels, 1945). Although experiments in California showed that the virus spread throughout the summer, neither *M. persicae* nor any of the many other insects tested transmitted the virus (Jones and Wilson, 1951).

Peach mosaic was also reported in a number of countries in Europe including Bulgaria, Czechoslovakia, Holland, and England (Atanasoff, 1934). The virus was transmitted by budding from peach, plum, and cherry to peach: from apricot, cherry, and plum to plum; and from plum to cherry. Also there was evidence that *Anuraphis padi* transmitted the mosaic virus from plum to plum.

There have been various other reports of mosaic disease of stone fruits, but there is no assurance that all are caused by the peach mosaic virus (Smith, 1957).

2. *Eriophyid Mites in Relation to Mosaic Symptoms*

Vasates cornutus (Banks), the peach silver mite, which is widespread in North America, causes a silvering of peach foliage, and since it was also associated with mosaiclike symptoms, Wilson and Cochran (1952) suspected that it could be transmitting a virus. In their experiments they found that the feeding of *V. cornutus* induced the "yellow spot" disease of peaches, which included vein-associated chlorosis, longitudinal rolling of leaves, and stem spotting as well as yellow spotting of leaves. A 24-hour feeding period by the mites was sufficient to produce symptoms, and there was a 10-day delay in their appearance suggestive of the behavior of a virus. However, patch bark grafting with precautions to assure that no mites were present failed to show that a virus was involved.

After more than 8000 unsuccessful tests with more than 150 species of insects and mites, Wilson *et al.* (1955) found a previously undescribed mite later named *Eriophyes insidiosus* (Keifer and Wilson, 1956). It occurred beneath bud scales and on rudimentary leaves and new growth

of peach and plum. Individuals of this species were transferred from naturally infested, mosaic-diseased field peach and plum trees to 65 healthy, potted, Rio Oso Gem peach seedlings in the greenhouse. Mosaic symptoms developed in 14 to 100 days on 16 of 56 seedlings that received mites from diseased peach and on 1 of 9 receiving mites from Hortulan plum. The presence of mosaic virus was confirmed by patch bark graft transmission to other healthy seedlings in the absence of mites. Check plants that had received equivalent numbers of mites from healthy peach and plum did not develop symptoms.

3. *Differentiation of Peach Mosaic from the Effects of Mites*

It appears that, except for the symptoms caused by *V. cornutus* on peaches, there was never a major difficulty in differentiating the symptoms of peach mosaic from the effects of eriophyid mites. The ability to transmit peach mosaic virus, but not the toxicogenic effects of the mites, by patch bark grafting in the absence of mites differentiated the effects of the virus from those of the mites. Since nonviruliferous *E. insidiosus* was available from mosaic-free peach and plum, and mites from eggs laid in buds of diseased trees but hatched on healthy seedlings were nonviruliferous, the effects of the virus transmitted by the mites could be readily differentiated from the effects of the mites.

One difficulty in assessing transmissibility of the virus either by grafting or mite transmission is the variability of the incubation period of the virus in peaches. In experiments done in the greenhouse, symptoms on some graft-inoculated seedlings may appear in 14 days but on others not until the next season. In the field, trees inoculated when breaking dormancy in spring may develop mosaic in 3 to 6 weeks, but if trees are in foliage when inoculated, symptoms may not develop until the next season.

4. *Eriophyes insidiosus in Relation to Peach Mosaic Virus*

Unpublished results of experiments by Wilson and Jones,* reported in personal communication, confirmed that *E. insidiosus* transmitted peach mosaic virus. With 1 mite transferred from diseased peach to each of 80 test plants, 2 plants, or 2.5%, developed mosaic symptoms. With increased numbers—up to 30 to 50 mites per plant—67 to 75% of the plants developed symptoms. Successful transmission resulted only when mites were transferred to buds rather than to leaf blades or stems. Infective mites retained the ability to transmit the virus for at least 2 days on glass slides in the absence of a virus source. Mites

* Entomology Research Branch, Agricultural Research Service, U.S. Department of Agriculture, Washington, D.C.

from eggs hatched on healthy seedlings did not cause mosaic symptoms on test plants.

5. Mites in Relation to Control of Peach Mosaic Virus

Peach mosaic eradication programs were in effect in several states long before *E. insidiosus* was found to be a vector of the virus. Despite problems created by other hosts, some of which are symptomless carriers of the virus, eradication of diseased trees greatly reduced mosaic spread (Bodine and Durrell, 1941).

The extent and effectiveness of the peach mosaic suppression program started in Mesa County, Colorado, in 1934 was indicated in a review by List *et al.* (1956). During 21 years, 125,000 trees were found infected. Of these, 36.5% were found and removed during the first 2 years. The average number of infected trees per 1000 inspected was 35.7 during the first 3 years and only 6.5 for the last 17 years, with no significant decrease during the latter period. These figures indicate that the major sources of infection were greatly reduced during the first 3 years. Through the remaining years the percentages of currently infected trees were about 10 times higher in orchards in which the disease had already been recorded than in orchards with no previous infections. Incidence was higher in orchards containing tolerant varieties because the disease may be present but not recognized on tolerant trees.

Evidently *E. insidiosus* occurs on trees not infected with mosaic but causes no apparent damage. There is little incentive for a program to control this mite except when the virus is present. No miticidal spray program that would be as effective as the present program to eradicate diseased trees has developed.

F. Ryegrass Mosaic

1. Recognition of Ryegrass Mosaic Virus and Its Vector

There was no long history between the discovery of ryegrass mosaic virus (RMV) in England and the recognition of an eriophyid mite as its vector. Mosaic symptoms were noted to be quite common on both perennial ryegrass (*Lolium perenne* L.) and Italian ryegrass (*L. multiflorum* Lam.) in 1956–1957 (Slykhuis, 1958) and a virus was readily transmitted manually, by the leaf-rubbing method, to ryegrass seedlings. The symptoms included light-green to yellow mottling and streaking. Some isolates affected some strains of ryegrass, causing severe brownish necrosis of leaves and reduced growth and vigor of plants.

Three species of eriophyid mites found on diseased ryegrass were tested as vectors by transferring them singly from naturally diseased

ryegrass to S.22 Italian ryegrass seedlings (Mulligan, 1960). Some of the mites transmitted the virus, and these were identified as *Abacarus hystrix* (Nalepa), which, like *A. tulipae,* reproduce by eggs without fertilization. All stages except eggs carried the virus. Nonviruliferous mites acquired RMV during acquisition access periods of 2 to 12 hours but lost infectivity within 24 hours on wheat immune to the virus.

2. Relation of Mites to the Epidemiology of Ryegrass Mosaic

Perennial ryegrass appears to provide a widespread and permanent reservoir of RMV and the mite *A. hystrix.* Some perennial ryegrass plants appear to be so susceptible they die, but many others, even in old leys, appear to be virus-free. However, plants with mild symptoms are common and probably are the main reservoirs of viruliferous mites. Susceptible strains of Italian ryegrass, a biennial, have been found heavily infected early in the second year, indicating that RMV had spread extensively the first summer. Mulligan (1960) reported diseased plants infected with mites in ryegrass leys only 4 months old.

3. Differentiation of Viruses Causing Mosaics of Ryegrass

After a survey for virus diseases of grasses in Northern Europe in 1957, the present author reported observing mosaic symptoms on ryegrass in 7 of 8 countries visited (Slykhuis, 1958). Mosaic symptoms were also observed on cocksfoot (*Dactylis glomerata* L.) in 6 of the countries. Some of these symptoms were probably caused by the aphid-transmitted cocksfoot streak mosaic virus (Smith, 1957). However, Mulligan (1960) found that cocksfoot streak virus readily infected ryegrasses, and RMV infected cocksfoot. It is not known which virus caused symptoms observed on different ryegrass and cocksfoot plants during the general field surveys. The diagnosis is further complicated by another quite distinct virus causing ryegrass mosaic in Germany (Ohmann-Kreutzberg, 1963). This virus has a spherical particle and is transmitted by nematodes and not by aphids or mites (Schmidt *et al.*, 1963).

A virus causing a mosaic of ryegrass and orchard grass (*D. glomerata*) in Washington was reported by Bruehl *et al.* (1957). We found a similar virus on ryegrass in British Columbia in 1964. These viruses, like English RMV, are associated with rod-shaped particles, but the natural vectors and other relationships have not been determined.

G. Suspected Virus Transmission by Eriophyidae

Negative results are seldom reported; consequently, it is not known how often mites have been tested as vectors of plant viruses. However, there are some diseases which may be caused by mite-transmitted viruses, but doubt remains because evidence of the other workers has been negative, or confirmatory experiments have not been done or have yielded inconclusive results.

1. Vine Panaschüre

It was reported that one of three viruses attacking vines in Germany was transmitted by *Eriophyes vitis, Phylloxera vastatrix, Otiorrhynchus sulcatus, Doralis (Aphis) fabae, Myzus persicae,* and a number of root-attacking nematodes (Ochs, 1958a). In another report, this virus, designated as "Panaschüre (Virus 1), which produced a leaf mosaic of vine, was sap-transmitted to Samsun tobacco, cucumber, and *Chenopodium quinoa.* It was also transmitted from tobacco to tobacco by *Cuscuta campestris* and *Myzus persicae,* and from vine to other test plants by *Eriophyes vitis.* The virus had spherical particles and was considered to belong to the cucumber mosaic group (Ochs, 1958b).

The above claims were refuted by Niemeyer and Bode (1959) after all trials to transmit any virus from vines to herbaceous test plants failed. Tests with the same vines originally used by Miss Ochs were also negative. This would seem to negate the claim that eriophyid mites transmitted a virus as described, but in a later report by Ochs (1960), much of the original claim was repeated, i.e., that *Eriophyes vitis, Doralis (Aphis) fabae, Myzus persicae, Phylloxera vastatrix,* and *Otiorrhynchus sulcatus* can transmit Panaschüre virus from infected to healthy vine stocks. This claim is exceptional in that no other virus transmitted by an eriophyid mite has been found to have other vectors. At best, it must remain a suspected example of mite transmission until convincing experimental evidence is presented.

2. Cherry Mottle Leaf

Cherry mottle leaf virus causes mottling, distortion, and necrosis of leaves, and reduction in size, yield, and quality of fruit of sweet cherries (*Prunus avium*) in a number of the western states and in British Columbia, Canada (Smith, 1957). It has also been reported in Europe. Strains differing in severity occur. Sour cherry is highly resistant, and peach is a symptomless carrier.

In a personal communication Jones reported that in 1959 an unidentified eriophyid mite was found on wild bitter cherry (*Prunus emarginata*) and sweet cherry. Mites from diseased *P. emarginata* were moved to healthy Rio Oso Gem peach seedlings. Since peach is a symptomless host, no symptoms developed, but tissue from each of the 21 exposed peach trees and 5 check peach trees was implanted into healthy Bing cherry trees. Twelve of the cherry trees developed cherry mottle leaf symptoms, indicating that the mites had transmitted the virus to the peach seedlings. Since confirmatory results have not been published this must still be considered as suspected mite transmission.

3. Agropyron Mosaic

Agropyron mosaic virus (AMV) has been found on *Agropyron repens* (L.) Beauv. in several widespread locations in North America, including Virginia, Nebraska, South Dakota, Saskatchewan, Ontario, and other eastern provinces in Canada. In Ontario the virus causes mosaic symptoms on wheat resembling wheat streak mosaic (Slykhuis, 1962). The virus particle is indistinguishable in size from that of WSMV, and this similarity prompted Staples and Brakke (1963) to conclude that it was merely a mild strain of WSMV with a slightly different host range. However, our unpublished results show there are a number of important differences between AMV and WSMV, particularly the synergism between these viruses in cross-inoculation tests; the absence of close serological relationships indicates that they are not as closely related as might be concluded from the similarities of particles.

Like WSMV, AMV spreads rapidly in wheat under certain conditions in the field. Winter wheat experimentally seeded in early summer near diseased *A. repens* becomes infected. Infection has also resulted when wheat seedlings growing in pots covered with cages made of 72-mesh-per-inch screen that excludes insects down to the size of thrips were left in the field near naturally diseased *A. repens* or wheat for a few days. Most experiments have indicated that the vector must be small. Eriophyids are usually found on naturally infected plants but tests with these mites transferred manually from diseased to healthy plants have not given conclusive results.

4. Cadang-Cadang Disease of Coconuts

Cadang-cadang is a destructive degenerative disease of coconut that appears to be spreading slowly but relentlessly in the Philippines (Price, 1958). Although it has not been proved that cadang-cadang is caused by a virus, several reports favor this view. Since the spread of the disease is exceedingly slow, the recent research has been for either a very inefficient vector or one which is either sedentary or for some other reason rarely moves from one host to another. Eriophyid mites have been found on diseased coconut and on other apparently susceptible hosts and tests have been started to determine if any of these could be vectors (Briones and Sill, 1963). Since the disease appears to have a long incubation period, results of transmission tests cannot be expected quickly (Sill *et al.*, 1963).

5. *Pigeon Pea Sterility*

Seth (1962) has reported that pigeon pea sterility is caused by a virus transmitted by an unidentified eriophyid mite in India.

The disease was characterized by mosaic symptoms on the leaves and partial sterility of flowers of the pigeon pea [*Cajanus cajan* (Linn.) Millsp.]. The cause was not transmitted by sap inoculation, but was successfully transmitted by wedge grafting in 35 out of 99 plants of pigeon pea variety N.P. 69. None of the leafhoppers, aphids, white flies, or thrips tested transmitted a causal virus, but three species of mites were found on leaves of diseased pigeon pea and successful transmissions were obtained with one of these, which was an eriophyid. Two types of mite-transfer tests were done. For one, detached leaves of infected plants bearing mites were placed on the healthy test plants and covered with a glass chimney for 24 hours to force the mites to move to the fresh leaves of the test plants from the withering leaves. For the other, mites were transferred individually from diseased leaves, 5–20 being placed on each test plant. After 4 days the plants were regularly sprayed with 0.1% Ekatox to kill the mites. Disease symptoms appeared in 3–5 weeks on 31 of 136 plants inoculated. Similar methods were used in another experiment to compare the effects of eriophyid mites from healthy pigeon pea with the effects of the ones from infected plants. The mites from healthy plants did not cause the symptoms on any of 53 test plants, but mites from diseased plants evidently infected 11 of 54 test plants.

This report indicates that eriophyid mites from leaves with disease symptoms induced symptoms on test plants, but these symptoms of pigeon pea sterility disease could as readily be attributed to toxicogenic effects of the mites as to a virus. There is no proof that mites were not present on scions used for graft transmission, or that mites were eliminated by the Ekatox sprays used 4 days after mites were transferred to test plants. Mites from healthy plants cannot be unconditionally considered adequate controls for mites from diseased plants because they could have been a different strain or species. There was no evidence that nonviruliferous mites, which will cause symptoms only after they have fed on diseased plants, can be obtained.

IV. Virus Transmission by Tetranychidae

A. *Potato Virus Y*

1. *The Disease and Its Distribution*

Recent volumes of *Review of Applied Mycology* contain abstracts of reports on potato virus Y (PVY) in potato, tomato, tobacco, and other plants, most of which are Solanaceae, on all continents. According to Smith (1957), this virus is common on potato in southeastern Ireland and in the south and east of England, where the symptoms are of the leaf-drop streak type or acropetal necrosis. In severe epidemics affected fields appear as though attacked by "blight." On the other hand, it is rare in Scotland and the best potato-growing districts of Ireland. It is common in many parts of North America where it is known as "vein-banding virus." It is of great economic importance either alone or in combination with potato virus X.

There are many strains of PVY which differ widely in virulence and cause diseases in Majestic potato ranging from severe leaf-drop streak to mild mosaic.

The virus is transmitted readily by artificial inoculation and except for one strain (potato virus C) it is transmitted efficiently by *Myzus persicae* and some other aphids. It has a nonpersistent relationship with the aphid vectors, which acquire it during short feeds and can transmit it immediately. The virus particles are long and flexuous.

2. *The Two-Spotted Spider Mite, Tetranychus telarius (L.)*

Tetranychus telarius (L.) is a common pest on a wide range of plants in the greenhouse and in the field (Metcalf *et al.*, 1952). Damaging infestations have been reported on potatoes in the Fraser Valley, British Columbia (Fulton, 1957).

3. *Relation of Tetranychus telarius to Potato Virus Y*

While experimenting with PVY in the greenhouse, Schulz (1963) noticed that infestations by *T. telarius* may have been responsible for the transmission of the virus to a single potato plant. In subsequent controlled experiments the mite transmitted two isolates of PVY to Red Pontiac potato. Optimum transmission was obtained when acquisition and test-feeding periods of 5 minutes were used. In a series of tests, with 5 mites per test plant, 18/37 potato plants were infected by mites given 5-minute acquisition and test-feeding periods. It appears the PVY was transmitted by the mite *T. telarius* from potato and *Nicotiana glutinosa* to potato in a manner characteristic of nonpersistent viruses.

More investigations are required to determine the role of *T. telarius* as a vector of different strains of PVY, and to assess its significance as a vector in the field.

B. Suspected Virus Transmission by Tetranychidae

Tetranychids have sometimes been found associated with plant diseases and have been suspected as vectors of viruses (Heinze, 1959). Schuster (1963), studying transmission of tobacco ringspot virus in the lower Rio Grand Valley, demonstrated transmission of the virus by the tobacco flea beetle, but not by white flies or tetranychid mites that were present. The possible transmission of a cause of cacao cushion gall in Costa Rica by two species of mites was suggested by Lara and Shenefelt (1962), but no experimental evidence was presented.

In other instances there have been indications of closer causal relationships between mites and disease symptoms.

1. Cotton Curliness

A virus causing "cotton curliness" disease in Azerbaijan was the first reported to be transmitted by a tetranychid mite (Moskovets, 1941). This virus was manually transmitted by infusing infected sap into decapitated stems of cotton plants (*Gossypium barbadense*). It was transmitted by aphids including *Aphis gossypii* (85–100%), *A. laburni* (6.1–16.6%), *Myzus persicae* (12.5–25%), and by the mite *Epitetranychus althaeae* (7.7–10%). The incubation period of the virus varied from 35 to 56 days. There was some evidence of seed transmission.

Since the incubation period of the virus was so long, it appears that the low percentage of transmissions attributed to the mites could have been caused by one of the indicated alternative means of transmission. These uncertainties and the lack of subsequent confirmation cast doubt on this claim of mite transmission of a virus.

2. Mosaic Symptoms on Wheat

In 1961 a new species of spider mite, *Tetranychus sinhai* Baker, became very abundant on barley in southern Manitoba (Wallace and Sinha, 1961). The mites caused a darkening, followed by yellowing and withering along the midrib and in patches. Occasionally the leaf edges were crinkled. Light infestations were also observed in rye, corn, and wheat. In barley and rye, symptoms appeared to be caused by the toxic effect of mite feeding. In wheat, fewer mites were found, but mosaiclike yellowing of the leaves suggested the possibility that the mites transmitted a virus. However, no experimental results were reported to support the hypothesis.

V. Conclusions

Only 1 tetranychid and 5 eriophid mites have been proved to be vectors of plant viruses (Table I), but others are suspected. Although these vectors all feed with piercing and sucking mouth parts, the relations of the mites in the two families to hosts, and to the viruses they transmit, differ greatly. *Tetranychus telarius* has a wide, nonspecific range of hosts. It also appears to be a nonspecific or casual vector of potato virus Y, which is readily transmitted manually and by aphids and has a nonpersistent relationship with both the aphids and the mite vector. In contrast, each of the eriophid mites has a restricted host range and each is the only vector known for the virus or viruses it transmits. WSMV and RMV are the only eriophyid-transmitted viruses that have been also transmitted by artificial sap inoculation. Of these, RMV appears to be nonpersistent in that its vector loses infectivity within 24 hours after removal from diseased leaves, but WSMV can persist up to 9 days in its vector on an immune host. WSMV and WSpMV persist in *A. tulipae* through the molts between instars. There are indications that the other eriophyids that are vectors also have very specific relationships with the viruses they transmit as well as with their hosts.

Although all the mite-transmitted viruses except WSpMV have been reported as causes of disease in a number of countries, only one, WSMV, has been shown by many investigators to be mite-transmitted in several agricultural regions and countries. Despite the wide geographical distribution of fig mosaic and peach mosaic, mite transmission of each of the causal viruses has been reported only in California. Similarly, currant reversion and ryegrass mosaic have been observed in different countries in Europe, but mite transmission of the viruses has been demonstrated only in southern England. However, it must be remembered that different viruses may cause similar symptoms. For example, similar mosaic symptoms may be caused on ryegrass by the aphid-transmitted cocksfoot streak virus and a nematode-transmitted virus as well as by the mite-transmitted RMV. Therefore, the identification of the above diseases in each different situation should, if possible, include the verification of mite transmissibility of the causal virus.

Usually, the most important initial difficulty in the investigation of mite transmission of plant viruses is that of differentiating symptoms of virus disease from possible toxicogenic effects of mites. The differentiation can be achieved readily if the virus can be transmitted by manual sap inoculation. Grafting diseased buds or shoots to determine if a graft-transmissible virus is present can be hazardous because of the

TABLE I
PLANT VIRUS TRANSMISSION BY MITES

	Currant reversion	Wheat streak mosaic	Wheat spot mosaic	Fig mosaic	Peach mosaic	Ryegrass mosaic	Potato virus Y
Disease recognized	Europe, 1904	United States, 1922–1932	Canada, 1952	United States, 1933	United States, 1932	England, 1956	United States, 1920
Virus identified	England, 1927	United States, 1932	Canada, 1952	United States, 1933	United States, 1932	England, 1956	United States, 1920
Distribution	British Isles, north Europe	Western United States, Alberta (Can.), Rumania	Alberta (Can.)	United States, British Isles, Europe, India, Australasia	Western United States Europe,	British Isles, Europe, Washington (United States) British Columbia (Can.)	North and South America, Europe, Asia, Australasia
Virus hosts	*Ribes nigrum* *R. vulgare* *R. sanguinea*	*Triticum* spp., some other Gramineae	*Triticum* spp., some other Gramineae	*Ficus* spp.	*Prunus* spp.	*Lolium* spp., some other Gramineae	Mainly *Solanaceae*, *Chenopodium* spp.
Disease symptoms	Reduced sepal hairs, altered leaf shape, veination, and serration	Chlorotic streaking, stunting	Spotting, chlorosis, stunting	Leaf mosaic and distortion, leaf and fruit fall	Leaf mosaic, crinkling and deformity, stunting	Mosaic, streaking, browning, necrosis	Mosaic, vein banding, leaf drop, streak, necrosis
Incubation period	1–3 years	5–10 days	3–10 days	10–90 days	14–100 days	10–14 days	7–28 days
Nonmite transmission	Graft	Sap	None	Graft	Graft	Sap	Sap, aphids

TABLE I (*Continued*)

	Currant reversion	Wheat streak mosaic	Wheat spot mosaic	Fig mosaic	Peach mosaic	Ryegrass mosaic	Potato Virus Y
Mite vector	*Phytoptus ribis*	*Aceria tulipae*	*Aceria tulipae*	*Aceria ficus*	*Eriophyes insidiosus*	*Abacarus hystrix*	*Tetranychus telarius*
Mite hosts	*Ribes* spp.	Gramineae, Liliaceae	Gramineae, Liliaceae	*Ficus* spp.	*Prunus* spp.	Gramineae	Numerous spp. in many families
Mite toxicogenic effects	Big buds, leaf distortion	Leaf roll	Leaf roll	Chlorosis and browning, leaf distortion, defoliation	—	—	Spotting, discoloration, necrosis
Mite vection reported	England, 1927; 1952	Canada, 1952; United States, 1953; Rumania, 1962	Canada, 1952	California, 1955	California, 1955	England, 1960	North Dakota (United States), 1963
% mites transmitting	—	34%	65%	70%	2.5%	30%	40% infection, 5 mites/plant
Egg transmission	—	0	0	0	0	0	—
Virus acquisition	—	30 minutes	1 day	—	—	—	5 minutes
Virus retention	—	9 days	13 days	—	2 days	12 hours	—
Disease control	Eradication, miticidal sprays	Eliminate diseased wheat before seeding	Eliminate diseased wheat before seeding	Resistance	Eradication	Resistance	Eradication

difficulty of ascertaining that the tiny mites are absent. Patch bark grafting or implanting has been useful for tree viruses like peach mosaic virus, because mites can more readily be eliminated from such scion material than from buds. Differentiation of mite symptoms from virus symptoms can also be achieved if nonviruliferous mites can be obtained by hatching eggs on healthy plants. Particularly difficult problems arise in attempts to differentiate symptoms when symptoms caused both by mites and virus develop slowly, and if the host is difficult to protect from accidental infestation by mites or other possible vectors.

The extremely small size of eriophyid mites and the preferences of many for feeding in protected places within buds or in leaf whorls create difficulties in timing inoculation feeds on test plants. It is almost impossible to observe such mites sufficiently closely while they are feeding. Furthermore, it is difficult to terminate the feeding of mites hidden within buds and leaf whorls, even with the use of miticides, without damaging the plants.

References

Ainsworth, G. C. (1935). *J. Roy. Hort. Soc.* **60**, 532–533.
Amos, J., and Hatton, R. G. (1927). *J. Pomol. Hort. Sci.* **6**, 167–183.
Amos, J., Hatton, R. G., Knight, R. C., and Massee, A. M. (1927) *Ann. Rept. East Malling Res. Sta. Kent,* Suppl. II, 126–150.
Anonymous. (1927). *Brit. Min. Agr. Leaflet* **377**, 10 pp.
Anonymous. (1929). *Tidsskr. Planteavl* **35**, 420–471.
Ashworth, L. J., Jr., and Futrell, M. C. (1961). *Plant Disease Reptr.* **45**, 220–224.
Atanasoff, D. (1934). *Ann. Univ. Sofia* **13**, 9–42.
Atanasoff, D. (1935). *Phytopathol. Z.* **8**, 197–223.
Atkinson, T. G., and Grant, M. N. (1964). *Can. Plant Disease Survey* **44**, 259–264.
Atkinson, T. G., and Slykhuis, J. T. (1963). *Can. Plant Disease Survey* **43**, 154–159.
Atkinson, D. E. (1949). *Phytopathology* **39**, 2.
Atkinson, D. E. (1952). *Dissertation Abstr.* **12**, 375.
Baker, E. W., and Wharton, G. W. (1952). "An Introduction to Acarology," pp. 211–219. Macmillan, New York.
Bodine, E. W. (1934). *Plant Disease Reptr.* **18**, 123.
Bodine, E. W. (1936) *Bull. Colo. Agr. Expt. Sta.* **421**, 11 pp.
Bodine, E. W., and Durrell, L. W. (1941). *Phytopathology* **31**, 322–333.
Brandes, J., and Wetter, C. (1959) *Virology* **8**, 99–115.
Briones, M. L., and Sill, W. H., Jr. (1963). *FAO Plant Protect. Bull.* **11**, 25–30.
Bruehl, G. W., and Keifer, H. H. (1958). *Plant Disease Reptr.* **42**, 32–35.
Bruehl, G. W., Toko, H., and McKinney, H. H. (1957). *Phytopathology* **47**, 517.
Burnett, H. C. (1961a). *Rev. Appl. Mycol.* **40**, 582.
Burnett, H. C. (1961b). *Proc. Fla. State Hort. Soc.* **73**, 316–320.
Chiu, R. J., and Sill, W. H., Jr. (1963). *Phytopathology* **53**, 1285–1291.
Cochran, L. C., and Hutchins, L. M. (1938). *Phytopathology* **28**, 890–892.
Condit, I. J., and Horne, W. T. (1933). *Phytopathology* **23**, 887–889.
Connin, R. V. (1952). *U.S. Dept. Agr. Spec. Rept.* **H-14**, 32 pp.

Connin, R. V. (1953). *U.S. Dept. Agr. Spec. Rept.* **W-13**, 10 pp.
Connin, R. V. (1956). *J. Econ. Entomol.* **49**, 1–4.
Cropley, R., Posnette, A. F., and Thresh, J. M. (1964). *Ann. Appl. Biol.* **54**, 177–182.
Daniels, L. B. (1945). *Science* **101**, 87–88.
Del Rosario, M. S. E. (1959). *Dissertation Abstr.* **20**, 863–864.
Dounin, M. S. (1939). *Rev. Appl. Mycol.* **18**, 697–700.
Fellows, H. (1949). *Plant Disease Reptr.* **33**, 356–358.
Finley, A. M. (1957). *Plant Disease Reptr.* **41**, 589–591.
Flock, R. A., and Wallace, J. M. (1955). *Phytopathology* **45**, 52–54.
Fulton, H. G. (1957). *Proc. Entomol. Soc. Brit. Columbia* **54**, 44.
Gibson, W. W., and Painter, R. H. (1957). *J. Kansas Entomol. Soc.* **30**, 147–153.
Glaeser, G. (1961). *Pflanzen schutz Ber.* **26**, 19–28.
Graniti, A. (1954). *Riv. Frutticolt. Viticolt. Orticolt* **16**, 23–25.
Heinze, K. (1959). "Phytopathogene Viren und ihre Überträger," 292 pp. Duncher und Humblot, Berlin.
Ho, W. T. H., and Li, L. Y. (1936). *Lingnan Sci. J.* **15**, 67–78.
Holmes, N. D., Swailes, G. E., and Hobbs, G. A. (1961). *Can. Entomologist* **93**, 644–647.
Houston, B. R., and Oswald, J. W. (1952). *Phytopathology* **42**, 12.
Hutchins, L. M. (1932). *Science* **76**, 123.
Hutchins, L. M., Bodine, E. W., and Thornberry, H. H. (1937). *U.S. Dept. Agr. Circ.* **427**, 48 pp.
Jones, L. S., and Wilson, N. S. (1951). *Bull. Calif. Dept. Agr.* **40**, 117–118.
Jørstad, I. (1928). "Beretning om Plantesykdommer i Land- og Hagebruket. V. Hagebrukets Nyttevekster," 68 pp. Grøndahl & Søns Boktykkeri, Oslo.
Kantack, E. J., and Knutson, H. (1958). *J. Econ. Entomol.* **51**, 68–72.
Keifer, H. H. (1952). *Bull. Calif. Insect Survey* **2**, 123 pp.
Keifer, H. H., and Wilson, N. S. (1956). *Bull. Calif. Dept. Agr.* **44**, 145–146.
King, C. L., and Sill, W. H., Jr. (1959). *Plant Disease Reptr.* **43**, 1256–1257.
Kristensen, H. R., and Christensen, M. (1957). *Tidsskr. Planteavl* **61**, 1–16.
Lara, E. F., and Shenefelt, R. D. (1962). *Rev. Appl. Mycol.* **41**, 219–220.
Lees, A. H. (1925). *Ann. Appl. Biol.* **12**, 199–210.
Li, L. Y., and Procter, C. H. (1944). *New Zealand J. Sci. Technol.* **A26**, 88–90.
Lihnell, D. (1949). *Sveriges Pomol. Fören Arsskr.* **50**, 36–50.
List, G. M., Landblom, N., and Sisson, M. A. (1956). *Colo. Agr. Expt. Sta. Tech. Bull.* **59**, 28 pp.
McKinney, H. H. (1937). *U.S. Dept. Agr. Circ.* **442**, 23 pp.
McKinney, H. H. (1944). *J. Wash. Acad. Sci.* **34**, 322–329.
McKinney, H. H., and Fellows, H. (1951). *Plant Disease Reptr.* **35**, 441–442.
McLarty, H. R. (1942). *22nd Ann. Rept. Can. Plant Disease Survey,* p. 82.
McNeal, F. H., and Dubbs, A. L. (1956). *Plant Disease Reptr.* **40**, 517–519.
Massee, A. M. (1928). *Bull. Entomol. Res.* **18**, 297–309.
Massee, A. M. (1952). *Ann. Rept. East Malling Res. Sta. Kent,* pp. 162–165.
Metcalf, C. L., Flint, W. P., and Metcalf, R. L. (1962). "Destructive and Useful Insects," 4th ed., 1087 pp. McGraw-Hill, New York.
Moskovets, S. N. (1941). "Plant Virus Diseases and Their Control," Transactions of the Conference on Plant Virus Diseases, Moscow, 4–7/II 1940, pp. 173–190. Moscow-Leningrad Acad. Sci. U.S.S.R.
Mulligan, T. (1960). *Ann. Appl. Biol.* **48**, 575–579.

Nagaich, B. B., and Vashisth, K. S. (1962). *Curr. Sci. (India)* **31**, 166–167.
Niemeyer, L., and Bode, O. (1959). *Z. Pflanzenkrankh.* **66**, 640–644.
Noble, R. J. (1934). *Intern. Bull. Plant Protect.* **8**, 3–5.
Ochs, G. (1958a). *Naturwissenschaften* **45**, 193.
Ochs, G. (1958b). *Z. Pflanzenkrankh.* **65**, 11–17.
Ochs, G. (1960). *Z. Angew. Zool.* **47**, 485–491.
Ohmann-Kreutzberg, G. (1963). *Phytopathol. Z.* **47**, 1–18.
Pop, I. (1962). *Phytopathol. Z.* **43**, 325–336.
Price, W. C. (1958). *FAO Rept.* **850**, 50 pp.
Razvyazkina, G. M., Kopkova, E. A., Belyanchikova, Y. V. (1963). *Zashchita Rast Moskva* **8**, 54–55.
Schmidt, H. B., Fritzsche, F., and Lehmann, W. (1963). *Naturwissenschaften* **50**, 386.
Schulz, J. T. (1963). *Plant Disease Reptr.* **47**, 594–596.
Schuster, M. F. (1963). *Plant Disease Reptr.* **47**, 510–511.
Seth, M. L. (1962). *Indian Phytopathol.* **15**, 225–227.
Sill, W. H., Jr., and Connin, R. V. (1953). *Trans. Kansas Acad. Sci.* **56**, 411–417.
Sill, W. H. Jr., Bigornia, A. E., and Pacumbaba, R. P. (1963). *FAO Plant Protect. Bull.* **11**, 49–58.
Slykhuis, J. T. (1952). *S. Dak. Agr. Expt. Sta. Tech. Bull.* **11**, 29 pp.
Slykhuis, J. T. (1953a). *Phytopathology* **43**, 537–540.
Slykhuis, J. T. (1953b). *Can. J. Agr. Sci.* **33**, 195–197.
Slykhuis, J. T. (1955). *Phytopathology* **45**, 116–128.
Slykhuis, J. T. (1956). *Phytopathology* **46**, 682–687.
Slykhuis, J. T. (1958). *FAO Plant Protect. Bull.* **6**, 129–134.
Slykhuis, J. T. (1961a). *Can. J. Plant Sci.* **41**, 211–216.
Slykhuis, J. T. (1961b). *Can. J. Plant Sci.* **41**, 304–308.
Slykhuis, J. T. (1962). *Can. J. Botany* **40**, 1439–1447.
Slykhuis, J. T. (1964). *Can. Plant Disease Survey* **44**, 242–243.
Slykhuis, J. T., and Bell, W. (1961). *Phytopathology* **53**, 236–237.
Slykhuis, J. T., Andrews, J. E., and Pittman, U. J. (1957). *Can. J. Plant Sci.* **37**, 113–127.
Smith, B. D. (1959). *Ann. Rept. Agr. Hort. Res. Sta. Long Ashton Bristol*, pp. 130–136.
Smith, B. D. (1960). *Ann. Rept. Agr. Hort. Res. Sta. Long Ashton Bristol*, pp. 129–133.
Smith, B. D. (1961). *Ann. Rept. Agr. Hort. Res. Sta. Long Ashton Bristol*, pp. 170–172.
Smith, B. D. (1962). *Ann. Rept. Agr. Hort. Res. Sta. Long Ashton, Bristol*, pp. 124–129.
Smith, F. F. (1952). *Yearbook Agr. (U.S. Dept. Agr.)*, pp. 652–656.
Smith, K. M. (1957). "Textbook of Plant Virus Diseases," 2nd ed., 652 pp. Churchill, London.
Staples, R., and Allington, W. B. (1956). *Univ. Neb. Agr. Expt. Sta. Res. Bull.* **178**, 41 pp.
Staples, R., and Brakke, M. (1963). *Phytopathology* **53**, 969–972.
Swarbrick, T., and Berry, W. E. (1937). *Ann. Rept. Agr. Hort. Res. Sta. Long Ashton Bristol*, pp. 124–132.
Thresh, J. M. (1963). *Ann. Rept. East Malling Res. Sta. Kent*, pp. 99–100.
Thresh, J. M. (1964a). *Nature* **202**, 1028.

Thresh, J. M. (1964b). *Nature* **202**, 1085–1087.
Thresh, J. M. (1964c). *Jubilee Ann. Rept. East Malling Res. Sta. Kent*, 1963, pp. 184–189.
Thresh, J. M. (1964d). *Proc. Brit. Insecticides Fungicides Conf., Brighton*, 1963. In press.
Timian, R. G., and Bissonnette, H. L. (1964). *Plant Disease Reptr.* **48**, 703.
Wadsworth, D. F. (1949). *Plant Disease Reptr.* **33**, 482.
Wallace, H. A. H., and Sinha, R. N. (1961). *Can. J. Plant Sci.* **41**, 871.
Wallin, J. R. (1950). *Plant Disease Reptr.* **34**, 211–212.
Walters, H. J. (1954). *Plant Disease Reptr.* **38**, 836–837.
Wilson, N. S., and Cochran, L. C. (1952). *Phytopathology* **42**, 443–447.
Wilson, N. S., Jones, L. S., and Cochran, L. C. (1955). *Plant Disease Reptr.* **39**, 889–892.

GENETICS OF PATHOGENICITY IN VIRUSES AND OF RESISTANCE IN HOST PLANTS

Francis O. Holmes

The Rockefeller Institute, New York, New York

I. Introduction 139
II. Natural Selection for the Most Favorable Level of Pathogenicity in Viruses . 140
III. Natural Selection for Increased Resistance in Host Plants 143
IV. Viral Diseases of Plants for Which Single or Multiple Genes Have Been Found to Confer Resistances 146
V. Viral Diseases for Which Resistances Have Been Noted without Disclosure of Genic Controls 156
References 158

I. Introduction

In nature, viruses and their host plants are constantly subjected to selective competition. Selection among viruses tends to raise or lower their degrees of pathogenicity so as to reach the most favorable level for their action in each host. Selection among host plants gradually increases the levels of resistance in all the hosts. It is interesting to note this distinction. An intermediate level of pathogenicity is ordinarily optimal for the parasite but the host is favored most, other things being equal, by an unlimited increase in its level of resistance. Perhaps this fact is partly responsible for the fortunate circumstance that for any particular parasite in nature we find the great majority of living organisms completely immune to attack.

Far less is known about the hereditary mechanisms and genetic constitutions of phytopathogenic viruses than about those of their hosts. Attempts, however, to learn how such viruses behave in the course of their reproduction have been made in many ways.

Strains of several viruses have been thought to hybridize when brought into mixture. Best (1954, 1961) as well as Best and Gallus (1955) concluded that two strains of spotted wilt virus (*Lethum australiense* H.) in a single host cell might exchange determiners and produce new strains. Watson (1960) presented evidence of genetic recombination between potato viruses Y and C (strains of *Marmor upsilon* H.) in infected *Nicotiana glutinosa* L. plants. Thomson (1961) reported the appearance of one or two new strains of potato virus X (*Annulus dubius* H.) after mixed infection of host plants by two virus X strains;

he concluded, however, that the evidence was inadequate to prove that genetic recombination between strains had occurred.

Holoubek (1962) reported a gain in infectivity when tobacco mosaic virus (*Marmor tabaci* H.) was reconstituted by adding ribonucleic acid derived from strains of the virus that were initially of low infectivity to protein derived from an ordinary field-type strain of high infectivity.

Whatever may prove eventually to be the status of hybridization between strains of viruses, we can be sure that viruses do *mutate*, and then, if isolated, generally breed essentially true as mutant lines, much as higher organisms do (Kunkel, 1940). Moreover, it has been shown clearly that an initial mutation in a virus tends to remain unaltered by subsequent mutations (Holmes, 1936, 1952 Norval, 1938). This independence of one mutational event from subsequent mutations in viruses seems comparable to the independence of mutations in spatially distant parts of the genetic structures of higher organisms.

II. Natural Selection for the Most Favorable Level of Pathogenicity in Viruses

Very few viruses are strictly confined to single host species. Even those that may be so confined are likely to infect a number of physiologically different varieties of their host. Spread of viruses from one species of host to another, or from variety to variety, in most cases tends to upset such equilibria as have arisen in the past (Holmes, 1953).

When a virus becomes established in a single host species, or in a single host plant, it begins soon to produce more of its own kind in very large numbers. Experimentally it can be shown that even within a few hours some variants of the virus are also formed; later, no doubt, more are produced. Many of the variants are promptly suppressed because they are less able than the original form of the virus to reach the uninvaded tissues that are constantly being formed by continued growth of the host plant. These suppressed variants will reach high concentrations only if they are subsequently transferred, by accident or design, to some new environment in which they will be alone or in which they prove to be competitively superior to the original viral strain. This may happen, for example, for some attenuated and poorly moving, but thermally superior, strains if the next transfer is to a host growing at so high a temperature that the original strain of the virus is unable to multiply in it, or able to multiply only at a greatly reduced rate (Holmes, 1934). Some variants, of course, are not suppressed but grow in mixture with the original strain and multiply in the same parts of the host.

Occasionally a variant may be slightly more capable of reaching young tissues near the growing tips of the plant than was the original strain. This is especially likely to happen if the introduced strain has become adapted earlier to a less resistant variety of the host plant. In such a case, the virus may produce large amounts of a new and more invasive strain in developing tissues of the host just before these tissues become old enough to be occupied in force by the original kind of virus. The new strain may, in turn, produce a few variants characterized by still further invasiveness. This, in essence, is a picture of one phase of the evolutionary process that goes on constantly in nature and that probably has made the viruses, which have shorter generation times than any other known parasites, the most intensively selected of all living entities.

If a partially adapted strain of a virus is transferred in succession to a large number of additional host plants of the same kind, the selection for greater and greater virulence eventually will be checked. Some level will be established at which the ability of the virus to move freely through the tissues of the host (and thus to provide abundant sites from which it may be extracted by insects or other vector agencies) is not so great as to involve excessive damage to the host. Any variant that stunts its host excessively, or kills portions of it, is likely not to be widely available to spread to new host plants as frequently as do milder strains. On the other hand, exceedingly mild strains commonly do not spread into a large enough proportion of the host tissues to take maximal advantage of their opportunities to multiply and be ready for most efficient dispersal.

Because each virus is transferred from time to time from one kind of host plant to another, sometimes from variety to variety within a host species but frequently also from a species in one family to a host species of entirely different taxonomic relationships and greatly different physiological attributes, a viral strain that has become almost ideally adapted to one kind of host plant may find itself poorly adapted to its next host. This requires a repetition of part or all of the process of adaptation.

Continued experimental transfers of the leafhopper-transmitted potato yellow dwarf virus [*Aureogenus vastans* (H.) Black] by rubbing-type mechanical inoculations in *Nicotiana rustica* L. have selected strains of this virus well-adapted to the experimental conditions but no longer leafhopper-transmissible (Black, 1953). A vectorless strain of wound tumor virus (*Aureogenus magnivena* Black) also has been found under experimental conditions. These conditions involved long-continued rooting of cuttings to provide vegetative propagation of the host and to avoid the necessity for repeated insect transfers of the stock virus to a succession of healthy seedlings. When injected into the appropriate leafhopper vector the vectorless strain of wound tumor virus seemed unable to multiply there as the field-type strain can (Black *et al.*, 1958).

Spontaneous increases in pathogenicity of viruses that have met experimentally selected types of resistant hosts have not yet caused as much trouble to plant breeders as has been experienced in connection with plants that are resistant to various fungal infections. Nevertheless there have been a few cases in which plants bred for resistance to viruses have been invaded eventually by more virulent strains than those originally studied.

The Virginia Savoy variety of spinach (*Spinacia oleracea* L.), which proved useful for many years because of its resistance to blight caused by American cucumber mosaic virus (*Marmor cucumeris* H.) (not to be confused with the so-called English cucumber mosaic virus or cucurbit mosaic virus, *M. astrictum* H.) has been found susceptible to some strains of the virus for which its resistance was intended (Webb *et al.*, 1960).

The Katahdin potato (*Solanum tuberosum* L.), once regarded as immune to potato virus A (*Marmor solani* H.), was shown by MacKinnon and Bagnall (1962) to be susceptible to infection by this virus as carried by the aphid *Myzus persicae* (Sulz.).

In lines of sugarbeet (*Beta vulgaris* L.) bred for resistance to sugar beet curly-top virus (*Ruga verrucosans* Carsner and Bennett), an isolate of the virus known as strain 11 was found to be seriously injurious to the formerly resistant sugar beet lines (Giddings, 1954).

A new strain of bean mosaic virus (*Marmor phaseoli* H.) was found capable of overcoming the formerly adequate recessive-type resistance of the Great Northern bean (*Phaseolus vulgaris* L.) but not capable of overcoming the dominant-type resistance of Idaho Refugee or Idaho Bountiful beans (Dean and Wilson, 1959). No strain of this virus has been found to overcome the monogenic dominant type of resistance (Buttenschön, 1963).

A newly isolated strain of bean virus 2 (*Marmor manifestum* Frandsen) was reported by Schroeder and Provvidenti (1962) as capable of overcoming the resistance of peas (*Pisum sativum* L.) characterized by the *mo mo* genotype. The mosaic pattern is, however, milder in the *mo mo* plants than in ordinary susceptible lines infected by previously known strains of the virus. Provvidenti and Schroeder (1963) report that the typical strain of the virus is suppressed by hot weather and that thermally more stable strains tend to supplant it and to induce symptoms on resistant plants.

A strain of turnip mosaic virus (*Marmor brassicae* H.) has been found by San Juan (1961) to overcome the formerly high resistance of the Christmas Red variety of annual stocks (*Matthiola incana* R. Br. var. *annua* Voss).

Recently an unusual strain of potato virus X (*Annulus dubius* H.) has been found to become systemic and to produce mottling and flecks of necrotic tissue in potato plants bearing the genes *Nb* and *Nx*, which together confer field-type immunity through necrotic-lesion formation when infected by the other known strains of this virus (Cockerham and Davidson, 1963). This unusual strain fails, however, to infect the potato varieties Saco and 41956, which possess full immunity to all known strains of the X virus.

The ability of phytopathogenic viruses to mutate in such a way as to overcome some resistances to diseases in plants and the possibility of interaction of mixed viral strains to produce new combinations of characteristics make it desirable to give special attention to the kinds of host resistance that may become available for the protection of crop plants against damage by viral diseases.

III. Natural Selection for Increased Resistance in Host Plants

Plants that are susceptible to viral diseases may acquire resistances of several kinds and many levels of efficiency by mutation followed by selection or by exchange of genetic characters with related species or varieties through hybridization. The kinds of resistance that may be acquired differ from each other in many ways. They cannot be arranged in a single linear series, but their different levels of resistance permit us to choose, when considering pairs of such levels, which types of resistance to infection shall be regarded as representing susceptibility and which shall be thought of as representing resistance. This choice ordinarily depends on the simple and practical criterion of degree of crop reduction and is thus an arbitrary one. Other motivations of a scientist might change the choice of terms but would not affect the genetic behavior of hybrids between differing parents.

If we arbitrarily select a viral disease that displays moderate chlorotic mottling of foliage and slight malformations of stems, roots, or leaves, with some reduction of crop if the plant is agriculturally useful, we may study variants of the process of disease. If, in the course of this study, we find a variety of the host plant in which the pathogen induces a more serious form of the disease, we may view the disastrous effect as an indication of greater inherent susceptibility of the host variety. The originally selected disease would then be viewed as a distinguishing mark of a slight but possibly useful resistance. A search for additional kinds of resistance may disclose varieties or breeding lines of the host in which symptoms are less conspicuous, or even demonstrable only by special studies. The various levels of resistance of this kind can be classed as degrees of *tolerance after infection.* In some cases at least, tolerance seems to imply an ability on the part of the host to reduce the rate of viral multiplication or to restrain the spread of virus through its tissues, with consequent escape of immature parts of the plant from severe disease until they are old enough to be but little changed in form or obvious function by viral invasion. This kind of resistance tends to be attractive to plant breeders because it usually is not accompanied by conspicuous death of invaded tissues. Tolerance as a form of resistance to disease presents some hazards, however. One hazard is that the infected but more or less symptomless host commonly does show an important reduction in yield, although this may not be recognized or readily detected. Moreover, the tolerant but infected plant constitutes an inconspicuous but dangerous reservoir of the pathogen. Another hazard is even more serious. The tolerant host provides a filtering mechanism by which the viral pathogen can increase its pathogenicity through development of variants and rapid multiplication of some of these as dominating types in mixture with the original form of the virus.

A characteristic that is thought to be largely independent of tolerance but that may be associated with any one of its apparently innumerable degrees or levels is the *level of susceptibility to infection.* This, like the level of tolerance, seems to have an unlimited number of variations. Some plants are infected with great ease by mechanical inoculation or by the feeding processes of insects. Others can be infected, but only by the application of more virus, by more abrasion, or by more exposure to viruliferous insects.

There is a practical aspect to the selection of these two characteristics of relative tolerance and relative ease of infection. One of the most effective of all means for control of viral diseases is the practice of roguing, which is the removal and destruction of infected plants. In order to be able to remove recently infected plants promptly, it is desirable that they should not be so highly tolerant as to be difficult to distinguish from nearby healthy plants. Moreover, removal of new cases of disease is most effective for diseases that do not spread very rapidly. Hence a praiseworthy objective in breeding is to produce plants that show a disease clearly but that become infected relatively rarely.

A third kind of resistance that seems to be to some extent independent of the level of tolerance and the level of susceptibility to infection is *decreased ease of recovery of virus from infected tissues.* Here, too, there seem to be many possible levels. Spread of disease among plants from which virus is not readily extractable, by mechanical means (Holmes, 1938) or by insect vectors (Daubeny and Stace-Smith, 1963), may be almost completely suppressed even in plants that are easily infected (i.e., highly susceptible to infection) and by no means tolerant (i.e., highly susceptible to conspicuous injury). This is true, for example, of varieties of tobacco (*Nicotiana tabacum* L.) carrying the dominant gene *N* from *N. glutinosa.* These plants are readily infected from outside sources by tobacco mosaic virus and they are so hypersensitive to infection that they may show conspicuous necrosis in infected leaves or stems, but so little virus can be extracted from their affected parts that spread of disease from plant to plant within the crop is virtually impossible, because no highly efficient mechanism for extraction and transmission of virus exists within tobacco fields. The *N. glutinosa* type of resistance has now proved highly effective in use. The single dominant gene that controls it has been incorporated into many locally satisfactory varieties of tobacco (Henderson *et al.*, 1957; Apple *et al.*, 1962).

Solymosy *et al.*, (1959) concluded that an accumulation of quinones is responsible for necrotic-lesion formation in hypersensitive tobacco plants infected by tobacco mosaic virus. Solymosy and Farkas (1962) noted a marked accumulation of the enzymes glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase around necrotic lesions in White Burley tobacco after infection by tobacco mosaic virus. They expressed the opinion that this causes a high phenol to quinone ratio, which hinders the growth of the lesions.

Weintraub and Ragetli (1961) noted that pectinase does not release cells from their attachments to each other in areas around necrotic lesions caused by tobacco mosaic virus in tobacco leaves so readily as it does in healthy areas, because calcium pectate is present instead of mixtures of pectic acid interspersed with calcium pectate.

Sunderland and Merrett (1963) reported that leaves of *Nicotiana glutinosa* that show necrotic lesions as a result of infection by tobacco mosaic virus possess increased levels of the reduced forms of nicotinamide-adenine dinucleotide and nicotinamide-adenine dinucleotide phosphate.

If a plant breeder can combine the kind of field-type resistance that is a result of *low transmissibility of virus from affected tissues* with *ease of recognition of disease* and with *relatively high resistance to infection* from outside sources of virus, he will have an ideal combination of characteristics. It is the opposite set of characteristics that causes greatest agricultural losses, often without attracting much attention because of the insidious nature of disease in highly susceptible but rather tolerant hosts that become effective reservoirs from which spread of disease may occur readily.

It has seemed necessary for plant pathologists and plant breeders, despite this reasoning, to accept tolerant varieties of many crop plants as temporary defenses against some viral diseases and to use them in preference to more severely damaged varieties of the same plants for lack of better kinds of resistant varieties. In some cases, also, technical difficulties have prevented the identification of the exact types of resistance in hand. Obviously it is difficult to analyze the relative parts played by tolerance to infection and by resistance to becoming infected unless techniques are available for quantitative determination of the level of resistance to becoming infected. Whenever the tolerant varieties can be replaced by immune varieties or even by varieties that rarely become infected and that can be readily rogued or that tend not to become highly effective reservoirs of virus for further spread of disease, this replacement would seem to be advantageous.

IV. Viral Diseases of Plants for Which Single or Multiple Genes Have Been Found to Confer Resistances

A broad field for activity in understanding the mode of inheritance of various resistances now challenges the intelligence of plant pathologists and plant breeders. Very many viral diseases have been studied sufficiently to show that useful resistances of major and minor importance do exist among varieties of their host plants or in close relatives of them. A substantial beginning has been made in learning how some of these resistances are inherited in successive generations after genetic crosses and hybridizations. The published literature seems to represent an increasing number of investigations that have as their goal the determination of which genotypes provide satisfactory resistances to numerous viral diseases.

A decade ago, a detailed review of studies dealing with genes that were then known to confer resistances to viral diseases of plants was published by Holmes (1954a). During the intervening years, results of research have accumulated at an increasing rate. They are described here, together with some earlier work that was not included in the above-mentioned review.

Timian and Sisler (1955) reported that a symptomless response (i.e., a tolerance or an immunity) in barley (*Hordeum vulgare* L.) inoculated with the California E strain of barley stripe mosaic virus was inherited by one fourth of the progeny of second-generation hybrids. Inouye (1962) reported that resistance is recessive in Imperial, but dominant in Wien, barley.

Suneson (1955) reported that a single recessive gene in barley confers moderate resistance to barley yellow dwarf disease (presumably a tolerance to the disease after infection). Rasmusson and Schaller (1959) found that an incompletely dominant gene Yd_2 confers a high degree of tolerance and assures a fairly satisfactory yield of grain in barley varieties infected by barley yellow dwarf virus. A recessive gene yd_1 confers a lower level of tolerance; presumably this is the recessive gene that was studied by Suneson. Resistance to barley yellow dwarf disease was found by Schaller (1960) only in Abyssinian varieties of barley. Damsteegt (1963) found that tolerance to this disease is especially common in Ethiopian (i.e., Abyssinian) varieties of barley, although it is not confined to these. One Chinese variety was found to be resistant (Schaller *et al.*, 1963). Damsteegt and Bruehl (1964) reported that ten resistant varieties tested by them could be crossed with Rasmusson and Schaller's resistant varieties with no segregation for resistance in the second filial generation. Crosses of locally adapted, but susceptible, barley varieties gave some F_3 lines that yielded better than either parent in the presence of barley yellow dwarf disease (Bruehl and Damsteegt, 1964).

Baggett (1956) noted that resistance to strains of bean yellow mosaic virus (*Marmor manifestum*) in an interspecific cross between *Phaseolus vulgaris* L. and *P. coccineus* L. is recessive and controlled by two, or perhaps three, major genes.

Diachun and Henson (1959) found that tolerance to infection by bean yellow mosaic virus is controlled by a single dominant gene in crosses involving tolerant and mottling-type varieties of red clover (*Trifolium pratense* L.); a necrotic-spotting type of response, which would tend to imply lack of easy spread of virus from the infected host, is controlled by a gene that is dominant in crosses with mottling-type clones of red clover but that does not function visibly in the presence of the epistatic gene for tolerance.

Yen and Fry (1956) reported that a single recessive gene in homozygous condition (*mo mo*) confers immunity in peas exposed to infection by "pea mosaic virus." The strains of virus that were used in this work were able to infect the bean, *Phaseolus vulgaris*, and were said to be similar in this respect to a strain recovered from gladiolus. In another paper (Schroeder and Provvidenti, 1962), the gene pair *mo mo* was said to confer resistance to ordinary strains of bean yellow mosaic virus (BV2). Thus the resistance conferred by the gene pair *mo mo* may be *to infection* or *to symptom expression* after exposure to bean yellow mosaic virus (*Marmor manifestum*), rather than after exposure to ordinary pea mosaic virus (*M. leguminosarum* H.). Barton *et al.* (1964) believed pea virus 2 to be a strain of bean yellow mosaic virus, because clones of garden pea in progenies segregating the gene *mo* always resist both if they resist either of these. Johnson and Hagedorn (1958) also stated that a single recessive gene in peas controls resistance (in the sense of failure to develop chlorotic mottling) to "pea mosaic" incited by bean virus 2 (bean yellow mosaic virus). Schroeder *et al.* (1960) reported the single-gene resistance to bean virus 2 in peas to be inherited as a recessive characteristic above 80°F. but as a dominant character below 65°F.

Schroeder and Barton (1958) reported that a single dominant gene in the garden pea confers resistance (to expression of symptoms) after infection by pea enation mosaic virus (*Marmor pisi* H.).

Hubbeling (1959) reported that tolerance to "stippelstreep" (stipple streak) of beans (*Phaseolus vulgaris*), caused by tobacco necrosis virus (*Marmor lethale* H.), is recessive to severe browning of leaf veins. The tolerance depends on the presence of two or more recessive genes in homozygous condition.

Miyake (1938) reported that a single dominant gene protects wheat (*Triticum aestivum* L.) from infection or visible damage by a mosaic disease.

Johnson and Barnhart (1956) found that a high degree of resistance to infection by turnip mosaic virus (*Marmor brassicae*) in flowering stocks (*Matthiola incana*) was dependent on the presence of a single recessive gene, *rm*, in homozygous condition.

Tolerance to turnip mosaic virus (referred to as virus A) in cabbage (*Brassica oleracea* L.) is said to be inherited polygenically, with incomplete dominance of the tolerant character (Pound and Walker, 1951). Pound (1952) reported that the tolerance is expressed best at 16° and 20°C., and less well at higher temperatures, although one line of cabbage, 25-823, proved tolerant even at 28°C., and in this line the virus remained relatively low in titer. Walker *et al.* (1953, 1957) have reported that a partial resistance to mosaic in cabbage, caused by turnip virus 1 (*Marmor brassicae*) and cauliflower virus 1 (*M. cruciferarum* H.), is controlled in polygenic fashion, but how many genes are involved has not yet been determined.

Despite earlier reports that three or more genes in cucumber (*Cucumis sativus* L.) control resistance to cucumber mosaic, Wasuwat and Walker (1961a) showed that currently used cucumber-mosaic-resistant varieties of cucumber, such as Wisconsin SMR 12, SMR 15, and SMR 18, possess only a single major dominant gene that is absent from susceptible varieties, such as National Pickling and Wisconsin SR 6. The Chinese Long variety of cucumber also possesses this major dominant gene for resistance. The resistance mechanism in the cucumber

partially suppresses, but does not entirely exclude, multiplication of cucumber mosaic virus (*Marmor cucumeris*) and systemic spread of this virus in the resistant plants (Wasuwat and Walker, 1961b). Respiration is not increased in infected plants of the resistant cucumber as it is in the susceptible (Menke and Walker, 1963). Resistant cucumber plants have been found to produce a higher percentage of unmottled fruits than do susceptible plants, after infection in the field, and such mottling as does occur is less severe in fruits of resistant than in fruits of susceptible plants (Sinclair and Walker, 1956).

Martin (1960) reported that resistance to extensive systemic spread of cucumber mosaic virus in *Cucurbita pepo* L. is controlled by two recessive genes in breeding lines 176959 and 174192 but that only one of these genes is shared by the two resistant lines; resistance in *C. maxima* Duschesne and *C. moschata* Duschesne var. Butternut is also recessive, but in *C. moschata* var. Batangas Native it is dominant to susceptibility.

Webb *et al.* (1960) showed that line P.I. 179590 of spinach owes its resistance to infection by many strains of cucumber mosaic virus to a single dominant gene not found in the highly susceptible variety Bloomsdale; it resists infection by a number of strains of cucumber mosaic virus that infect and damage the older resistant variety Virginia Savoy although it, like Virginia Savoy spinach and the fully susceptible variety Bloomsdale, becomes infected readily by strain 6 of cucumber mosaic virus.

In the T.I. 245 variety of tobacco (*Nicotiana tabacum*), Fulton (1953) found a moderate degree of resistance to becoming infected by cucumber mosaic virus. Troutman and Fulton (1958) believed that inheritance of this trait was polygenic.

Sinclair and Walker (1955) reported that a single dominant gene governs the resistant-type response, which is characterized by formation of necrotic local lesions as opposed to systemic mottling, in cowpeas [*Vigna sinensis* (L.) Endl.] infected by cucumber mosaic virus. The resistance in this case is so effective that the protected plant will survive grafting to a systemically infected and mottled recessive-type plant without itself becoming visibly affected.

Susceptibility of cowpea to systemic mottling disease caused by cucumber mosaic virus was found by deZeeuw and Crum (1963) to be linked with resistance to systemic invasion by tobacco ringspot virus (*Annulus tabaci* H.) with about 25% crossover. Both resistances to systemic invasion are to some extent dominant, plants of the F_1 generation being resistant to both viruses in crosses between lines that were resistant to each separately. A single, partially dominant gene in cowpea was reported by deZeeuw and Ballard (1959) to give a useful degree of hypersensitivity to infection by tobacco ringspot virus. This hypersensitivity is expressed by the formation of small necrotic primary lesions, with localization of the invading virus near the site of inoculation; absence of the more or less dominant gene permits formation of large necrotic lesions at the site of infection, followed by top necrosis and death of the plant. In heterozygotes, primary lesions are somewhat larger than in homozygous resistant plants.

Tolerance after infection by peanut rosette virus (*Marmor arachidis* H.) in peanut (groundnut), *Arachis hypogaea* L., is controlled by two recessive genes; resistant plants, of constitution *aabb*, resemble susceptible plants in being attacked by the aphid vector *Aphis leguminosae* Theob., but are said to produce an antiviral substance that localizes virus in chlorotic patches or leaves plants healthy in appearance (de Berchoux, 1960).

Jennings (1963) reported that a gene A_1 causes the raspberry variety Malling Landmark to resist infestation by the aphid *Amphorophora rubi* Kalt., which transmits the virus of raspberry mosaic disease.

Tolerance to infection by bhindi yellow vein mosaic virus (*Ochravena hibiscae* Capoor and Varma) in *Hibiscus esculentus* L. (okra) was found by Singh *et al.* (1962) to require the presence of two recessive genes, yv_1 and yv_2, in homozygous condition.

Walter (1956b) reported that a single recessive gene in the P.I. 183692 tomato (*Lycopersicon esculentum* Mill.) confers a resistance to infection by tobacco etch virus (*Marmor erodens* H.) that may constitute complete or nearly complete immunity.

Greenleaf (1956) reported that the recessive genes et^{l} and et^{a} give etch resistance (masking of symptoms) in peppers (*Capsicum frutescens* L. and *C. annuum* L.) when either is present in the absence of its corresponding dominant gene, et^{l+} or et^{a+}. Whether the two recessive genes are allelic is not known. The resistance in this case seems to be a result of slowing multiplication of tobacco etch virus in infected plants and thus reducing the intensity of symptoms. Modifying genes for higher resistance were suspected of occurring in some individual plants.

The Tabasco pepper (*Capsicum frutescens*) differs from ordinary garden peppers in that it shows distinct wilting following infection by tobacco etch virus. This wilting effect is associated with the presence of a single dominant gene not present in nonwilting peppers (Greenleaf 1959a,b).

Stover (1951) found that the two White Burley genes, g_1 and g_2, cause tobacco plants to be more severely injured by tobacco etch virus than do their dominant alleles.

Cook (1961) found an individual bell pepper (*Capsicum annuum*) plant that remained uninfected after graft and mechanical inoculations with potato virus Y (*Marmor upsilon*). This plant owed its immunity to a single recessive gene that was closely associated with a gene governing susceptibility to tobacco etch virus.

Subsequently, a mutant strain of potato Y virus appeared in one of three pepper varieties, all of which possessed a single recessive gene for resistance to the normal strain (Cook, 1962). The mutant strain, however, could affect only the single variety in which it was discovered.

In later studies (Cook, 1963a), it was found that some strains of potato virus Y were adequately resisted by single recessive genes in pepper, but that at least three genes had some effect on the responses of pepper plants to infection by one or another of the many tested strains of Y virus.

Simmonds and Harrison (1960) reported that two loci were concerned in the reactions of pepper to infection by "pepper vein-banding virus" (potato virus Y). The doubly recessive $v_1v_1v_2v_2$ plants were difficult to infect, showed only mild mosaic when infected, and grew well as diseased plants. The presence of one incompletely dominant gene, V_1, did not modify resistance to infection, but in infected plants it tended to induce abscission of part of the leaves and to cause stunting of growth. The other dominant, V_2, increased susceptibility to infection and plants carrying it showed severe mosaic and poor growth. When the two genes V_1 and V_2 were both present, plants were easily infected and developed veinal necrosis after infection; subsequently these highly susceptible plants lost their leaves and died.

In *Solanum stoloniferum* Schlechtd. (formerly known as *S. antipoviczii* Buk.) and in hybrids of this species with the cultivated potato *S. tuberosum*, Ross (1958a, 1960) found three alleles in a descending order of dominance; *Ry* controlling extreme resistance (immunity) to infection by virus Y, *Ryn* providing hypersensitivity in the sense of a tendency of infected tissues to die with localization of the invading virus, and *ry* giving susceptibility in the sense of readiness to become infected, although often the induced mosaic-type disease was mild or nearly symptomless.

An additional gene of undetermined allelism in *Solanum stoloniferum* is epistatic or dominant to *ry* but not to *Ryn* or *Ry* (Ross, 1961); this gene *Rym* permits mosaic mottling accompanied by faint necroses when plants carrying it are infected with Y virus. It appears also that the four genes of this series control disease types produced by virus A (potato mild mosaic virus, *Marmor solani*), *Ry* causing immunity, *Ryn* or *Rym* formation of local necrotic lesions followed by mosaic, and *ry* a simple mosaic reaction.

In *Solanum demissum* Lindl., Cockerham (1958) found three allelic genes, in a descending order of dominance; one caused a necrotizing response to infection by either potato virus Y or potato virus A, a second caused a similar response to virus A but not to virus Y, and the third failed to do this with respect to either virus.

Ross (1958b) has reviewed recent studies of the heritable resistances to damage by infection of potato and potato hybrids with four viruses, namely, potato leaf roll virus (*Corium solani* H.) (polygenic dominant resistance to infection), potato virus Y (polygenic moderate resistance to infection), potato virus A (monomeric dominant extreme resistance to infection), and potato virus X (*Annulus dubius*) (dimeric dominant hypersensitivity and monomeric as well as dimeric immunity). Efforts to combine resistance to the four viruses are also reported in the same publication.

Bagnall and Bradley (1958) reported that potato varieties responding to infection with potato virus Y by mottling showed a larger total number of infected plants each year but that varieties that reacted by death of affected tissues tended to show less total infection in successive years, the disease being to some extent self-eliminating, affected plants eventually dying without producing tubers.

Endemann (1955) found that resistance in many lines of tobacco with respect to the strains of potato virus Y that cause browning along veins was controlled by recessive genes, and probably by no more than two recessive genes.

Partial resistance to infection by Y virus in tobacco was reported by Koelle (1958) in a mutant of a susceptible tobacco called Virgin A; the mutant proved very susceptible to infection by *Peronospora tabacina* Adam (Koelle, 1961); Koelle (1962) attributed the resistance to infection by Y virus to a single recessive gene in which Virgin A and its mutant differ. Koelle (1963) stated that Y virus-resistant varieties of tobacco showed higher polyphenol oxidase activity than susceptible varieties.

The variety V20 of tobacco was reported by Schmelzer and Klinkowski (1959) to be fully resistant to infection by some strains of virus Y; presumably this means complete or nearly complete immunity with respect to infection.

Henderson and Troutman (1963) reported recently that a disease believed to have been caused by virus Y in tobacco was most severe, with vascular necrosis, in plants carrying a gene for root-knot resistance.

In the allotetraploid *Solanum acaule* Bitt., Ross (1954) found that some plants were easily infected by potato X virus (i.e., potato mottle virus) and subsequently became mottled. Others showed necrosis, largely localized in dark brown primary lesions at the site of inoculation.

Still others showed high resistance or immunity, in the sense that they failed to become infected and hence showed no symptoms. Immune plants crossed with mottling-type susceptibles gave resistant F_1 progeny, approximate 3:1 ratios of resistant to susceptible seedlings in F_2 generations, and half of the F_2 plants that showed segregation gave 3:1 ratios but the other half much higher ratios, 20.8:1 to 35:1. This suggested that some of the S. *acaule* plants of the F_2 generation had one and others more than one dominant gene capable of producing the highly resistant condition.

Cockerham (1958) showed that three alleles in a descending order of dominance control the immune, necrotizing, and tolerant responses of *Solanum acaule* to infection by potato virus X.

Kishore (1960) found a recessive type of immunity to infection by "virus X" (potato mottle virus) in the cultivated potato *Solanum tuberosum.* This recessive type of immunity is controlled by a gene *c*, not needed for the previously known immunity in potato that depends on the simultaneous presence of two dominant genes, but that in its turn does not need the dominant genes and behaves independently. When present as a homozygous recessive, gene *c* imparts immunity but it does not otherwise affect the expression of the dominant complementary genes.

A combination of three resistances in line P11 of pepper (*Capsicum annuum*) was reported by Cook and Anderson in 1959. This combination involved a hypersensitive response to most strains of tobacco mosaic virus (*Marmor tabaci*), a tolerance to most strains of tobacco etch virus (*M. erodens*), and a failure to permit multiplication of potato Y virus (*M. upsilon*). Cook and Anderson (1960) attributed the resistance to Y virus of P11 pepper to a single recessive gene y^a. Cook (1961) reported a combination of two resistances in a single pepper plant, YRP10; this plant occurred in a Y virus-susceptible line, but sublines derived from it contained a single recessive gene in homozygous condition that did not segregate following crosses with the P11 pepper line and that seemed to confer immunity to infection by Y virus; he concluded that lines of pepper derived from line YPR10 were homozygous for the recessive gene y^a; the pepper line derived from YPR10 also showed the l^il^i genes that condition the imperfect localization reaction to tobacco mosaic virus.

Cook (1963b) reported that a plant of P.I. 183441, a variety of *Capsicum annuum*, showed no symptoms after inoculation by tobacco mosaic virus; in F_1 hybrids with other pepper lines localized necrotic lesions appeared; in some F_2 plants, necrotic lesions were not clearly demarcated and abscission of inoculated leaves was retarded. Cook expressed the opinion, however, that a gene in the original resistant pepper plant was similar to, or the same as, the Tabasco gene, which normally implies a sharply demarcated necrotic local-lesion response with prompt and effective abscission of inoculated leaves.

Lutes (1954) confirmed the earlier reported behavior of genes L (localized necrosis), l^i (imperfectly localized necrosis) and l (absence of necrotic local lesions and subsequent systemic chlorotic mottling) in peppers (*Capsicum frutescens* and *C. annuum*) inoculated with tobacco mosaic virus.

Thompson *et al.* (1962) reported that lines of Scotia bean (*Phaseolus vulgaris*) capable of responding to inoculation with tobacco mosaic virus by production of localized necrotic lesions differed from completely immune lines of the same variety by possessing a single recessive gene *tm* in homozygous condition.

Endemann and Egerer (1962) reported three recessive genes as conferring resistance to tobacco mosaic virus in derivatives of Ambalema tobacco; usually tolerance in Ambalema tobacco has been described as depending on two recessive genes in homozygous condition.

In 1955, Holmes reviewed the successive increases in resistance to tobacco mosaic disease in tobacco that had been gained by accumulation of genes to that date. Clayton (1953) indicated that tobacco mosaic virus is unable to maintain itself in available resistant varieties and that such crops are completely free of this virus.

Holmes (1958) pointed out that each gene for resistance earlier studied had been specific for the constituent strains of a single virus and had failed to be applicable to resistance against multiple viruses; hence a presumption of viral relationship would be provided if a single segregating gene could be shown to influence the type of disease produced by two viral isolates. In contrast to this, Troutman and Fulton (1958) showed that a line of tobacco known as T.I. 245 tended to escape infection not only by cucumber mosaic virus (*Marmor cucumeris*) but also by tobacco necrosis virus (*M. lethale*), turnip mosaic virus (*M. brassicae*), tobacco streak virus (*Annulus orae* H.), and tobacco mosaic virus. Subsequently this T.I. 245 tobacco was found to have a similar tendency to escape infection by a number of additional viruses and incorporation of two recessive genes from it was effective in raising the level of resistance to infection by tobacco mosaic virus in tobacco mosaic-resistant stocks of tobacco (Holmes, 1960). At the same time tobacco mosaic-resistant derivatives of T.I. 245 tobacco, but not susceptible sister lines, were shown to have acquired resistance to six viruses other than tobacco mosaic virus; to all of these T.I. 245 tobacco was known to be moderately resistant (Holmes, 1961). In this case, at least, a sort of nonspecificity of resistance seemed to exist, as though the genes in question were effective in reducing slightly the efficiency of the inoculation process or some other factor common to all the attempts at infection by the tested viruses.

The development of lines of tomato resistant to tobacco mosaic disease has proved relatively difficult.

Watson *et al.* (1954) produced tomatolike derivatives from crosses of tomato with *Lycopersicon hirsutum* Humb. and Bonpl. These derivatives showed a tendency to resist infection by tobacco mosaic virus and to suppress multiplication of the virus after it was established in them.

Holmes (1954b) reported that a single dominant gene in *Lycopersicon esculentum* notably reduced the level of susceptibility to infection by tobacco mosaic virus; the introduced tendency to escape infection in the field was adequate to protect tomato lines against internal browning disease, to which no other tested line of tomato had ever proved resistant. At first, although backcrosses of resistant plants gave approximate 1:1 ratios of resistant and susceptible plants, ratios were deficient after self-pollination of resistant-type plants, usually being approximate 2:1 rather than 3:1 ratios. Eventually, however, true-breeding resistant lines were produced (Holmes, 1957).

Walter (1956a) developed tomato lines carrying three recessive genes for resistance to tobacco mosaic and combined this resistance with a single recessive gene resistance to tobacco etch infection (Walter, 1956b).

Murakishi (1960) reported that Holmes' and Walter's tobacco mosaic-resistant tomato lines resisted both gray wall and internal browning disease.

Murakishi (1962) found that the mosaic-resistant tomato, N-32-66-1a, did not show the usual systemic necrosis (double virus streak) when simultaneously infected by tobacco mosaic virus and potato virus X, the tobacco mosaic virus remaining at a minimum detectable level even though it stimulated a fourfold increase of potato virus X over singly infected plants. Murakishi and Honma (1963) reported the same resistance to double virus streak in several other of the known tobacco mosaic-resistant tomato lines.

Alexander (1963) transferred a resistance to four strains of tobacco mosaic virus from *Lycopersicon peruvianum* (L.) Mill. to *L. esculentum*. A single dominant gene was believed to control the resistance, but resistant-type plants were found to be more numerous than expected in backcross generations.

Soost (1963) described a dominant gene, Tm_2, which occurred only in heterozygous condition in normally green plants from a complex tomato hybrid, all plants homozygous for this gene being virescent. The presence of this dominant gene conferred resistance to expression of mottling symptoms after inoculation with tobacco mosaic virus, except that symptoms were noted late in the season in some heterozygous plants.

V. Viral Diseases for Which Resistances Have Been Noted without Disclosure of Genic Controls

Numerous resistances have been noted by plant pathologists and plant breeders without any corresponding knowledge of breeding behavior. It is obvious that future studies of the mode of inheritance will be needed in many cases.

Bagnall (1960) noted dominance of a high degree of resistance to potato virus F in the cross *Solanum tuberosum* var. Epicure × 4*n* S. *chacoense* Bitt., but did not learn how many genes were involved in inheritance of the resistance. Kollmer and Larson (1960) also noted resistance to infection by potato virus F in S. *chacoense* and in several interspecific hybrids with that species, such as F_1 S. *goniocalyx* × S. *chacoense*, S. *verrucosum* × *chacoense*, and S. *demissum* × S. *chacoense*, which completely resisted sap inoculation and developed only slow and often incomplete top necrosis upon graft inoculation. Nineteen introductions of the species S. *chacoense* had resisted both sap inoculation and top-graft inoculation.

Bagnall and Young (1960) reported that immunity to infection by virus S in potato was inherited in multigenic fashion, at least one significant gene being recessive.

In crosses between varieties of cacao (*Theobroma cacao* L.) that are respectively sensitive and tolerant to infection by cacao swollen shoot virus (*Marmor theobromae* Posnette), the sensitive reaction has been reported as dominant (Longworth and Thresh, 1963), but the number of genes responsible for this characteristic is not known.

The variety of tobacco known as V20 has been reported by Schmelzer and Klinkowski (1959) to be relatively resistant to infection by tobacco etch virus (*Marmor erodens*) and by potato virus A (*M. solani*). The mode of inheritance of this resistance is not yet known, nor is it certain whether virus fails to infect or fails to spread systemically.

Resistance to top yellows in peas was thought by Hubbeling (1956) to have a dominant type of inheritance but the number of genes involved was not disclosed.

Resistance to subterranean clover stunt virus was found dominant in crosses between susceptible and resistant varieties of subterranean clover (*Trifolium subterraneum* L.) by Peak *et al.* (1963), but the

nature of resistance is not yet fully understood and more than one major gene may be involved.

Brierley and Smith (1946) found that of 27 tested varieties of onion (*Allium cepa* L.) only the variety Nebuka resisted damage when exposed to infection by the shallot strain and 3 other strains of onion yellow dwarf virus (*Marmor cepae* H.). This same resistance was shared by the amphidiploid Nebuka × White Portugal, which showed that the resistance is heritable and dominant in effect. Whether a single dominant gene is adequate to account for this resistance has not yet been determined.

In muskmelon (*Cucumis melo* L.), plants in some lines were found to be susceptible to mottling-type infection by watermelon mosaic virus (*Marmor citrulli* Anderson) but some plants in other lines seemed to be immune to the virus strain used for mechanical inoculation (Foster and Dennis, 1963). Future breeding operations are likely to disclose a genetic mechanism responsible for these different responses to inoculation.

All tested varieties of alfalfa (*Medicago sativa* L.) seem to be susceptible to infection by alfalfa dwarf disease virus (*Morsus suffodiens* H.), but some individual plants were selected by Houston and Stanford (1954) as more tolerant than others; a resistant line, California Common 49, is based on these selections.

Tomato varieties that are more or less resistant to damage by sugar-beet curly top virus (*Ruga verrucosans*) have been introduced. The Owyhee tomato, developed in Idaho, and line 193, developed in Utah, have proved less resistant when grown in each other's areas than in their own (Martin, 1963). The genic constitution of these varieties has not yet been established, but the difference in behavior in different areas suggests that each possesses some factor or factors for resistance lacking in the other.

Resistance to damage by curly top virus has been noted also in *Solanum pennelii* Dun. and an effort is being made to transfer the genes responsible for this resistance to curly top-resistant breeding lines of tomato to complement resistance already available (Martin, 1962).

Some breeding now has been done in sweet potatoes [*Ipomoea batatas* (L.) Lam.] to establish resistance to internal cork virus (*Lethum rhizopilum* Hildebrand). Nielsen (1956) stated that the wide range of symptoms among sweet potato varieties indicates that a number of genetic factors affect symptom development. Nielsen and Pope (1960) pointed out that resistance to virus multiplication occurs in seedlings of the sweet potato variety HM-15 and that a condition of tolerance occurs also. Harmon (1960) reported that resistance to internal cork disease is dominant in crosses of resistant varieties, such as HM-15 and HM-36, with several susceptible varieties.

Simons (1960) found the Italian El pepper resistant to infection by potato Y virus (*Marmor upsilon*). Later, Simons and Moss (1963) reported that the resistance in this variety, in respect to infection by both aphid feeding and mechanical inoculation, was associated with the presence of an inhibitory substance in the leaves. The inhibitor is believed not to affect the virus but merely to reduce the susceptibility of the host plant.

Some wheat varieties did not become infected by prairie wheat yellow mosaic virus (*Marmor tritici* H. var. *fulvum* McK.) or by wheat mosaic rosette virus (*M. tritici* var. *typicum* McK.) when exposed to infested soil in the field, but were found to be susceptible on mechanical inoculation. Others were found to be susceptible both in the field and to experimental inoculation (McKinney, 1948). Immunity in the field may be to a vector, rather than to the virus as such. The mode of inheritance of the immunity is still to be learned by future breeding experiments.

Resistances to wheat streak mosaic virus (*Marmor virgatum* McK.) have been noted in some wheat × *Agropyron* and wheat × rye crosses (Bellingham *et al.*, 1957). The mode of inheritance is unknown.

Studies of resistance to oat red leaf, the disease caused by barley yellow dwarf virus in oats (*Avena sativa* L.), have been summarized by Bruehl (1961); nothing is known of the genes involved.

In sorghum (*Sorghum vulgare* Pers.), a few varieties produce necrotic lesions systemically as a result of infection by sugarcane mosaic virus (*Marmor sacchari* H.); some are tolerant, and others show only mosaic mottling (Dean and Coleman, 1959). The mode of inheritance of these different responses is not yet known.

References

Alexander, L. J. (1963). *Phytopathology* **53**, 869. Abstr.
Apple, J. L., Chaplin, J. F., and Mann, T. J. (1962). *Phytopathology* **52**, 722. Abstr.
Baggett, J. R. (1956). *Plant Disease Reptr.* **40**, 702–707.
Bagnall, R. H. (1960). *Phytopathology* **50**, 460–464.
Bagnall, R. H., and Bradley, R. H. E. (1958). *Phytopathology* **48**, 121–125.
Bagnall, R. H., and Young, D. A. (1960). *Am. Potato J.* **37**, 311. Abstr.
Barton, D. W., Schroeder, W. T., Provvidenti, R., and Mishanec, W. (1964). *Plant Disease Reptr.* **48**, 353–355.
Bellingham, R. C., Fellows, H., and Sill, W. H., Jr. (1957). Phytopathology **47**, 516. Abstr.
Best, R. J. (1954). *Australian J. Biol. Sci.* **7**, 415–424.
Best, R. J. (1961). *Virology* **15**, 327–339.
Best, R. J., and Gallus, H. P. C. (1955). *Enzymologia* **17**, 207–221.
Black, L. M. (1953). *Phytopathology* **43**, 466. Abstr.

Black, L. M., Wolcyrz, S., and Whitcomb, R. F. (1958). *Intern. Cong. Microbiol., 7th, Stockholm, 1958* p. 255. Almqvist and Wiksells, Uppsala.
Brierley, P., and Smith, F. F. (1946). *Phytopathology* **36**, 292–296.
Bruehl, G. W. (1961). *Am. Phytopathol. Soc. Monograph* **1**, 52 pp.
Bruehl, G. W., and Damsteegt, V. D. (1964). *Plant Disease Reptr.* **48**, 470.
Buttenschön, H. (1963). Z. Pflanzenzuecht. **50**, 9–20.
Clayton, E. E. (1953). *Phytopathology* **43**, 239–244.
Cockerham, G. (1958). *Proc. 3rd Conf. Potato Virus Diseases, Lisse-Wageningen, 1957* pp. 199–203. H. Veenman & Zonen, Wageningen.
Cockerham, G., and Davidson, T. M. W. (1963). *Scot. Plant Breeding Sta. Record,* pp. 26–29.
Cook, A. A. (1961). *Phytopathology* **51**, 550–552.
Cook, A. A. (1962). *Plant Disease Reptr.* **46**, 569.
Cook, A. A. (1963a). *Phytopathology* **53**, 720–722.
Cook, A. A. (1963b). *Plant Disease Reptr.* **47**, 783–786.
Cook, A. A., and Anderson, C. R. (1959). *Phytopathology* **49**, 198–201.
Cook, A. A., and Anderson, C. R. (1960). *Phytopathology* **50**, 73–75.
Damsteegt, V. D. (1963). *Dissertation Abstr.* **23**, 4492.
Damsteegt, V. D., and Bruehl, G. W. (1964). *Phytopathology* **54**, 219–224.
Daubeny, H. A., and Stace-Smith, R. (1963). *Can. J. Plant Sci.* **43**, 413–415.
Dean, J. L., and Coleman, O. H. (1959). *Plant Disease Reptr.* **43**, 522–527.
Dean, L. L., and Wilson, V. E. (1959). *Plant Disease Reptr.* **43**, 1108–1110.
de Berchoux, C. (1960). *Oleagineux* **15**, 229–234.
deZeeuw, D. J., and Ballard, J. C. (1959). *Phytopathology* **49**, 332–334.
deZeeuw, D. J., and Crum, R. A. (1963). *Phytopathology* **53**, 337–340.
Diachun, S., and Henson, L. (1959). *Phytopathology* **49**, 537.
Endemann, W. (1955). *Ber. Inst. Tabakforsch. Dresden* **2**, 57–76.
Endemann, W., and Egerer, A. (1962). *Ber. Inst. Tabakforsch. Dresden.* **9**, 230–245.
Foster, R. E., and Dennis, H. E. (1963). *Phytopathology* **53**, 600–602.
Fulton, R. W. (1953). *Phytopathology* **43**, 472. Abstr.
Giddings, N. J. (1954). *Phytopathology* **44**, 123–125.
Greenleaf, W. H. (1956). *Phytopathology* **46**, 371–375.
Greenleaf, W. H. (1959a). *Phytopathology* **49**, 317. Abstr.
Greenleaf, W. H. (1959b). "Highlights of Agricultural Research," Vol. 6, No. 1. Agricultural Experiment Station of the Alabama Polytechnic Inst., Auburn, Alabama.
Harmon, S. A. (1960). *Dissertation Abstr.* **21**, 411–412.
Henderson, R. G., and Troutman, J. L. (1963). *Plant Disease Reptr.* **47**, 187–189.
Henderson, R. G., Sears, R. D., and Spasoff, L. (1957). *Virginia J. Sci.* **8**, 267.
Holmes, F. O. (1934). *Phytopathology* **24**, 845–873.
Holmes, F. O. (1936). *Phytopathology* **26**, 896–904.
Holmes, F. O. (1938). *Phytopathology* **28**, 553–561.
Holmes, F. O. (1952). *Indian Phytopathol.* **5**, 8–16.
Holmes, F. O. (1953). *34th. Ann. Rept. Quebec Soc. Protection Plants, Quebec, 1952*, pp. 116–127.
Holmes, F. O. (1954a). *Advan. Virus Res.* **2**, 1–30.
Holmes, F. O. (1954b). *Phytopathology* **44**, 640–642.
Holmes, F. O. (1955). *Ann. Appl. Biol.* **42**, 129–139.
Holmes, F. O. (1957). *Phytopathology* **47**, 16–17. Abstr.

Holmes, F. O. (1958). *Virology* **5**, 382–390.
Holmes, F. O. (1960). *Virology* **12**, 59–67.
Holmes, F. O. (1961). *Virology* **13**, 409–413.
Holoubek, V. (1962). *Virology* **18**, 401–404.
Houston, B. R., and Stanford, E. H. (1954). *Phytopathology* **44**, 493. Abstr.
Hubbeling, N. (1956). *Euphytica* **5**, 71–86.
Hubbeling, N. (1959). *Recent Advan. Botany* **1**, 438–443.
Inouye, T. (1962). *Ber. Ohara Inst. Landwirtsch. Biol. Okayama. Univ.* **11**, 413–496.
Jennings, D. L. (1963). *Hort. Res.* **2**, 82–96.
Johnson, B. L., and Barnhart, D. 1956. *Proc. Am. Soc. Hort. Sci.* **67**, 522–533.
Johnson, K. W., and Hagedorn, D. J. (1958). *Phytopathology* **48**, 451–453.
Kishore, H. (1960). *Dissertation Abstr.* **20**, 4249–4250.
Koelle, G. (1958). *Tabak-Forsch.* **25**, 90–92.
Koelle, G. (1961). *Zuechter* **31**, 90–93.
Koelle, G. (1962). *Zuechter* **32**, 369–371.
Koelle, G. (1963). *Zuechter* **33**, 81–84.
Kollmer, G. F., and Larson, R. H. (1960). *Wisconsin Univ. Agr. Expt. Sta. Res. Bull.* **223**, 40 pp.
Kunkel, L. O. (1940). *Publ. Am. Assoc. Advan. Sci.* **12**, 22–27.
Longworth, J. F., and Thresh, J. M. (1963). *Ann. Appl. Biol.* **52**, 117–124.
Lutes, D. D. (1954). *Dissertation Abstr.* **14**, 1900–1901.
McKinney, H. H. (1948). *Phytopathology* **38**, 1003–1013.
MacKinnon, J. P., and Bagnall, R. H. (1962). *Phytopathology* **52**, 718–719.
Martin, M. W. (1960). *Dissertation Abstr.* **20**, 3462.
Martin, M. W. (1962). *Phytopathology* **52**, 1230–1231.
Martin, M. W. (1963). *Plant Disease Reptr.* **47**, 119–120.
Menke, G. H., and Walker, J. C. (1963). *Phytopathology* **53**, 1349–1355.
Miyake, M. (1938). *Japan. J. Genet.* **14**, 239–242.
Murakishi, H. H. (1960). *Phytopathology* **50**, 408–412.
Murakishi, H. H. (1962). *Phytopathology* **52**, 22. Abstr.
Murakishi, H. H., and Honma, S. (1963). *Euphytica* **12**, 27–31.
Nielsen, L. W. (1956). *Phytopathology* **46**, 22. Abstr.
Nielsen, L. W., and Pope, D. T. (1960). *Plant Disease Reptr.* **44**, 342–347.
Norval, I. P. (1938). *Phytopathology* **28**, 675–692.
Peak, J. W., Morley, F. H. W., and Grylls, N. E. (1963). *Australian J. Agr. Res.* **14**, 639–645.
Pound, G. S. (1952). *Phytopathology* **42**, 83–88.
Pound, G. S., and Walker, J. C. (1951). *Phytopathology* **41**, 1083–1090.
Provvidenti, R., and Schroeder, W. T. (1963). *Phytopathology* **53**, 886. Abstr.
Rasmusson, D. C., and Schaller, C. W. (1959). *Agron. J.* **51**, 661–664.
Ross, H. (1954). *Proc. Intern. Congr. Genet., 9th, Bellagio, Italy, 1953, published* in *Caryologia* **6** (suppl.), 1128–1132.
Ross, H. (1958a). *Proc. 3rd Conf. Potato Virus Diseases, Lisse-Wageningen, 1957* pp. 204–211. H. Veenman & Zonen, Wageningen.
Ross, H. (1958b). *European Potato J.* **1**(4), 1–19.
Ross, H. (1960). *European Potato J.* **3**(4), 296–306.
Ross, H. (1961). *Proc. 4th Conf. Potato Virus Diseases, Braunschweig,* 1960 pp. 40–49. H. Veenman & Zonen, Wageningen.
San Juan, M. O. (1961). *Dissertation Abst.* **22**, 1789–1790. San Juan, M. O., and Pound, G. S. (1963). *Phytopathology* **53**, 1276–1279.

Schaller, C. W. (1960). *Phytopathology* **50**, 653. Abstr.
Schaller, C. W., Rasmusson, D. C., and Qualset, C. O. (1963). *Crop. Sci.* **3**, 342–344.
Schmelzer, K., and Klinkowski, M. (1959). *Züchter* **29**, 229–237.
Schroeder, W. T., and Barton, D. W. (1958). *Phytopathology* **48**, 628–632.
Schroeder, W. T., and Provvidenti, R. (1962). *Phytopathology* **52**, 751. Abstr.
Schroeder, W. T., Provvidenti, R., Barton, D. W., and Mishanec, W. (1960). *Phytopathology* **50**, 654, Abstr.
Simmonds, N. W., and Harrison, E. (1960). *Genetics* **44**, 1281–1289.
Simons, J. N. (1960). *Phytopathology* **50**, 424–428.
Simons, J. N., and Moss, L. M. (1963). *Phytopathology* **53**, 684–691.
Sinclair, J. B., and Walker, J. C. (1955). *Phytopathology* **45**, 563–564.
Sinclair J. B., and Walker, J. C. (1956). *Phytopathology* **46**, 519–522.
Singh, H. B., Joshi, B. S., Khanna, P. P., and Gupta, P. S. (1962). *Indian J. Genet.* **22**, 137–144.
Solymosy, F., and Farkas, G. L. (1962). *Nature* **195**, 835.
Solymosy, F., Farkas, G. L., and Király, Z. (1959). *Nature* **184**, 706–707.
Soost, R. K. (1963). *J. Heredity* **54**, 241–244.
Stover, R. H. (1951). *Phytopathology* **41**, 1125–1126.
Sunderland, D. W., and Merrett, M. J. (1963). *Nature* **200**, 921.
Suneson, C. A. (1955). *Agron. J.* **47**, 283.
Thompson, A. E., Lower, R. L., and Thornberry, H. H. (1962). *J. Heredity* **53**, 89–91.
Thomson, A. D. (1961). *Virology* **13**, 507–514.
Timian, R. G., and Sisler, W. W. (1955). *Plant Disease Reptr.* **39**, 550–552.
Troutman, J. L., and Fulton, R. W. (1958). *Virology* **6**, 303–316.
Walker, J. C., Larson, R. H., and Pound, G. S. (1953). *Phytopathology* **43**, 649–650.
Walker, J. C., Larson, R. H., and Pound, G. S. (1957). *Phytopathology* **47**, 269–271.
Walter, J. M. (1956a). *Phytopathology* **46**, 513–516.
Walter, J. M. (1956b). *Phytopathology* **46**, 517–519.
Wasuwat, S. L., and Walker, J. C. (1961a). *Phytopathology* **51**, 423–428.
Wasuwat, S. L., and Walker, J. C., (1961b). *Phytopathology* **51**, 614–616.
Watson, M. A. (1960). *Virology* **10**, 211–232.
Watson, R. D., Heinrich, E. C., and Harvey, W. R. (1954). *Idaho Univ. Agr. Expt. Sta. Res. Bull.* **27**, 20 pp.
Webb, R. E., Perry, B. A., Jones, H. A., and McLean, D. M. (1960). *Phytopathology* **50**, 54–56.
Weintraub, M., and Ragetli, H. W. J. (1961). *Phytopathology* **51**, 215–219.
Yen, D. E., and Fry, P. R. (1956). *Australian J. Agr. Res.* **7**, 272–280, plus 1 plate.

INTRODUCTION, TRANSLOCATION, AND DISTRIBUTION OF VIRUSES IN PLANTS

Irving R. Schneider

Plant Virology Laboratory, Crops Research Division, U.S. Department of Agriculture, Beltsville, Maryland

I. Scope of Review 163
II. Problems in Studying the Translocation of Viruses 164
A. Quantitative Aspects 164
B. Activities Concurrent with the Translocation of Viruses 165
C. Following the Translocation of Virus Particles 165
III. Introduction of Viruses into Different Regions and the Effect on Distribution 166
A. Introduction of Viruses 166
B. Effect on Distribution of Viruses 171
IV. Intracellular Movement and Distribution of Viruses 175
A. Movement 175
B. Distribution 180
V. Transport from Cell to Cell 184
A. Introduction 184
B. Short-Distance Transport 184
C. Long-Distance Transport 189
D. Intermediate-Distance Transport 199
VI. Distribution 202
A. Distribution of Tissue-Limited Viruses 202
B. Distribution Patterns of Mosaic Viruses 203
C. Meristematic Tissues 206
D. Seed Transmission 208
E. Absence of Translocation in Some Necrotic-Responding Hosts . . . 211
VII. Summary 213
References 215

I. Scope of Review

The subject of this chapter was reviewed in detail by Bennett (1956). The present writer reviews primarily research from 1956 through June 1964. Select phases have been covered by others (Esau, 1956; Esau *et al.*, 1957; Yarwood, 1957; Wildman, 1959; Crafts, 1961; Mundry, 1963; Siegel and Zaitlin, 1964). Several of the reviews are limited exclusively to tobacco mosaic virus (TMV) in leaves.

The introduction of viruses into different tissues and portions of the plant is considered especially in relation to their subsequent translocation and distribution. The infection by viruses and their multiplication are treated in relation to translocation and distribution but are not discussed in detail.

Recently, data have been obtained about early phases of virus infections, and the writer is summarizing that part which concerns the intracellular movement and distribution of viruses.

Movement from cell to cell may be via different pathways, and advances in our knowledge about translocation of viruses along these pathways, together with some remaining questions, are indicated. Movement of a virus refers to the movement of unknown form(s) of virus particles which leave one cell and initiate infection in another cell. The evidence for possible translocating forms of a virus is discussed. The possible mechanisms of transport of viruses are part of the general problem concerning the transport of solutes. The differing mechanisms of transport hypothesized, together with supporting data, are treated in other recent reviews (Esau *et al.*, 1957; Crafts, 1961; Thaine, 1962; Kursanov, 1963; Canny, 1963; Zimmermann, 1964).

Finally, the distribution of viruses within plants may be extensive or restricted, and data concerning different distribution patterns are reviewed here. The conclusions presented in this chapter are considered tentative because evidence is frequently circumstantial rather than unequivocal.

II. Problems in Studying the Translocation of Viruses

A. *Quantitative Aspects*

The translocation of viruses within plants cannot be placed on a quantitative basis (Mitchell *et al.*, 1960). The translocation of exogenously applied compounds includes both absorption and translocation. Even though the two processes are different, the two together result in the transfer of a portion of a compound applied to the plant's external surface from the region of application to various places within the plant. To learn about this, one should know what portion of the applied compound entered the plant and the subsequent distribution of the compound in different regions of the plant at different intervals of time on a quantitative basis. The compound may be poorly or readily absorbed and may be more or less rapidly metabolized into different metabolites, but some quantitative accounting may be given because some portion of the applied compound or derivatives of it are recoverable.

Serious difficulties arise when we try to gain similar data about plant viruses. The analogy of "absorption" is the introduction (through

wounds) of virus particles into plant cells. Some particles initiate an infection process, but most of the particles applied do not. The virus particles that are successfully introduced into cells are not usually the very same particles that move appreciable distances from introduction sites. Rather, some intermediate product or part of a new "generation" of virus particles may now move into nearby cells where the process is presumably repeated for every new cell infected. An unknown number of virus particles begins a process responsible for the formation of an unknown number of translocated particles. The bulk of the infectious particles appears to remain in the cell in which they were formed.

Some of the translocated particles may move only to an adjacent cell; some that enter specialized cells may move farther. The effect created when one observes symptom spread and detects large quantities of virus in various uninoculated portions of the plant is that large numbers of virus particles have moved. This may be an illusion, however, because thus far it has been impossible to detect moving particles themselves, as distinguished from virus particles formed intracellularly, and to determine the number that contribute to the rapid spread. Since viruses are not motile but are dependent upon the plant's own translocation systems, it is not likely that viruses are translocated more readily than other large particles such as nucleic acid, protein, or nucleoprotein. However, little information has been published concerning the translocation of such particles.

B. *Activities Concurrent with the Translocation of Viruses*

Since the recovery and detection of the translocated particles are usually impossible, the data obtained concerning movement have been affected by asynchronous cycles of infection, multiplication, and survival which occur concurrently. It thus has been difficult or impossible to study factors that influence the translocation of particles without also influencing the interrelated phases of virus formation. The results may thus not be explainable on the basis of translocation alone. In effect, most studies follow the accumulation of newly formed virus particles in new regions rather than the translocation of virus particles from inoculated to uninoculated regions.

C. *Following the Translocation of Virus Particles*

We have been unable to obtain data concerning the direction of virus movement within specific regions during very short periods of time. This inability results because we cannot follow the movement of radioactively labeled virus particles. As stated previously in this section, the infecting particles are not the same particles that move appreciable distances. Furthermore, it is rarely possible to distinguish between virus translocated from primarily infected loci and virus translocated from secondarily infected loci.

Translocation alone, without infection and multiplication preceding, probably can occur in the phloem of nonhosts, which may receive virus by organic union with dodder and/or an infected host and may possibly occur when virus is introduced directly by a vector into sieve tubes (Bennett, 1934) and probably occurs when virus is introduced into dead water-conducting cells of the xylem (Schneider and Worley, 1959a).

There is still considerable doubt about what moves between cells. Whatever moves must carry with it the ability to initiate an infection and the multiplication of specific nucleoprotein (virus) in cells not originally inoculated. This will be discussed in more detail in Section V,C.

III. Introduction of Viruses into Different Regions and the Effect on Distribution

A. *Introduction of Viruses*

The introduction of virus into a cell determines the primary sites from which translocation may begin. Introduced virus particles may be translocated before primary infections occur (Bennett, 1934; Schneider and Worley, 1959a). However, in most diseases, primary infections do precede the spread of virus from the cells in which the virus particles were introduced.

Viruses may be introduced into different portions of the plant; viz., into the leaf, stem, or root. It has been usual to study movement that originates from primary sites in a leaf. However, the importance of soil transmission in the initiation of primary infections has become more evident (Harrison, 1960; Cadman, 1963). Pollen can introduce viruses into an unusual entry site when it transmits viruses to mother plants via their flowers (Das and Milbrath, 1961; George and Davidson, 1963).

Viruses may be introduced into a superficial layer or alternately may be introduced into cells located below the epidermis by vectors or by tissue union with infected sources.

1. *Introduction into the Epidermis by Abrasion (Mechanical Inoculation)*

a. Inefficiency. The introduction of virus into the superficial layer of a leaf by mechanical inoculation is an inefficient process. Yarwood found that about 6% of TMV applied by rubbing remained on the leaf after washing (Yarwood, 1952). This bound virus conceivably could be the

portion of the inoculum that takes part in the infection process; but to result in a single visible infection site (a lesion), as many as 50,000 particles (Steere, 1955) to a million or more of TMV (Bawden, 1964) may be necessary. With tobacco ringspot virus (TRSV), one infection per 120,000 particles is possible (Steere, 1955). It appears that most of the particles that remain on the surface do not take part in infection if one particle per infection is required. Even if only 1.5% of the TMV rods is capable of initiating infection, as has been suggested (Rappaport and Siegel, 1955), the inefficiency of mechanical inoculation has not been satisfactorily explained.

Individual susceptible regions appear to vary in susceptibility. Thus, different numbers of virus particles are necessary to produce infection at different sites (Kleczkowski, 1950). Areas which were more difficult to infect on *Nicotiana glutinosa* L. leaves, and which required a greater concentration of TMV than easily infected areas, were those near the petiole and near the leaf tip (Rappaport and Wildman, 1959). It has been estimated that of 10^7 to 10^8 cells on the surface of a tobacco leaf, only 10^3 may be infected at one time under the usual conditions of inoculation (Wildman, 1959). Other cells must be susceptible inasmuch as many are later infected by cell-to-cell movement of virus. Not all susceptible points of entry are invaded with a single rubbing; additional rubbings may add to the total number of infections (Yarwood, 1957).

b. Wound Requirement. The possibility that undamaged cells of tissue cultures can sometimes be infected with TMV was suggested (Kassanis *et al.*, 1958). Apparently, tissue cultures lack a cuticle and their thin walls (which may be primary only) might differ in permeability from those of intact plants. However, the number of infections that may have occurred without wounds was low; the number of permanent infections depended on the number of injuries as well as on the type (Bergmann, 1959).

Root infections, presumably in the absence of vectors, do not occur unequivocally without wounds; they may occur naturally in root growth and development.

Most virus workers agree that wounds are required for introduction of virus particles into plant tissues, but the kind of wound or the particular points of entry are less certain. Because of the low success of cell-injection inoculations (Sheffield, 1936; Benda, 1956) and the comparable high success from rubbing, Yarwood questions the necessity or desirability of puncturing the susceptible cell (Yarwood, 1957). Wounding must not be too drastic; otherwise, it will prevent rather than favor infection. Most of the wounds made by rubbing are soon repaired and susceptibility decreases rapidly (Harrison, 1961; Furumoto and

Wildman, 1963). However, early changes do not always result in a reduction of susceptibility. The early reports of a rapid decrease in the susceptibility of injured leaves to TMV was confirmed (Jedlinski, 1956); but when the study was extended to other viruses and hosts, susceptibility was found to increase during the first 10 minutes, to decrease, or to remain constant, depending upon the virus-host combination used.

c. Where Is Virus Introduced? Early results suggested that leaf hairs were not points of entry (Boyle and McKinney, 1938). Wildman (1959) questioned the significance of leaf hairs as entry points for starting infection when the leaf is rubbed with virus because usually a low percentage of cells become infected after injecting large quantities of virus into hair cells. He found that most of the larger leaf hairs were destroyed by rubbing the leaf surface in the absence of virus. Yet when the leaf was rubbed with virus 24 hours later, the same number of lesions appeared as on control leaves which did not receive the rubbing pretreatment (Wildman, 1959). Recent work has suggested the basal septa of broken trichomes in *Nicotiana* as possible infection sites for TMV (Kontaxis and Schlegel, 1962). But virus accumulation on ruptured epidermis and broken trichomes of nonhosts and hosts indicated that susceptibility is determined by other factors (Herridge and Schlegel, 1962). Yarwood (1962) concluded that trichomes aid but are not necessary for contact transmission of virus. Mundry (1963) considered the relationship between the concentration of virus and the saturation of all susceptible sites and suggests that a susceptible site may be close to a few microns in diameter. In agreement with this view, the relative susceptibility of leaves toward infection with TMV correlated with the average number of ectodesmata (Zech and Vogt-Köhne, 1956).

Direct introduction of virus into single cells of trichomes (of tobacco) is possible, and it provides direct evidence that cells in trichomes can be ports of entry. In such a procedure, a drop of cell content was extruded and apparently retracted (Hildebrand, 1943). Virus may enter wounded cells in these droplets. Hirai and Hirai (1964) reported recently that 30–80% infections resulted when they injected TMV into a large cell at the base of a trichome from tomato stems.

It is probable that mechanical inoculations do not introduce virus any deeper than the epidermal layer. Fry and Matthews (1963) found that less than 1% of the disks of leaves from which the epidermis was stripped at the time of inoculation became infected, indicating that the virus was rarely introduced into the mesophyll.

d. Number of Particles per Cell for Infection. In spite of the inefficiency of introducing viruses by abrasion, the dilution curves of many plant viruses fit the assumption that a single particle is sufficient to

initiate an infection. The variation in host susceptibility is explained by the differences in numbers of susceptible sites (Lauffer and Price, 1945). The exponential survival curve during the first phase of the infection process is independent of inoculum concentration; this was interpreted to mean that an exclusion mechanism is operative, permitting only one infectious particle to initiate an infection (Siegel and Wildman, 1956). The specific interference between strains of the same virus and the lack of specific interference between TMV and tobacco necrosis virus (TNV) (two unrelated viruses) was interpreted to mean that strains of the same virus utilize the same site to institute infection, but that unrelated viruses use different sites (Wu and Hudson, 1963). However, when a mixture of two strains of TMV was injected into a single hair cell of a seedling leaf of *Nicotiana glutinosa* L., both strains were found in 5 of the 20 lesions that resulted (Benda, 1956). Results with the effect of dilution on necrotic ringspot virus infectivity and the enhancement of infectivity by noninfective virus suggested that a dose of two or more particles at an infection site is necessary to initiate infection and that the dose requirement may be satisfied partly by virus that is unable to infect alone (Fulton, 1962).

e. Detection of Infection. Detection of infection is dependent upon subsequent virus multiplication. This may result in the appearance of a lesion. Results with heat activation of virus infection and other studies suggest that inoculation with viruses may produce more local infections than lesions (Yarwood, 1951, 1958; Helms and McIntyre, 1962). The appearance of more lesions on heated than on nonheated leaves may be the result of the activation of these otherwise latent infections (Yarwood, 1958).

2. *Introduction of Viruses by Vectors*

Virus may be introduced into superficial layers by vectors as well as mechanical inoculation. No data are known which compare the number of particles required per infection site by a vector with that required in mechanical inoculations. It may be that a vector introduces fewer particles per cell in a less severely damaged cell than introduction by mechanical inoculation. Commonly, translocation begins from a more limited number of primary infections and in a more limited area than with mechanical inoculation.

a. Epidermis. Myzus persicae rarely becomes infective with potato virus Y and rarely transmits it after the stylets penetrate beyond the first layer of plant cells (Bradley, 1956). This probably applies to many aphid-borne "nonpersistent" viruses. The time spent in feeding can determine the depth of penetration. In short feeding periods, the stylets rarely passed the epidermis; but in prolonged feedings, they penetrated into subepidermal tissues (Bradley, 1952).

b. Introduction into Epidermis and Deeper Tissue. Some viruses may be introduced into a variety of cells. For example, sugarbeet yellows virus infections may result from introduction of virus by aphids into phloem as well as into parenchyma and epidermal cells (Bennett, 1960).

c. Introduction into Phloem and Xylem. Some viruses appear to be transmitted exclusively by introduction into the phloem and others by introduction into the xylem (Bennett, 1956; Esau, 1956). Leafhoppers may introduce viruses into the phloem. Of 26 viruses transmitted by leafhoppers, only 3 have been transmitted mechanically (Black, 1954), and 2 of the 3 viruses were transmitted poorly by pinpricks through solutions into the crowns of plants. Apparently, viruses in this group must reach functioning cells of the phloem.

Not all viruses that are introduced into the phloem are introduced into the same kind of cell. For example, when the curly top virus was found to move 15 cm. in the first 6 minutes after introduction (Bennett, 1934), the virus was probably introduced directly into the sieve tube element. On the other hand, although much evidence indicates that the potato leaf roll virus is also introduced by its vector into the phloem (Bennett, 1956), the virus may take 24 hours or longer to pass from an inoculated leaf to the stem (Beemster, 1959). This suggests that the virus is not brought directly into the sieve tubes but into some other part of the phloem. Demonstrations that phloem tissue has been penetrated by aphids are relatively common but not demonstrations of the puncturing of sieve elements (Esau *et al.*, 1961).

3. *Introduction into Roots*

Root introduction of virus results frequently in a distribution of the virus different from aerial introduction of the same virus. Virus may be introduced into roots by root contacts (Roberts, 1948), the soil (Roberts, 1950; Miyamoto, 1959; Yarwood, 1960a; Menon and Shanta, 1962), or circulating water (Sturgess, 1962). Various vectors are known for viruses that appear to be introduced into the superficial layers of roots. These agents include nematodes and fungi (Harrison, 1960; Cadman, 1963).

4. *Introduction by Dodder or Graft Union*

Dodder or graft union can introduce large numbers of virus particles into different cells, including those of the vascular system (Bennett, 1956).

B. *Effect on Distribution of Viruses*

The rate of symptom development and the severity of symptoms in systemically invaded tissue commonly tends to be greater as the inoculum concentration is increased. This response plausibly results from the larger number of particles egressing from the increased number of primary infection sites; the particles more quickly and completely invade and infect uninoculated portions of the plant. The number of primary infection sites may also determine if long-distance transport occurs in some virus-host combinations; this may be an indirect effect since increasing the number of infections will also increase the chances of their occurrence at appropriate locations. Different symptoms may result from insect and mechanical inoculations but have not been explained (Costa *et al.*, 1958; Bennett, 1960).

The location of the inoculation may have a profound effect on the subsequent spread of virus. Viruses that are not very restricted in their tissue-invading capabilities may still be more or less restricted in their spread, depending upon the tissues inoculated or on the part of plant inoculated.

1. *Influence of Primary Infections*

Factors which affected the frequency of occurrence of systemic U2 infections after mixed inoculation with U1 and U2, two strains of TMV, were studied (Cohen *et al.*, 1957). It was postulated that systemic infection arises either from the systemic egress of only a limited number of the virus particles present in the inoculated leaf or from the contribution of virus particles from only a limited number of primary infections (Cohen *et al.*, 1957). It may be that systemic egress arises both from a limited number of virus particles and from a limited number of primary infections. Bennett (1960) observed a correlation between the number of local lesions produced as a result of infection by the sugarbeet yellows virus (on *Chenopodium capitatum*) and the probability of systemic infection. Only about 10% of the plants with 25 lesions or less became infected systemically. Systemic infection of 100% was obtained only after the number of local lesions reached 200 per plant. Bennett (1960) suggested that the virus is limited to parenchyma cells in the majority of lesions, but the virus may escape into the phloem in a low percentage of instances and produce systemic infection.

The writer has found that a similar phenomenon occurs with tobacco ringspot infections in Pinto bean plants (Schneider, 1961). All plants became systemically infected when their primary leaves were inoculated with concentrated inoculum. Inoculation with dilutions of the same inoculum resulted in plants without detectable virus in uninoculated leaves although primary leaves attached to the same plants had a small number of lesions. The time required for symptom development increased when the concentration of potato virus X inoculated on leaves of *Datura tatula* L. was decreased or the area inoculated was restricted (Hooker and Benson, 1960).

Differences in symptoms that result from different methods of virus introduction have been noted. For example, Costa *et al.* (1958) obtained transmission of sowbane mosaic virus to sowbane by means of the leaf miner, but local lesions (that normally result from mechanical inoculations with the same virus) were not observed around many hundreds of insect-feeding punctures in the leaves. Furthermore, local lesions do not result from introduction of the sugarbeet yellows virus into leaves of *Chenopodium capitatum* by the green peach aphid, but local lesions are often evident after introduction of the virus by abrasion (Bennett, 1960). Usually, with local-lesion symptoms, most virus is associated with the necrosis, but whether less virus particles per infection locus resulted from infections by the vectors than from infections of the same viruses when introduced mechanically is not known.

2. *Tissue Reached and/or Part of the Plant Inoculated*

With viruses that are restricted to certain tissues, such as Pierce's disease virus in the xylem and the curly top virus in the phloem, the establishment and spread of the virus may depend on whether it is introduced into the appropriate tissue. For example, when the vector of Pierce's disease virus was limited to the bark of the grapevine by a metal plate inserted between the bark and the wood, no transmission occurred. When, however, insects were placed on wood freed from bark, the virus was successfully introduced into the plant (Houston *et al.*, 1947).

The infection and distribution of viruses which are not as restricted in their relationship to their tissues as are the curly top and Pierce's disease viruses may still be affected by the kind of tissue into which the viruses are introduced.

Reports indicate that *Myzus persicae* is more successful in transmitting the beet yellows virus during long periods of penetration of leaves than during short ones (Roberts, 1940; Bennett, 1960). The green peach aphid (*M. persicae*) is able to infect with beet yellows virus in a feeding time as short as 5 minutes. However, there is a marked increase in the percentage of infection in the 1-hour feeding period as compared to shorter periods (Bennett, 1960).

Both tobacco necrosis virus and southern bean mosaic virus (SBMV) will serve to illustrate how profoundly different the spread of a virus may be, depending upon the location of virus introduction both with respect to the tissue and the part of the plant. Smith found that TNV did not normally move from the roots. However, if the virus was inoculated by needle prick into the center of a bean stem, it multiplied and moved both up and down, as indicated by the necrosis of cells. The plant was almost invariably killed by a systemic necrosis, but sometimes the progress of necrosis, and presumably of the virus, was stopped by a node. Although TNV multiplied readily in inoculated leaves and roots of tobacco, it did not appear to multiply in stems of tobacco, and it could not be recovered from the inoculated zone a week or two later (Smith, 1951).

After inoculation with TNV, the leaves of many plants typically respond with necrotic lesions within which the virus remains localized. Some recent studies indicate that the leaves of plants may be free of symptoms but not of TNV when their roots are infected with the virus. Kassanis and Nixon (1961) found infectious TNV in apparently healthy tobacco leaves. Babos and Kassanis (1963) found that the leaves of *D. tatula* and *N. clevelandii*, although symptomless, often contained considerably more virus than that found in *N. tabacum*. There was considerably more virus in old than in young plants, and this was usually correlated with the amount in their roots. It was suggested that the virus moved from the roots into the leaves but did not multiply.

Yarwood (1959a) found the roots relatively more resistant to mechanical inoculations with TNV than the leaves of Prince bean seedlings. He compared different sites of inoculation in the root and found differences in susceptibility. Infections always failed in some regions. Apparently, the regions resistant to inoculation were also resistant to invasion of the virus from other parts. When inoculated directly, the upper part of the hypocotyl or the tip of inoculated young bean roots appeared to be free of TNV. However, the virus was found in these regions in greenhouse plants about 40 days after their inoculation as seedlings. Yarwood (1959b) summarizes the differences observed when TNV is introduced into different parts of the same plant: "It is injurious if introduced by mechanical inoculation into leaves, noninjurious if it reaches leaves as a result of inoculation of the roots, is relatively noninfectious if introduced into the stems, and stimulatory if introduced into the roots."

Information concerning the influence of the part of the plant inoculated or the tissue reached has been derived from studies with SBMV in a hypersensitive host (Pinto bean). When smooth expanded primary leaves are mechanically inoculated with SBMV, necrotic lesions, a few millimeters in diameter, result. Most of the virus particles remain localized in these necrotic regions (Schneider and Worley, 1958), and egress from the inoculated leaf is not detectable. When the same virus is introduced into the hypocotyl, also by mechanical inoculation, the early appearing necrosis is localized as in the leaf; but after penetrating into the phloem region, internal streaking upward and downward is associated with the fibers of the phloem (Mitchell *et al.*, 1956). Although the virus may extend vertically several centimeters in the stem, contrasting with the spread of only a few millimeters in the leaf, the virus has never been detected in any part of a mature plant above where the primary leaves join the stem (Schneider and Worley, 1960).

If the virus was introduced directly into water-conducting elements, however, a systemiclike necrosis in trifoliolate leaves above the site of introduction resulted (Schneider and Worley, 1959a). The growing region was rarely affected; necrosis failed to continue to appear indefinitely as more leaves expanded at higher and higher nodes.

When the virus was introduced into the same region of the stem by approach-grafting with Black Valentine bean plants inoculated after approach grafting, the most extensive invasion of the receptor bean plant (Pinto variety) occurred. This, together with the rapid two-directional movement, suggested that the virus had invaded the phloem of the Pinto variety more extensively than it was able to do when introduced by any of the other methods.

The distribution of many viruses differs when virus is introduced by graft union rather than by mechanical inoculation. Hypersensitive hosts, which typically respond with necrotic local lesions when mechanically inoculated, will respond with a systemic necrosis when the same virus is introduced by means of a systemically infected donor. This has been found to occur with SBMV and common bean mosaic virus in bean (Bridgmon and Walker, 1951; Grogan and Walker, 1948), TMV in *N. glutinosa* (Smith, 1951), potato virus Y in potato (Hutton and Bald, 1945), potato virus X in hypersensitive potato varieties (Bawden, 1964), and carnation mosaic virus in clones of *Dianthus barbatus* (Weintraub *et al.*, 1961). It appears that the virus reaches and moves in cells in the stem of the hypersensitive host when introduced by the graft union, but the virus does not ordinarily reach these same cells when introduced into the leaves by mechanical inoculation.

The question that remains is why viruses mechanically inoculated into leaves of hypersensitive hosts rarely egress from these leaves and reach cells in the stem through which they can be more extensively

distributed. Weintraub *et al.* (1961, 1963) stated that the entry of virus into the vascular system may not suffice for systemic infection in *Nicotiana glutinosa* L. grafted to tobacco systemically infected with TMV. They favored an earlier suggestion (Walker, 1953) that when a continuously high inoculum enters the hypersensitive host, the virus can become systemic. The observations of Zaitlin (1962) led him to suggest that the systemic infection of hypersensitive hosts which are the result of grafting is due to the introduction of the virus into the vascular elements (probably the phloem). According to Zaitlin, entry into the vascular system is normally a rare event in a hypersensitive host.

The general problem of why viruses do not normally egress from mechanically inoculated leaves of hypersensitive responding hosts will be considered in Section VI. From the experimental work reviewed to this point, it is apparent that virus particles that reach the internally located cells of the stem can spread appreciable distances in hypersensitive hosts, and systemic necrotic symptoms result.

Other experimental evidence suggests that virus invades more extensively when it is introduced by means of a graft union than when it is introduced by a vector (Bennett, 1953). Tobacco plants that recovered from curly top appeared to be immune to infection with yellow-vein virus introduced by means of the beet leafhopper. They were resistant but not immune to infection with the same virus introduced by means of diseased scions (Bennett, 1957).

Several viruses that are transmitted by ectoparasitic nematodes are also transmitted through the seed of various kinds of plants (Cadman and Lister, 1961). A feature of the infected progeny from infected plants of several kinds was the absence of any of the symptoms typical of their infection with the viruses concerned. Many seemed normal or nearly so; others showed symptoms quite unlike any seen in artificially infected plants. It is likely that invasion by the virus present in the seeds of these plants is different in time and extent from mechanical introduction of the same virus into the corresponding host.

IV. Intracellular Movement and Distribution of Viruses

A. *Movement*

1. *Speculative Basis*

One can speculate about the possible intracellular movement involved in the passage of virus from the initial entry ports to the various sites of activity where production and assembly of new virus particles may occur. Let us assume that there are at least three sites of activity: nucleic acid synthesis, protein synthesis, and assembly of nucleic acid and protein. Let us assume further that nucleic acid is responsible for the replication of more virus nucleic acid and is also responsible through its provision of a template for the synthesis of virus-specific protein. Then the occurrence of these processes at different sites would require, at the minimum, that virus nucleic acid should move to the site of virus nucleic acid synthesis and to the site of virus protein synthesis; and that ultimately the newly synthesized nucleic acid and protein must rendezvous at assembly sites. If the virus particle is intact upon entrance into the cell, it may be freed of its protein shell before reaching the nucleic acid replication site and move as free nucleic acid to that site, or it may move as nucleoprotein and be freed of protein after reaching the nucleic acid replication site.

The various parts of complete virus particles which may move intracellularly are not known. It may be assumed, however, that infectious nucleic acid, protein, a replicative unit, if such occurs, as well as complete virus particles probably do move intracellularly. Zech (1960) reported structures that may be intermediary products formed during TMV reproduction.

2. Early Movement and Removal of Protein

Intact virus, after introduction by mechanical inoculation, probably separates into nucleic acid and protein (Bawden, 1964). Diener (1962) detected virus nucleic acid in TMV-infected cells after intact virus was inoculated. It is not known when and where in the cell the separation of nucleic acid and protein occurs. Free nucleic acid is more sensitive to inactivation by ultraviolet (UV) radiation than are intact virus particles (McLaren and Takahashi, 1957). During the first few hours, however, the sensitivity to UV radiation remains constant. It has been suggested that separation may not occur during the first few hours (Siegel *et al.*, 1957). But when sensitivity does change, it decreases rather than increases; this may mean that the separation of protein and nucleic acid occurs behind or inside UV-absorbing material (e.g., the nucleus) (Harrison, 1961). The sensitivity to UV changes earlier when nucleic acid serves as inoculum, but it also decreases. This fits with Bawden's (1964) view that irradiation of virus-infected leaves reflects changes produced by infection but does not show the nature of the changes. Bawden and Kleczkowski (1960) found that irradiation of leaves at different intervals after inoculation with intact TMV did not increase the susceptibility of infection centers to inactivation. Also, no enhanced

effect of photoreactivation was detected. Both results show that although virus may separate into nucleic acid and protein, ultraviolet radiation has not revealed when this occurs.

Benda (1959) found that the nucleus may move toward a wounded portion of the cell. Conceivably, intracellular movement may be movement of the nucleus toward virus entering wounds rather than virus moving toward the nucleus. Hirai and Wildman (1963) noted the migration of nuclei toward the transverse wall of the basal hair cell nearest the epidermis after TMV was inoculated into a strip of epidermal tissue. Presumably, in this example, the cell in which the nucleus migrated had not been wounded, but the adjacent infected cell had somehow induced the migration of the nucleus.

3. Nuclear Activity and Possible Movement of Viruses

Other studies concerned with the early sequence of events, from inoculation onward through multiplication of virus particles, have provided some evidence of possible intracellular virus movement. Observations of living material, coupled with histochemical data, have been concerned primarily with infections of TMV. They have contributed early sequential images of cell activities induced as a result of virus infection; they thus provide circumstantial evidence of possible sites of the formation of virus nucleic acid, protein, and possible sites of assembly. The continuous observations of living material can provide a more precise sequential picture than is possible with fixed material alone, and it can avoid the artifacts and dislocations of structures which may result from fixation.

The nucleus is the site of earliest activity detected in studies by various workers of TMV-infected cells (Zech, 1952, 1954; Bald and Solberg, 1961). Wettstein and Zech (1962) considered this the first phase of TMV reproduction which lasted from 30 minutes to 2 hours after inoculation, during which the nucleic acids in the nucleus and the nuclear mass increased. Formations of the nuclear membrane indicated that nuclear material, enveloped in a double nuclear membrane, was pinched off from the nucleus and transported through channels into the cytoplasm. Bald (1964) noted cycles of change in the nucleolus of infected cells. Phase microscopy revealed apparent emission of material optically less dense than the nucleoplasm. Images of stained sections indicated that this material was ribonucleic acid (RNA). Hirai and Wildman (1963) described a granular body, which was different from X-bodies and which maintained a close association with the nucleus of TMV-infected hair cells. It was found to be rich in RNA, a fact which suggests that viral RNA of the nucleus may have been entering the cytoplasm via the granular body. They suggested the possibility that as viral RNA is released from the nucleus, it is surrounded by the sphaerosomes and then transported to other regions of the cell via the protoplasmic streaming. The observations of Bald and Solberg (1961) indicated a possible functional association between nucleus and plastids. Chloroplasts became tightly adherent to the nucleus and remained so in spite of rapid streaming of cytoplasm around them. Approaching the nucleus before adhering to it, a chloroplast may put out a fine protrusion that attains a length of 5 to 30 μ. A second protrusion from the opposite side of the same chloroplast occasionally appeared and became surrounded by spherosomes, mitochondria, etc. More than 30 chloroplasts have been seen adherent to a large nucleus. Bald and Solberg (1961) interpreted the bubbling they observed around the nucleus as an indication of a radical change in permeability of the nuclear membrane and nearby endoplasmic reticulum.

Hirai and Hirai (1964) used a fluorescent antibody to follow this early stage of TMV infection. Soon after injection of TMV, specific fluorescence was sometimes observed near the position where the virus was injected into the base cells, but this disappeared within 1 to 3 hours after injection. Six hours after injection a new fluorescent antibody reaction could be detected in about 50% of the cells injected with TMV. It was mainly confined to the nucleus and was less frequently found in both the nucleus and the cytoplasm adhering to the nucleus. With time, the percentage of cells that showed the TMV antibody reaction increased, but the reaction was less apparent in the nuclei and more prominent in the cytoplasm immediately surrounding the nuclei. A similar sequence was induced when TMV-RNA was injected, except that no early reaction with antibody as in TMV injections was evident in the cytoplasm immediately after injection. Although nuclei sometimes reacted with the fluorescent antibody after inactivated TMV was injected into hair cells, the reaction disappeared 6 hours after injection, and no further antibody stain either in the nuclei or other cell components was seen at later periods. This suggests the possibility that inactivated TMV can enter the nucleus although no further development of new virus proceeds. Specific antigen of TMV was not detected in the nucleus by Schramm and Röttger (1959), who found that only the cytoplasm around the nucleus exhibited the specific fluorescence. They concluded that the synthesis of virus protein seemed to take place in the cytoplasm, but they did not detect specific fluorescence at the earliest until 45 or more hours after infection. The location of fluorescence approaches that of the later images rather than the early images of Hirai and Hirai (1964).

Other evidence for the early activity of the nucleus in TMV-infected cells is that tritiated uridine was selectively incorporated into the nucleus and nucleolus of tobacco leaf disks infected with TMV that had been treated with actinomycin D (Smith and Schlegel, 1964a).

Although most of the evidence for early nuclear activity has been observed in TMV infections, other early images of nuclear activity associated with virus infections have been observed with infections of *Beta vulgaris* with beet mosaic virus (Reitberger, 1956) and of NN Burley tobacco with spotted wilt virus (Bald, 1964).

4. *Other Possible Sites of Activity and Movement*

Movement of TMV into and out of the nucleus would not be involved if it could be shown that the formation of the complete virus occurs in some other organelle or at some other site. The highest rate of incorporation into cytoplasmic protein was observed 6 hours after infection, but no virus was found at this time (Hayashi, 1963). Later, the rate of incorporation increased in nuclear and chloroplast fractions. It was suggested that, possibly, virus protein is synthesized on the surface of the nuclei and/or chloroplasts. Chloroplasts were suggested as possible sites of TMV formation (Zaitlin and Boardman, 1958; Boardman and Zaitlin, 1958). Some workers believe that virus crystals may indicate the site of virus synthesis; they argue that crystallization would probably occur where concentrated relatively pure particles occur (Fukushi *et al.*, 1962; Koshimizu and Iizuka, 1957); and except for a few strains of TMV, TMV inclusions are found in the cytoplasm rather than in the nucleus (Woods and Eck, 1948; Gold'in and Vostrova, 1959). Inclusions associated with TMV have not been demonstrated within intact chloroplasts, but virus particles have been described in association with chloroplasts (Leyon, 1953; Shalla, 1964a). The development of vacuoles within disrupting chloroplasts conceivably could lead to entrapment of particles of virus (Sorokin 1927; Shalla, 1964a). Wettstein and Zech (1962) presented evidence that the synthesis and assembly of TMV protein is carried out in localized areas of the cytoplasm with a folded differentiation of the endoplasmic reticulum. More recent microscopy revealed (Kolehmainen *et al.*, 1965) fluid crystals (which may be the gray plates of Bald and Solberg) within a network of the endoplasmic reticulum and ribosomes.

The distribution of virus within the cell appears to vary considerably with different viruses. Many of the observations reported do not provide any early sequential information that would necessarily apply to intracellular movement and will, therefore, be considered in the next section concerned with intracellular distribution.

B. *Distribution*

1. *General*

Earlier studies indicated that the cytoplasm contains most of the virus particles present in living cells, and few, if any, virus particles are present in vacuolar sap (Bennett, 1956). Support for this view is contained in much recent microscopy, but the intracellular location of virus within the cytoplasm may vary with different viruses and strains of the same virus. The distribution may also vary, depending on the stage of infection considered, as indicated in the previous section. Several workers have postulated that virus crystals may indicate the site of virus reproduction (Koshimizu and Iizuka, 1957; Fukushi *et al.*, 1962). The postulation may not apply to viruses if their inclusions are mobile (Resconich, 1961); however, the microscopy of inclusions associated with virus infections can at least reveal the location of large virus concentrations when such inclusions are known to be largely composed of virus. This was first shown to be true for TMV (Steere and Williams, 1953).

2. *Virus in Discrete Areas or within Inclusions in the Cytoplasm*

Extensive examination of ultrathin sections from different portions of plants systemically infected with TMV failed to demonstrate the presence of rod-shaped particles that resembled TMV, except in cells containing crystalline inclusion bodies (Shalla, 1959; Wettstein and Zech, 1962). Preparation methods may shatter inclusions and result in sections with dispersed particles which originally may have been part of a crystal (Gold'in, 1960). Although crystals of the common strain of TMV are found in the cytoplasm of all living cells of systemically invaded plants (Esau, 1941), the occurrence of crystals of other strains, such as aucuba or enation strains, in certain cells may be less frequent (Smith, 1958). These inclusions appear mobile, particularly at elevated temperatures; crystals apparently different in fixed sections may be different views of the same crystal (Resconich, 1961).

The absence of inclusions cannot be taken to mean the absence of virus even with viruses that do form crystals. The host environment may or may not favor their formation (Bawden, 1964).

In contrast to TMV, rod-shaped particles resembling those associated with barley stripe mosaic virus were easily demonstrated in the absence of inclusions in the cytoplasm of various cells of barley leaves (Shalla, 1959). Particles were never found in the vacuole or in association with nuclei or chloroplasts. Many viruses consisting of long filamentous particles are commonly distributed randomly in the cytoplasm (Ferreira and Borges, 1958; Herold and Weibel, 1962).

In leaf cells of *D. stramonium* infected with potato virus X, the particles believed to be the virus occurred usually in fibrous masses within the cytoplasm (Kikumoto and Matsui, 1961). The chloroplasts, nuclei, and mitochondria within the virus-infected leaf cells appeared to be intact; no virus or viruslike particles were detectable within them. These observations agree with others (Brandes, 1956; Borges and Ferreira, 1959), who also did not detect potato virus X-like particles within chloroplasts, nuclei, or mitochondria.

Small spherical particles of plant viruses are difficult to distinguish in the cytoplasm. Smith (1956) reported that he found groups of particles in regular alignment on the inner edge of the cytoplasm of certain cells of bushy-stunt infected plants. The spacing of this crystalline arrangement was 30 mμ and is the same as in crystals of the tomato bushy stunt virus.

Characteristic particles of wound tumor virus were found inside the cytoplasm of virus-infected tumor cells in defined structures. However, these structures could not be identified with certainty (Shikata *et al.*, 1964).

Particles of rice dwarf virus were frequently arranged in rows in the endoplasmic reticulum (Fukushi *et al.*, 1962). Large inclusions showed fluorescence when treated with a fluorescent antibody (Hirai *et al.*, 1964).

3. *Virus in Plastids?*

The effects of virus diseases of the mosaic type of plastids have been described. Plastids have been postulated as possible sites of TMV multiplication (Zaitlin and Boardman, 1958; Boardman and Zaitlin, 1958). Electron microscopic examination revealed particles of TMV (as well as of beet yellows virus) which were frequently in association with chloroplasts and appeared to have been extruded from them (Leyon, 1953). The possibility of secondhand attachment of particles to fragmented chloroplasts, or particle assembly in ground cytoplasm and subsequent envelopment by chloroplasts (Shalla, 1964a) was not excluded. Further studies of beet yellows virus indicate that abundant inclusions accumulate and may almost fill the cells (Esau, 1960a). Many cells with inclusions seem to retain normal nuclei, but plastids degenerate rather rapidly. Inclusions were found to be the most consistent indicators of the presence of the virus in plants. They could be found in the absence of any other internal symptom. The inclusions did not stain with the chlorazol pink B and trypan blue combination (Esau, 1960b).

It was reported later that some chloroplasts of sugarbeet plants infected with either beet yellows virus or Western yellows virus contain inclusions with a regular pattern (Engelbrecht and Esau, 1963), and inclusions with a regular linear or cross-hatched pattern were described in the chloroplasts of sugarbeet plants infected with tomato ringspot virus (Engelbrecht and Weier, 1963). In proplastids of the young developing leaves, the hexagonal inclusions were enclosed in a membrane. The presence of the inclusions which are closely associated with the yellows and ringspot diseases in beet is evident, but the significance with respect to the location of virus awaits further information.

Two different viruses of white clover, each inducing a different type of cytoplasmic inclusion, have been described (Koshimizu and Iizuka, 1957). Both inclusions appeared to originate from plastids; since both types could be found in the same cell, the authors considered that each virus was formed in separate plastids. The inclusions contained both protein and nucleic acid. Although inclusions associated with some virus diseases do occur within plastids, characteristic virus particles within these inclusions have not been detected to date.

4. Virus in X-Bodies and Relation to Distribution

All the X-bodies that have been tested have given the usual protein reactions and have been Feulgen-negative (Bawden, 1964). Furthermore, it has been possible to demonstrate particles characteristic of the virus in the X-bodies associated with many diseases; however, in other diseases, few, if any, particles characteristic of the virus can be found in the X-bodies.

Some X-bodies change into obvious fibrous or semicrystalline forms. These include those associated with henbane mosaic virus, severe etch virus (Bawden, 1964), aucuba mosaic virus, and petunia ringspot virus (Rubio-Huertos, 1962). Granular X-bodies formed by sugarbeet yellows virus later become fibrous or banded and faintly birefringent (Esau, 1960b).

The inclusions formed by tomato aucuba mosaic virus (Bawden, 1964), tobacco etch (Sheffield, 1946), cabbage black ring, turnip yellow mosaic (Rubio, 1956), and petunia ringspot virus (Rubio-Huertos, 1962) contain particles of the same shape and size as the virus particles. Electron micrographs of inclusions in cabbage black ring infected plants reveal particles aligned in parallel (Rubio, 1956), which suggests that the common development of birefringence as X-bodies age probably means that the elongated particles are initially at random but later become increasingly concentrated and oriented (Bawden, 1964). Electron micrographs of the X-bodies induced by infections with the broad bean

mottle virus show they are composed mainly or entirely of spherical particles (Rubio and van Slogteren, 1956), which, both in size and shape, are apparently identical with the broad bean mottle virus in purified preparations (Bawden *et al.*, 1951). In contrast, it was rather difficult to find particles of cauliflower mosaic virus (Rubio, 1956), or *Phaseolus* virus 2 (Rubio and Van Slogteren, 1956), or TMV rods within an X-body (Matsui, 1959).

It is thus apparent that although X-bodies of different viruses may be similar when viewed by light microscopy, the bodies may indicate large concentrations of virus in some diseases, but in others they do not.

5. Intranuclear Distribution of Inclusions

Although the common strain of TMV induces the formation of cytoplasmic crystals, other strains of TMV have been reported which induce the formation of intranuclear crystals (Woods, and Eck, 1948; Gold'in and Vostrova, 1959). Both strains of TMV produced cytoplasmic as well as nuclear inclusions. The nuclear as well as the cytoplasmic inclusions induced by the yellow mottle strain reacted in all tests, as did the various cytoplasmic inclusions found in cells infected with the type virus (Woods and Eck, 1948).

Crystalline intranuclear inclusions of tobacco etch virus did not stain with acridine orange. Purified TMV and TNV, dried on a glass slide, fluoresced flame-red after staining with acridine orange (Hooker and Summanwar, 1964). Takahashi (1962) found that intranuclear inclusions of tobacco etch virus did not stain with nucleic acid stains but did stain with fast green. In electron microscopic studies of *Datura stramonium* L. leaves with fully developed external symptoms, virus particles were found in a mass within the cytoplasm but not within the nucleus (Matsui and Yamaguchi, 1964). Although virus or viruslike particles could not be found within the intranuclear crystals, some crystals did exhibit a regular internal pattern. More recently, elongated particles were found within the nuclei of tobacco infected with severe etch virus, which the authors believed were virus particles (Rubio-Huertos and Hidalgo, 1964).

Intranuclear crystals have also been reported to be associated with other virus-induced diseases; for example, the viruses causing pea mosaic and yellow bean mosaic (McWhorter, 1941), leaf crumple virus (Erwin and Meyer, 1961), and dahlia mosaic virus (Robb, 1963, 1964). In epidermal cells of *Dahlia variabilis* Cav. infected with the latter virus, inclusion bodies appear to form in the nuclei of immature tissue and to be extruded into the cytoplasm. They are spherical and hyaline (spherule type), similar to bodies described by Littau and Black (1952) in wound tumor-infected tissue and different from amorphous X-bodies. The granules stain positively for RNA and protein. It was postulated that the inclusion bodies undergo a cycle of development which may be related to virus multiplication.

In one of two virus diseases described in white clover, intranuclear inclusions were reported (Koshimizu and Iizuka, 1957). The intranuclear inclusions were shown to develop from the nucleoli.

It is evident that inclusions associated with different diseases may be found within various bodies and various organelles of the cell. The explanation for this large variation sometimes in the same virus disease is not clear. Perhaps more precise time studies of early events in sequence will help to clarify the steps involved in movement and subsequent distribution of particles with a cell.

V. Transport from Cell to Cell

A. Introduction

Transport from cell to cell is conventionally divided into two categories (Bennett, 1956). One is the slow, short-distance transport system, and the other is the much more rapid long-distance transport system. Within the latter system, the xylem and phloem are included. Published data indicate that the phloem is the more important pathway in the long-distance system as far as viruses are concerned.

A third pathway, included in this section, is designated "intermediate distance transport." The separation is merely for convenience to permit the writer to introduce and discuss movement more rapid than the characteristic slow movement in parenchyma (Bennett, 1956). The examples discussed resemble slow transport since they appear to involve a continuous cell-to-cell progression. Nevertheless, movement in specialized living cells which has received little attention in regard to translocation may play a significant role in the complete and rapid distribution of viruses concurrent with long-distance transport.

B. Short-Distance Transport

1. *Pathway between Living Cells*

The pathway followed by viruses, at least in tissue culture, has been questioned (Kassanis *et al.*, 1958; Brants, 1961). No plasmodesmata were found to join cells in tissue cultures. The technique of Crafts (1931) which resulted in the demonstration of plasmodesmata in tobacco stems, leaves, potato tubers, and stems was used. Electron microscopy revealed small holes in normal tissue culture walls, but none in the conditioned tissue cultures (Kassanis *et al.*, 1958). Brants (1961) found thin walls in tissue cultures, but no plasmodesmata. Although no plasmodesmata were found TMV moved between cells at a rate of about 1 mm. per week (Kassanis *et al.*, 1958). Lambertz (1954) concluded from light miscroscope studies of plants that plasmodesmata are tentacle-like extensions of protoplasts, at first independent of those derived from adjacent protoplasts but later uniting. He described them as being làbile structures periodically withdrawn from the cell walls. Others have concluded, however, that plasmodesmata are continuous cytoplasmic strands that connect adjacent protoplasts (Buvat, 1957; Strugger, 1957). In fact, the endoplasmic reticulum may connect one cell to another through the plasmodesmata (Buvat, 1957; Livingston, 1964).

2. *Continuity of Cell-to-Cell Spread*

The writer is inclined to believe that no appreciable movement occurs from cells which virus particles enter but do not infect after mechanical inoculation. If virus particles moved readily from cell-to-cell without intervening infections, one would find discontinuous spread from primary infections in the leaf, which does not seem to occur. When high concentrations of virus were introduced into hair cells, lesions usually developed only when the hair remained alive at least one day (Benda, 1956). Among the 463 hairs which had collapsed within one day, only a single lesion developed. Furthermore, the inoculated hair appeared to be approximately at the center of any lesion that developed. A virus particle that does not participate in the infection process may soon be destroyed in the cell. When leaves were inoculated with radioactive TMV, it was found that the P^{32} of the virus was largely converted to a nonvirus form during virus multiplication (Lashbrook and Loring, 1950).

Studies of virus infections in local-lesion hosts have provided information concerning the cell-to-cell spread of virus. Evidence indicates similarity in the rate of accumulation of virus in local lesion and systemic hosts of TMV (Taniguchi, 1963).

The amount of TMV in the affected areas may be proportional to the number of first cells invaded and infected by the virus. Rappaport and Wildman (1957) studied the spread of local lesions produced by three different strains of TMV as a function of time. Each strain had its own characteristic spreading rate, and the three rates were uniform with time. A direct proportionality was observed between lesion area and extractable infectious material associated with two of the three strains. The strict proportionality between extractable infectious material and area of involvement was interpreted as evidence in favor of a continuous and direct cell-to-cell invasion.

3. Rate of Cell-to-Cell Spread

Mundry (1963) reviewed recent data of Zech (1963) that indicate that within 30 minutes after inoculation of the terminal cell of a leaf hair with TMV, the ultraviolet extinction at 265 mμ of the nucleus of the adjacent uninjured cell increased significantly. Seven hours after inoculation an increase in ultraviolet absorption in the nucleus of the fifth cell from the terminal was detected. It remains to be shown if the responses noted precede or are coincident with the entrance of translocated virus particles.

It has been suggested that the rate of cell-to-cell spread may depend solely on velocity of cyclosis,* the rate of production of infectious units within a cell, and the number of available cell exits in the form of plasmodesmata (Rappaport and Wildman, 1957).

a. Cyclosis. It may be that virus infections indirectly affect the rate of their spread as well as the movement of other substances in the cell since changes in rates of cyclosis and in viscosity have been noted in virus-infected cells (Zech, 1952; Solberg and Bald, 1962).

b. Temperature—Movement from Epidermis to Mesophyll. Temperature is known to influence the rate of protoplasmic streaming as well as the rate of virus increase and/or spread from cell-to-cell (Kassanis, 1957). Welkie and Pound (1958) obtained results which suggest that temperature directly influences the rate of virus passage from epidermal cells into mesophyll cells. The most rapid rate of cucumber mosaic virus passage through the epidermis was at a temperature of 28°C. in contrast to the optimum temperature for maximum lesion number, 20°C. Bawden and Harrison (1955) defined the mean time that the virus requires to move from epidermis to mesophyll as the time after inoculation when irradiation halves the number of lesions, as compared to untreated leaves. On this assumption, Harrison (1956a) found that the movement of TNV depended on the temperature at which the plants were kept. The time required for the virus to move into the mesophyll was reduced from 30 to 6 hours when the temperature was increased from 10° to 22°C. At 30° the virus moved in from the epidermis in slightly less than the 6 hours required at 22°, although the rate of accumulation of virus at 30° was much less than it was at 22°C.

Benda (1955) found that UV radiation can penetrate beyond the epidermis of an irradiated leaf. The radiation could possibly affect virus in the mesophyll. Dijkstra (1962) found that with nucleic acid as inoculum, the dose of UV had no effect on lesion number after 5 hours, yet infection was not established in the mesophyll until 7 hours. The same investigation suggested that TMV passed through the epidermal layer into the mesophyll cells of *N. glutinosa* leaves in about 8 hours at 20°–22° and in about 10 hours at 17°–19°C. Kontaxis (1961) detected movement from epidermis into mesophyll in 3 hours after inoculation of the upper epidermis. Fry and Matthews (1963) found that infectious material of TMV migrated into the mesophyll about 4 hours after inoculation at 27°–28°C. whereas the first newly made virus appeared in the inoculated epidermis after about 7 hours.

* The circulatory movement of protoplasm within a cell.

c. Rate of Production of Infectious Units. It is difficult to evaluate the suggestion that the rate of cell-to-cell spread may depend in part on the rate of production of infectious units within a cell (Rappaport and Wildman, 1957), since the rate of multiplication data has been obtained coincident with the addition of newly infected cells (Wildman, 1959; Bawden, 1964). If the translocating particles leave the infected cell in 2 hours (Wettstein and Zech, 1962) or in 4 hours (Wildman, 1959; Fry and Matthews, 1963), which is before multiplication is detectable, it appears that the rate of cell-to-cell spread should depend on the rate of formation of translocating units. If rate of spread is proportional to number of multiplied particles per cell, then the rate of spread of a virus in a systemic host should be faster than the rate of spread of the same virus in a local lesion host.

d. Strains. Strains of TMV appear to differ in their rate of spread (Rappaport and Wildman, 1957). One strain (U1) was introduced into a new cell every 4 hours, and the other strain (U2) was introduced every 6 hours. The rates found corresponded approximately to earlier estimates (Bennett, 1956).

Mutants produced as a result of nitrous acid treatment always produced a lesion smaller than the parent (Siegel, 1960). This was true even when the parent strain itself induced a lesion smaller than that induced by other strains. The observation suggests that nitrous acid mutants invariably spread less than their parental type.

e. Rate between Cells in Different Regions. Although many of the more recent estimates of the rate of cell-to-cell spread agree with those of earlier investigators, Zech (1952) has detected TMV moving at more rapid rates in the leaf by using cytological abnormalities as a guide. He calculated a rate as fast as 0.18 mm. per hour in interveinal areas, 0.4 mm. per hour in elongated epidermal cells along the veins, and 20 times this rate in elongated bundle sheath cells. Köhler (1956) also related different rates of movement of potato viruses to the differences in length of parenchyma cells. When the potato virus was inoculated over the main lateral veins, the rate of spread was more than twice that of inoculation over the interveinal area.

f. Directional Spread. Cell-to-cell movement is probably symmetrical in a lateral direction, as indicated by the roughly spherical shape of lesions (Wildman, 1959) and by the observation that an inoculated hair is the approximate center of the resulting lesion (Benda, 1956). Bennett (1960) observed that the spread of sugarbeet yellows virus in *Chenopodium capitatum* is usually more rapid in depth than in lateral spread. He suggested that this is probably due to the more rapid movement of virus in the elongated palisade cells.

Brants (1961) studied the directional spread of TMV in inoculated attached or detached tobacco leaves. Virus appeared to be transported toward and into a wounded region when the injuries were made during a period from 48 hours before, to simultaneously with, inoculation. When radioactive TMV was used to inoculate detached tobacco leaves in a narrow band perpendicular to the midrib, the direction of transport of the radioactivity was influenced by the location of wounding distant from the inoculated region (Brants, 1963). No evidence was presented that radioactivity was associated only with inoculated purified virus or that the radioactivity translocated was infectious virus rather than compounds derived from the virus after infection.

g. Root Culture. It is known that TMV spreads very slowly in tissue cultures (Kassanis *et al.*, 1958). It appears to move very slowly or not at all in root culture (White, 1934). The explanation for this and similar results may not be so much the absence of movement as the inability of cell-to-cell transported virus to infect meristematic cell regions. Kassanis *et al.* (1958) obtained permanent infection in isolated roots when the entire length of the root, including the tip, was rubbed with inoculum. They interpreted their results to indicate that the meristematic region, or at least tissues near to it, must become infected for the roots to be permanently infected. Results that may agree with this were obtained by Bergmann (1959) who inoculated root tips about 3–5 cm. long and tested for virus activity 2 weeks later. One group gave 200–300 lesions per leaf on *N. glutinosa;* the other group gave about 10 lesions per leaf. Permanently infected roots were obtained only from among those that showed a large number of lesions. Bergmann concluded that the virus in isolated tomato did not move or moved very slowly. The average growth of isolated tomato roots is of the order of 0.2 to 0.5 mm./hour, but estimates of the spread of TMV in parenchymatic tissues (Bennett, 1956) is of the order of 0.01 mm./hour. Because the growth rate of the root tip is faster, the virus could be lost (Bergmann, 1959).

C. Long-Distance Transport

Viruses, if they were translocated only in parenchyma tissue, would not be rapidly translocated throughout a plant. Although some viruses appear to be more or less restricted to cell-to-cell movement (Milbrath, 1963), a large number of viruses are not. In many virus-host combinations, egress, which does not occur, is associated with a hypersensitive host response that is heritable. This will be discussed in Section VI. Long-distance transport of many viruses occurs rapidly in the phloem and, in a few known examples, in the xylem. Transport of viruses in the phloem is thought to occur in the sieve tubes. However, the possible role played by other specialized cells within the phloem has not been investigated. Vectors may introduce a virus more or less directly in tissue of the long-distance transport system; but in many examples, the vector-introduced virus is not transported rapidly until after it has spread slowly through parenchyma and other cells. The physiology of phloem and the general problem of the translocation of solutes in plants are covered in various reviews (Esau *et al.*, 1957; Crafts, 1961; Thaine, 1962; Kursanov, 1963; Canny, 1963; Zimmerman, 1964).

1. Egress and Phloem Transport

a. Time Required for Egress. With mechanically inoculated viruses, there appears to be a considerable delay before a virus leaves the inoculated leaf. This delay is generally attributed to the slow movement through parenchyma, but a careful review of published rates suggests that we cannot yet fully explain egress rates. For example, it takes no longer than 24 hours for some particles of TMV (Bennett, 1956), or SBMV (Worley and Schneider, 1963), and probably other mechanically transmitted viruses to spread from an inoculated epidermis to the other epidermal layer. Yet, frequently these viruses require much more than 24 hours to egress from inoculated leaves. Viruses may require 2 to 5 or more days to leave an inoculated leaf (Beemster, 1958). The studies of Zech (1952) and of Köhler (1956) suggest viruses may move faster in certain elongate cells than in the ordinary parenchyma, but this appears to be parallel to the vascular bundles rather than toward the vascular bundles. It seems that the rate of movement somewhere within the vascular bundle is extremely slow and adds considerable time to that required for movement in epidermal and parenchyma cells. Perhaps a significant delay occurs in entering the long-distance transport system after it is almost reached rather than primarily while a virus is transported in the epidermis and mesophyll of the inoculated leaf.

b. Quantity of Virus. In Section III it was pointed out that the egress of several viruses from inoculated leaves showed a relationship to the number of primary infections (Bennett, 1960; Schneider, 1961). Beemster (1958) found that potato virus X moved out of the inoculated leaf of *Physalis floridana* within 40 to 48 hours after inoculation. Diluting the inoculum 5000-fold only lengthened the time of egress by 4 to 8 hours. The same virus in potato moved out of the inoculated leaf between the fifth and sixth day after inoculation. The amount of virus in the inoculated leaf before entering the stem was relatively high in potato and relatively low in *Physalis floridana.*

c. Mixed Virus Infections. In some combinations, the presence of one virus appears to favor the systemic spread of a second virus. It may be that in some of these combinations egress of the second virus is favored indirectly by its greater increase.

Potato virus X (PVX) alone barely multiplied at 31°C., and it failed to move out of the inoculated leaf (Close, 1964). The presence of potato virus Y, as well as TMV, cucumber mosaic virus (yellow strain), or henbane mosaic virus (all viruses that multiply at 31°C.) assisted the systemic spread of potato virus X. It was suggested that possibly these viruses aided potato virus X's movement between mesophyll cells and into the phloem (Close, 1964). Ford and Ross (1962) detected an increase of potato virus X in inoculated leaves with potato virus Y present at 30°C., and so egress in Close's experiments may have been favored indirectly by the increase of PVX. Benda (1957) found the frequency of the white spotting over leaves containing the yellow aucuba and ordinary strain of TMV depended on whether both strains were applied together. Benda explained this phenomenon by assuming that the ordinary strain affected the movement of the yellow aucuba strain through the plant, probably by facilitating its access to the phloem.

d. Comparison of the Egress Rates of Different Viruses. When the egress rates of different viruses are measured simultaneously, valid comparisons can be made. Beemster (1959) compared the egress of four viruses from an inoculated leaf of *Physalis floridana.* Leaf roll and potato virus X entered the stem about the same time; some hours later the turnip mosaic virus entered, and potato virus Y entered the stem 36 hours later. When X and Y were inoculated together on one leaf, both viruses required the same length of time to egress as they did when they were inoculated singly. But when virus X was introduced into plants already infected with leaf roll virus, the translocation from the leaf to the stem seemed to be retarded by about 24 hours. Köhler (1956) found that X-virus reached the upper leaves of the tobacco plant after

it was inoculated into old leaves, but it did so much more slowly than Y-virus. This agrees with Smith (1931) who found that in a tobacco plant inoculated with a mixture of X and Y, Y moved ahead and could be isolated from the growing point free of X. It would be interesting to know if the X-virus egressed from tobacco leaves faster than Y, as in Beemster's study in *Physalis floridana.* When both viruses were inoculated into the same old leaves, Y reached the top of the plant in the same short time whether applied as 1 or 50% of the inoculum. No difference was found in the latent period or multiplication rate of X-virus in old and young leaves inoculated directly. Köhler (1956) suggested that an inactivating factor may be present in the phloem of tobacco acting against X and not against Y, TMV, or cucumber mosaic virus.

e. Age of the Plant. Strain E of tomato spotted wilt virus will invade young *N. glutinosa* plants systemically. If the virus is inoculated onto plants that are older than a certain critical age, the virus will remain localized in the inoculated leaves (Best, 1956). As soybean plants age, the chances of their becoming systemically infected with tobacco necrosis virus decreases greatly (Resconich, 1963). The decrease in the translocation of many plant viruses from the inoculated leaf to the tuber as potato plants age, has been termed "mature plant resistance" (Bercks, 1952). A long delay in rate of egress from the inoculated leaf may be a factor. When the top of a potato plant is cut off and the leaves of the new shoot that develop are inoculated, the tubers become infected within a short time even though inoculation of a leaf of a comparable but unpruned plant seldom results in transport of virus to the tubers (Beemster, 1958). Also, when a leaf at the base of the stem was inoculated, translocation to the tubers was much slower than when apical leaves were inoculated (Beemster, 1958). Even though large quantities of virus were present in the inoculated leaf of potato plants, the amount of infection of tubers was low. On this basis, Beemster favored the idea that virus passes occasionally from the cells in which it multiplied to the sieve tubes.

f. Relation to Site of Inoculation. When a virus is directly introduced into the long-distance transport system, as may be the case with curly top virus, it can move as rapidly as 15 cm. in the first 6 minutes after its introduction (Bennett, 1934). Other vector-transmitted viruses which are introduced deeply may not egress as fast as curly top virus. but they do egress much more rapidly than the viruses introduced into the epidermal layer. An undue delay in the egress of both potato leaf roll virus (LRV) and turnip latent virus (TLV) (both of which persist in their aphid vector) led MacKinnon (1960) to suggest that the supposed site of inoculation (the phloem) should be reassessed.

LRV moved out of a few leaves within 24 hours, but in some cases it had not moved out in 48 hours and TLV had not moved out of any. When the feeding time was increased to 24 hours for TLV, the virus had moved out of only a few leaves in 24 and 48 hours; even after 72 hours, the virus had moved out of only one half as many as in the controls. The distance involved from the inoculation site to the stem was less than 5 cm. Thus, both viruses resemble mechanically inoculated viruses in their rate of egress rather than viruses introduced into the phloem.

g. *Direction of Virus Transport.* Much early work indicates that viruses move rapidly away from the point of entrance in the phloem toward growing regions or other regions of food utilization (Bennett, 1956). Research with potato viruses fits with this generalization. In potato plants that had not reached the stage of tuber formation, rapid movement of potato virus X up the stem occurred from inoculated basal leaves. With later inoculations, the virus did not move upward within 30 days but moved downward into the tubers (Beemster, 1958).

Brants (1961) attempted to locate the region responsible for the direction of virus-transport in the phloem. She found that removal of the stem tip did not influence either the direction or the rate of virus transport out of the inoculated leaf. An increase in the rate of spread of the virus occurred when the axillary buds or both axillary buds and stem tip were removed. Wounding of the stem also exercised an influence on the rate of virus transport, but only when the wound was inflicted 24 hours before, simultaneously with or up to 48 hours after inoculation of a middle leaf. Virus did not egress from the inoculated leaf before 48 hours. She believes that the directing action which is attributed by others to top meristems apparently does not derive from the meristems themselves but from the active young tissues formed by the primary meristematic tissues such as leaf primordia.

Brants (1961) also reported that meristems or stem tips added to callus tissue influenced the rate of transport indirectly by inducing a differentiation of the originally homogeneous callus mass.

2. *Phloem Transport and Mass Flow*

Previously reviewed studies provide impressive evidence of the control of directional movement and indicate a correlation between virus transport and food translocation (Bennett, 1956).

Relatively few measurements of the rates of phloem transport of viruses are available. The fastest rates reported for plant viruses are for those that can be directly introduced into phloem and are comparable to the rates reported for photosynthates and other compounds which were radioactively labeled. Most rates reported for mosaic viruses have been much slower. This may be due to the difficulty of measuring only that part of the transport that takes place in the phloem; however, it would be beneficial to have more accurate measurements for this group of viruses. Some data which do not clearly indicate a correlation between virus movement and food transport (Roberts, 1952) have also been reviewed (Bennett, 1956).

There is no reason to assume that viruses move in the phloem by a mechanism independent of that involved in the transport of other materials in the phloem; however, the mechanism of such transport remains unanswered (Esau *et al.*, 1957; Crafts, 1961; Thaine, 1962; Kursanov, 1963; Zimmerman, 1964). Since previous reviews present abundant evidence in favor of the simultaneous transport of virus and photosynthate in the phloem, it may be of some benefit if the writer, instead of adding more supporting information, plays the devil's advocate by focusing on some questions derived from translocation data.

One recent study impressively indicates a correlation between virus movement and food translocation, and also provides a valuable comparison of three viruses (Bennett, 1960). In triple crown beet plants, defoliation appeared to favor the rapid movement of all three viruses, whereas considerable delay ensued when each virus had to travel from the same inoculated shoot into a nondefoliated shoot. The symptoms in the nondefoliated shoot and presumably the virus movement varied considerably. The mosaic virus produced symptoms in the shortest time, averaging about 34 days; the yellows virus required an average of 57 days, whereas curly top had not produced symptoms in as long as 140 days. Previous studies indicated that the same results, at least with curly top, would be obtained if darkness rather than defoliation had been the treatment (Bennett, 1956).

Two portions of these data do not seem to be explained on the basis of our present knowledge. (*1*) The length of the delay of the movement of the three viruses—34 to 140 days. (*2*) The much shorter delay in the transport of the mosaic virus compared with that of the yellows virus, and the shorter delay in the transport of the yellows virus as compared with that of the curly top virus. Regarding the first point, it is true that the delay supports the correlation between virus and food translocation; but bidirectional movement of various compounds is known to occur in the phloem (Esau *et al.*, 1957). One may ask whether it occurs simultaneously or in the same phloem strand, but not whether it can occur. If it can occur, why do these viruses take so long to move into nondefoliated shoots? Unfortunately, defoliation and shading must result in many profound changes in addition to influencing the direction of food translocation.

Regarding the second point, Bennett (1960) explains the much shorter delay in the case of the mosaic virus by suggesting that it moves slowly through parenchyma outside the phloem. However, the mosaic virus delay was 23 days less than that of the yellows virus which is also presumed to be moving through parenchyma since it is not delayed as long as the curly top virus. Even if the mosaic virus were able to move (and multiply) in parenchyma as fast as 2 mm. per day, it would only gain 4.6 cm. over a virus which did not move in a 23-day period. It appears that the mosaic virus, and perhaps the yellows virus, are moving more rapidly than one would expect them to move in parenchyma cells. Perhaps they are moving in more specialized cells (see Section V,D).

The slow movement of viruses out of root systems of inoculated roots and the enhancement of movement by pruning or keeping plants in the dark are considered harmonious with the concept that virus movement is correlated with food transport (Bennett, 1956). However, Bawden (1964) suggests that some other factor besides the direction of food transport may explain the absence of movement, and he indicates that one possibility is that viruses usually do not reach high concentrations in the root. He also claims that viruses regularly move upward from stem bases when a virus is introduced below the lowest leaf, where, presumably, the flow through the sieve tubes is in the same direction as in the tap root.

Other results indicate that, with soil-transmitted diseases, viruses may move out of roots. For example, tobacco ringspot virus was introduced into the roots of cucumber seedlings by *Xiphinema americanum* and became systemic in 80% of the plants, remaining in the roots of the other 20% (McGuire, 1964). It remains to be shown in what tissue or tissues this and other soil-transmitted viruses move.

Other results indicate that a cherry virus will pass through prunus root grafts. Symptoms appeared in the inoculated (inserted buds at bud break) members of pairs 3 to 5 weeks after inoculation, and in the uninoculated members from 1 to 3 weeks later—30 root-grafted intraspecific pairs and 16 interspecific pairs. In this example, each member of the root-grafted pair would be comparable in physiological stage of development. The virus, starting from the inserted bud, moved upward and down to the roots in the inoculated tree, but it also moved from the roots of the receptor tree upward (Hobart, 1956).

Acropetal movement from roots has been indicated for other viruses, such as chlorotic streak of sugarcane (Bird *et al.*, 1958) and TNV (Yarwood, 1959b), but it is not known in what cells these viruses are moving. It may be that the former virus is translocated in the xylem.

The rapid movement of southern bean mosaic virus from one inoculated expanded primary leaf to its opposite member (Worley and Schneider, 1963; Schneider, 1964) does not coincide with a food transport pathway. The virus is moving from one leaf of equivalent physiological age to the other, and one leaf cannot be considered a "sink" or a "source" in translocation terms (Crafts, 1961) without the other leaf's being the same. Thus, movement here is not from source to sink. The rate of movement, the distribution of virus in petiole and uninoculated leaf, and the steaming of petioles all favor the view that some of the southern bean mosaic virus particles have reached the uninoculated leaf via the phloem transport system (Schneider, 1964).

The same study indicated that tobacco ringspot virus (TRSV) can egress rapidly from a leaf via the phloem; yet by the time it reached the petiole of the opposite leaf, it appeared to move (and multiply) at a rate much too slow for phloem transport. It is of interest to note that the delayed movement of TRSV between the two leaves fits with the concept of food transport better than the rapid movement of SBMV in the same plant does (Schneider, 1964). This may be an illusion, however, since TRSV does not appear to be moving in the sieve tubes of the petiole.

a. Form of the Translocated Particle. The form of the translocated particle has been a subject of increasing consideration by virus researchers, particularly since the demonstration of the infectivity of virus nucleic acid alone (Gierer and Schramm, 1956; Fraenkel-Conrat, 1956).

At one time the plasmodesmata were considered too small to allow complete virus particles to pass (Zech, 1952), but it appears now that the plasmodesmata are large enough to allow the passage of virus particles (Shalla, 1959; Livingston, 1964). Complete particles cannot be eliminated by the smallness of plasmodesmata; complete particles will have to be considered along with infectious nucleic acid or some other form prior to the complete virus as a possible translocated form of virus.

Many workers have suggested that TMV moves between cells in a form other than complete virus, possibly nucleic acid (Zech, 1952; Zech and Vogt-Köhne, 1956; Rappaport and Wildman, 1957). Arguments are derived from kinetic studies and from radiation inactivation experiments which suggest that intact virus particles are not present during the earliest stages of multiplication of the virus when, presumably, particles will have invaded adjacent cells (Rappaport and Wildman, 1957; Wildman, 1959; Fry and Matthews, 1963). The enzyme RNase prevented lesion formation when introduced into a cell through which the virus must pass to reach the leaf from the inoculated cell (Benda, 1958). One interpretation could be that TMV moved as nucleic acid; but it must be remembered that the enzyme can inhibit infection by TMV even though it does not destroy it (Loring, 1942).

Others who claimed an initial RNase-sensitive phase in the multiplication of TMV (Hamers-Casterman and Jeener, 1957) postulated that the virus does not move from cell to cell in an RNase-sensitive form; however, enzyme and virus were not introduced into separate cells.

It has been suggested that viruses other than TMV may move as nucleic acid, such as a tobacco rattle variant transmitted with difficulty (Cadman, 1962). An unstable variant of TNV occurs in leaf extracts largely in a form susceptible to inactivation by RNase, possibly free nucleic acid (Babos and Kassanis, 1962). However, extracts from leaves contained a few virus particles that were stable.

New evidence that indicates virus can move from cell to cell in the form of nucleic acid, but not in the same form in the phloem, has come from studies with defective TMV strains (Siegel *et al.*, 1962). One strain failed to synthesize virus protein, and the other synthesized a viral protein which failed to join with nucleic acid and form complete virus particles. Frequently, these strains did not egress from the inoculated leaf. When secondary infection did occur, spreading was slow and up the plant a leaf at a time; this does not indicate phloem transport. Young expanding leaves, which would normally develop symptoms rapidly when invaded by the common TMV strain, did not develop disease symptoms (Siegel *et al.*, 1962). Even when symptoms showed in shoots originating from topped plants, they were seen only on the oldest and largest leaves of the shoot, the apical leaves appearing normal. The results suggest that the infective agent can only spread in the host from cell to cell (since primary symptoms increased in diameter with time and followed a main vein after reaching it) but not through the conducting elements of the vascular system (Siegel *et al.*, 1962). The authors suggest that "proteinless" viruses are either incapable of entering the conducting elements or that movement takes place in the vascular parenchyma, but no anatomical studies in the regions of the veinal symptoms in the inoculated leaf and the yellowish streaking along the main stem were reported. This work introduces the possibility that although viral nucleic acid may be involved in slow cell-to-cell transport, some other form of the virus may be responsible for long-distance transport in the phloem.

Bald (1964) reported similar observations with respect to movement of the same strains studied by Siegel *et al.* but noted occasional evidence of erratic systemic translocation of virus. Strains originating as a result of deaminating or brominating RNA of TMV also spread very slowly up the plant (Veldee and Fraenkel-Conrat, 1962). Zech (1952) thought complete particles of TMV much too large to pass from cell to cell, and he considered the discontinuous spread in the phloem as evidence that noninfective precursory particles were incapable of combining into the complete particles within the enucleate sieve elements. To combine and result in infectious units, they would have to move into parenchyma cells. A double-stranded TMV-specific RNA has been identified and partially purified from tobacco leaves infected with TMV (Burdon *et al.*, 1964). It is resistant to high concentrations of RNase; its possible role in spread of the virus remains to be assessed.

One possible way of accumulating translocated particles, as distinguished from "multiplied" virus, is to allow them to move into the translocation system of a nonhost. In immune hosts, there may be a gradual disappearance of the virus introduced by grafting (Bennett, 1957). However, the question difficult to resolve is: Did the virus increase in very limited amounts, perhaps in very restricted regions, or did all the virus enter by translocation from the donor host? Characteristic TMV particles identified by sedimentation rate and electron microscopy have been recovered from dodder (*Cuscuta campestris*) grown on virus-infected *Nicotiana* spp. (Jensen, 1962). The virus was infectious but present in the dodder only in trace amounts. Since no evidence was found of virus multiplication in dodder, it was assumed that the nucleoprotein virus particles moved into the dodder from the tobacco plant (Jensen, 1962).

Some researchers believe that virus does not multiply in the sieve tubes (Bennett, 1956). If so, then exudate exclusively from these cells presumably would contain the translocating form of the virus. Vectors of phloem-limited viruses, which may introduce virus directly into sieve tube elements, may transmit a form of these viruses that is immediately translocated, without intervening multiplication, and which is the long-distance translocating form of the virus. Such would seem to be possible with the streak and curly top viruses which move 3 and 15 cm. only minutes after their introduction (Bennett, 1956).

b. Do Viruses Multiply in the Sieve Elements? It is believed that the long-distance transport of many viruses occurs in the sieve tubes of the phloem. Whether viruses are or are not able to multiply in the sieve tubes has not been resolved. The discontinuous distribution of virus during the initial systemic movement of virus (Esau *et al.*, 1957), and the rapid transport of curly top virus immediately after its introduction (Bennett, 1956), indicate that multiplication is not necessary for movement in the phloem to occur. Esau (1960b) has pointed out the absence of inclusions in mature enucleate sieve elements of beets infected with beet yellows virus. Inclusions are known to be absent also in mature sieve elements of tobacco infected with TMV (Esau, 1941) but present in young sieve elements still containing nuclei (Smith and McWhorter, 1957; Esau, 1941). Esau (1960b) suggests the possibility that the absence of inclusions in mature elements may indicate that the virus does not multiply in these enucleate cells. Specific staining with a fluorescent antibody provides evidence for the presence of antigen of SBMV in the sieve tubes of systemically invaded Black Valentine bean plants (Worley, 1964a), and indicates the possibility that a large number of particles may be present. The reaction may have resulted from the presence of virus antigen which may or may not be "complete virus," and cannot distinguish between "multiplied virus" and "translocated virus."

c. Xylem Transport

Previous reviews have summarized pertinent information about two diseases, phony peach and Pierce's disease, in which evidence points to a very close restriction of the viruses to the xylem (Bennett, 1956; Esau, 1956). For the latter disease, a relatively rapid upward transport occurred in alfalfa stems; this indicated movement with the water stream. If the vector introduced the virus directly into vessel elements, the virus must have been able to enter living cells after passing upward in the water-conducting cells. TMV was not able to follow such a pathway, and the hypothesis was that TMV, and presumably viruses in general, are too large to pass through a membrane into a living cell (Caldwell, 1930, 1931).

More recently it was shown that a virus (which is not xylem-limited and is able to move long distances in the phloem) is also able to induce infections in foliage after moving upward rapidly in the tracheary elements of Pinto bean plants (Schneider and Worley, 1959a,b; Jensen, 1962). The virus was introduced directly into the tracheary elements by stem puncture or through a graft union after it infected a systemic host (Schneider and Worley, 1959b). In the latter arrangement, virus had to enter the dead water-conducting cells from living cells as well as exit from dead cells and induce new infections in living cells. The direction of movement was rapidly upward for long distances, some particles moving as fast as 1 foot per minute (Schneider and Worley, 1960). This rate agrees well with rates reported for much smaller particles moving with the water stream (Matthews, 1959). SBMV is able to follow such a pathway in Black Valentine bean, a systemic host, as well as in Pinto bean, a local-lesion host (Schneider and Worley, 1960).

Although the inability of TMV to enter or leave tracheary elements fits with the view that it is probably too large to pass through a living membrane, if the virus is directly introduced in tracheary elements, it is not as readily translocated as SBMV (Schneider and Worley, 1959c).

Scott (1959) suggested that SBMV does not pass through living membranes but rather passes progressively through xylem elements in transitional stages between living and dead cells. It has been shown that vessels may remain alive longer than one might realize were he to study fixed tissues exclusively (Scott, 1949, 1959; Scott *et al.*, 1960).

In agreement with the hypothesis of Scott (1959), SBMV infection occurs only in young crinkled leaves and not in older smooth leaves, even though leaves of both ages are extensively invaded by virus initially introduced into the tracheary elements, and are at least equally susceptible to mechanical inoculation with the virus (Schneider and Worley, 1960). The transitional stages would be expected to occur more frequently in the younger than in the older leaves (Scott, 1959).

It is possible that many viruses cannot follow the same pathway as SBMV, but there may be many reasons for this inability besides the impermeability of a cell membrane to virus. In comparing the movement of four viruses in Pinto bean after their introduction into tracheary elements, we found that TMV moved very little in 24 hours but it could be recovered in the region of introduction many days later; that pod mottle virus could not be recovered even in the region of introduction after 24 hours; that both SBMV and TNV moved rapidly up the plant; that neither SBMV nor TNV infected smooth expanded leaves although both viruses invaded them; and that SBMV, but not TNV, infected younger leaves even though TNV entered them and was recoverable (Schneider and Worley, 1960). The possible connection between the presence of virus in natural guttation water (Johnson, 1951; Ohmann-Kreutzberg *et al.*, 1960) and water transport should be considered. The latter author detected infective virus in guttation fluid as early as 4 days after a unifoliate leaf of ryegrass was inoculated. Maximum infectivity was detected 10 to 14 days after inoculation. The translocation as well as the survival of viruses will most likely differ within the water-conducting vessels, and perhaps no single barrier will keep viruses from entering and leaving the long-distance transport system.

D. Intermediate-Distance Transport

Two transport systems are generally contrasted on the basis of rate and extent of transport. One is a slow and short-distance system; the other a fast and long-distance system. Movement through parenchyma is relatively slow, and movement through the phloem is relatively rapid.

With the few documented exceptions considered in the previous section, movement of many viruses in the xylem is in living cells and also is presumed to be a relatively slow process. If virus movement in parenchyma of the stem and leaf were 0.01 to 1 mm. per day, widespread distribution of virus could not occur after transport through the sieve tubes unless virus could escape into cells of the slow-transport system at closely spaced sites. Studies indicate that at least in the stem there may be big gaps between exit sites soon after invasion by virus (Bennett, 1934; Samuel, 1934; Capoor, 1949; Kunkel, 1939). A similar limit to distribution may not occur when virus enters young expanding leaves; one study suggests that many exit sites are present when a virus enters smooth well-expanded leaves after translocation in the phloem (Worley and Schneider, 1963). Returning to the stem, if all cells are very quickly invaded and fill in the original gaps, there may be a more rapid local distribution via certain living cells than in the parenchyma cells considered representative of cell-to-cell transport. Reference has already been made to the more rapid transport within certain cells of the leaf (Zech, 1952; Köhler, 1956). Mitchell and Worley (1964) described a rotational streaming of cytoplasm in immature and relatively mature fibers which was capable of carrying plastids a distance of 2 mm. in about 3 minutes. Zech (1952) and Köhler (1956) ascribed considerable importance to the length of cells in the leaf which move a virus more rapidly than the ordinary parenchyma. Similarly, the average phloem fiber in the hypocotyl of a relatively mature bean plant is approximately 2 mm. in length—about 25 times the length of the average phloem parenchyma cell in the same plant (Mitchell and Worley, 1964). When SBMV invaded the hypocotyl of young Pinto bean plants, the resulting rate of necrosis and virus spread in the region of the pericyle phloem tissue was 30 to 40 times as fast as before when virus moved inward through the cortex (Mitchell *et al.*, 1956).

Smith and McWhorter (1957) followed a sequence of primary symptom development after inoculation of *Vicia faba* with a strain of tomato ringspot virus. They noted that as the necrosis progressed rapidly down the stem into the roots, it followed the vascularization but did not at first involve the conducting tissues. Instead, the necrosis progressed downward in the parenchyma tissue external to the phloem or in the immature primary phloem fibers. Between leaves, the necrosis nearly always progressed downward in the immature primary phloem fibers or adjacent parenchyma. It was suggested that the cyclosis observed in these elongate fiber cells (in sections of living tissue) could cause the relatively rapid transport of the virus down the stem.

Another description of a disease that circumstantially incriminates

cells usually not considered significant in the transport of virus is the description of leaf-drop streak (Bawden, 1964). This disease, caused by potato virus Y, can result in lesions which spread along the veins into the petioles and main stems. The damage appears primarily in the collenchyma. Although the whole cortex may be destroyed, the vascular system remains intact. Yarwood (1959a) confirmed the Smith (1951) report that the movement of TNV in bean may be stopped by a node. This would not be expected if virus were moving through parenchyma cells. Phloem fibers may discontinue at nodes; and SBMV, when it is transported in these cells, may also be stopped by a node (Worley, 1965).

Perhaps in the case of other viruses, even when symptomatology is not so obviously localized, a rapid distribution of viruses in a longitudinal direction is greatly facilitated in elongated fibers or in similar cells in other parts of the plant. It may not be exclusively dependent upon the sieve tubes for movement in a longitudinal direction.

Our total knowledge about the transport between cells of viruses is most extensive in regard to two transport systems. Except for the few viruses in the xylem-limited group, we have generally attempted to divide the entire pathway followed by viruses between the part that involves a slow continuous cell-to-cell movement (parenchyma) and the part that involves a rapid discontinuous transport (sieve tubes). It would help to add some information concerning different aspects of the complete pathway followed. It would be desirable, for example, to be able to answer these questions, among others:

1. Are there a limited number of egress points available to viruses introduced into leaves, and, if so, where are they?
2. If one leaf of a plant is inoculated, how much of the systemic spread is provided by particles that move out from this one leaf, and how much by the egress of a virus from secondary infections that arise in uninoculated leaves and stems?
3. Can a large number of virus particles escape from a relatively unlimited number of closely spaced exit sites in sieve tubes of the stem and leaf; or is the number of translocated particles small, and are the exit sites relatively widely spaced ?
4. What cells and tissues are primarily important in the radial, longitudinal, and circumferential spread of virus particles external to the sieve tubes of the long-distance transport system?

An analogy can be drawn with a modern network of routes leading into a large city. In the analogy with virus transport, we have descriptions of an expressway connected together with a complex of minor arteries, but we have directed little attention to investigating whether major arteries are necessary to link the throughway and minor arteries for the complete and rapid distribution of a virus.

VI. Distribution

The developmental approach to anatomical studies, together with studies of virus transmission and translocation, have provided information about viruses limited to certain tissues of the host and other viruses more widely distributed (Esau, 1956, 1961; Bennett, 1956). The primary symptoms of some virus diseases are localized in the phloem; others in the xylem and the effects of other virus diseases are not generally tissue-restricted (mosaic group).

A. *Distribution of Tissue-Limited Viruses*

Within the phloem-limited group, two different development patterns are evident (Esau, 1956). A collapse and death of phloem cells that pass through an apparently normal course of development occurs (potato leaf roll; cereal yellow dwarf); in the other, abnormal growth phenomena and a profound disturbance of the pattern of differentiation of the phloem before necrosis occur (curly top; aster yellows). The internal symptoms, and presumably virus distribution, in the latter two diseases were not sharply distinguished from each other (Esau, 1956). More recently, however, it was found that in two ways the aster yellows-induced symptoms deviated from those of curly top. One was that in aster yellows the degenerative changes were found where the sieve elements were still immature, but in curly top the changes did not extend above those points where the elements were mature (Rasa and Esau, 1961). Second, in plants infected with aster yellows virus only, long needle-shaped crystalline inclusion bodies were found in mature and immature sieve tubes, companion cells, and parenchyma (Rasa and Esau, 1961; Struckmeyer, 1963). Thus, two phloem-limited viruses with generally similar internal disturbances may differ in two ways that suggest subtle differences in their effect and, possibly, in the distribution of the viruses.

In addition to viruses quite closely limited to the phloem are others that resemble them in this regard but which can become more widely distributed in different tissues in the way that members of the mosaic group do. The sugarbeet yellows virus is an example (Bennett, 1960).

An unusual distribution of virus occurs in the case of the wound tumor virus. Spherules associated with the wound tumor virus disease in the cytoplasm of tumor cells of sorrel (*Rumex acetosa* L.) are sometimes found in dead tumor tracheids. The spherules have never been observed in the nontumorous cells from which tumors arose (Littau and Black, 1952). By means of fluorescent antibody, Nagaraj and Black (1961) located specific antigen concentrated in the pseudophloem tissue of tumors and in a few thick-walled cells in the xylem region. No other tissues contained detectable amounts of virus antigen. The stem on which the tumor had developed did not contain detectable amounts of wound tumor virus antigen. Characteristic particles of wound tumor virus have also been found in tumors by electron microscopy (Shikata *et al.*, 1964).

B. *Distribution Patterns of Mosaic Viruses*

Although mosaic viruses are widely distributed in different tissues, there may be differing patterns of localization. Unfortunately, we have no satisfactory quantitative methods for a detailed examination of such patterns. To determine the concentration of virus per cell, and possible differences from cell to cell, would require quantitative procedures tailored to single-cell analysis. Some cells can be separated (Zaitlin, 1959; Nagaraj, 1962), but the loss of virus per cell and recovery after cell separation would have to be determined.

1. *Virus Content Per Cell*

Nixon (1956) estimated that the number of TMV particles in a single hair cell was 6×10^7 particles in a volume of $250{,}000\,\mu^3$. An estimate of the average virus content of TMV per mesophyll cell was 10^5 to 10^6 particles (Harrison, 1955). Both estimates suggest that the virus content of one cell would not be sufficient for the quantitative assay of infectivity unless more sensitive methods of detection can be developed. Even lower estimates have been made for the content of virus per cell in a hypersensitive host (Rappaport and Wildman, 1957; Weintraub and Ragetli, 1964). The virus antigen in a single cell can be detected by fluorescent antibody (Schramm and Röttger, 1959; Nagaraj and Black, 1961; Nagaraj, 1962; Worley and Schneider, 1963). Virus particles *in situ* can be detected by electron microscopy but not on a quantitative basis.

2. *Distribution of Inclusions*

Some indications of where virus particles may be more abundant have been indicated in observations of the distribution of inclusions, and they have been used as one criterion characterizing the histotropism of different viruses (Roland, 1957). Inclusions may be found in most tissues of roots, stems, and leaves, at least with mosaic viruses, but they are not always present in every infected cell (Bawden, 1964). Their formation may depend on their cellular environment (Bawden,

1964) so that one cannot equate the absence of inclusions with the absence of virus.

a. Distribution of Inclusions of TMV. Although inclusions of TMV can be found in all kinds of living tissues of the host (Esau, 1956), the distribution of inclusions has been observed to vary considerably. In observations of hair cells, it was observed that crystals and presumably virus appeared in the first two or three cells within a few days of each other; 25 to 30 days later nothing arose in the remaining cells that appeared to be alive (Gold'in and Vostrova, 1959). They also noted unequal distribution of crystals in epidermal cells which others noted also (Takahashi and Hirai, 1963). When the Sakaguchi reaction for arginine was used, the highest concentration of TMV was usually observed in epidermal and hair cells; less frequently virus was found to be most concentrated in the palisade parenchyma cells. The spongy parenchyma and vascular tissue contained the least virus (Rawlins *et al.*, 1956). The characteristic inclusions of the aucuba mosaic virus, on the other hand, are abundant in hairs; less so in the epidermis, and rare in the mesophyll (Sheffield, 1931).

b. Distribution of Inclusions of Other Viruses. The patterns of distribution of inclusions of different viruses may vary considerably. For example, the X-bodies of broad bean mottle virus were found only in the cells of the chlorotic areas of the leaf of *Vicia faba* (Rubio and van Slogteren, 1956). The amoeboid bodies associated with a turnip mosaic virus were restricted almost entirely to the subveinal epidermis (Berkeley and Weintraub, 1952). The inclusions of tomato ringspot virus in *Vicia faba* were found primarily in the parenchyma of the vascular tissue, in procambium, in the cells of the apical meristems, and, to a lesser degree, in parenchyma tissue adjacent to the vascular tissues (Smith and McWhorter, 1957).

3. *Distribution Differences between Epidermis and Mesophyll*

Other evidence has favored a more uniform distribution of TMV when epidermis and mesophyll were compared (Bawden *et al.*, 1954; Nixon, 1956; Hitchborn, 1958).

Even though the effects of UV irradiation of leaves infected with cabbage black ringspot virus, henbane mosaic virus, and sugarbeet mosaic virus (Bawden *et al.*, 1954) were interpreted to indicate that the epidermal cells contain more extractable virus than the deeper tissues, transmission studies with potato virus Y (Bradley, 1956), and with cucumber mosaic virus in *N. glutinosa* (Namba, 1962) did not support Bawden's interpretation. Hitchborn (1958) found that henbane mosaic virus, potato virus Y, as well as TMV are distributed similarly between epidermis and mesophyll.

Kikumoto and Matsui (1961) reported an uneven distribution of potato virus X in different tissues of the leaf. Electron microscopy of potato virus X-infected *Datura stramonium* leaves revealed that the fibrous masses of PVX were commonly found within the palisade and spongy tissue cells but rarely within the epidermal cells. Virus particles were not observed within the cells of the vascular bundle. TMV, on the other hand, was readily detected in the vascular bundle (Matsui and Yamada, 1958).

4. *Distribution in Uninoculated Portions*

There is some evidence that mechanically inoculated leaves or portions of leaves contain higher concentrations of virus than leaves or portions of leaves invaded by cell-transported virus. The concentration of SBMV in an inoculated primary leaf was about 10 times that reached in the opposite invaded primary leaf (Schneider, 1964; Worley, 1964b). Other viruses for which this general relationship between inoculated and invaded portions hold are: TMV (Cohen *et al.*, 1957), potato virus X (Ford and Ross, 1962; Stouffer and Ross, 1961), and TRSV in bean plants (Schneider, 1961).

In studies of the invasion of *Nicotiana glauca* Grah. by strains of TMV, all stages of foliar susceptibility to virus invasion were found (Solberg and Bald, 1963). Expansion and maturation of a leaf is basipetal, and symptom expression and particle counts of virus correlated with this pattern of maturation.

In some cases the difference in titer between inoculated and uninoculated foliage may be due to the incomplete invasion of the latter. Uninoculated virus-invaded leaves of tobacco which exhibit "recovery" symptoms in tobacco ringspot infections also contain less virus than the inoculated leaves (Price, 1936); but Benda and Naylor (1957) interpreted recovery to result from the complete invasion of very young leaves. If they are correct, then less virus in this example of an invaded leaf indicates that, on an average, less virus is in each cell of completely invaded leaves than is in each cell of the inoculated leaves.

5. *Miscellaneous Patterns of Distribution*

Virus distribution in leaves may occur in various patterns. Thus, potato virus X concentrations were consistently higher in apical halves of inoculated leaves than in basal halves (Ford and Ross, 1962). The concentration of potato viruses X and Y was lower in veinal than in interveinal tissue (Rochow and Ross, 1955). Interveinal regions were somewhat better than adjacent veins as a source of virus for aphids (Bradley, 1962). This was also true for SBMV in a hypersensitive host at low temperatures (about 21°C.); but at high temperatures (about

32°C.), the distribution pattern reversed (Schneider and Worley, 1958). Distribution patterns may also correlate with the particular external symptom pattern, as in the local lesions of hypersensitive hosts (Yarwood, 1957) and in the virus concentration in yellow-green versus green portions of rice dwarf-infected leaves (Kimura, 1962).

C. *Meristematic Tissues*

Bennett (1956) summarized the relationship of viruses to meristematic tissues. Certain viruses may be able to invade primary meristematic tissues with little evidence that serious injury results from such invasion. In other instances, growing points of affected plants appeared to be free of virus.

1. *Stem Apex*

Simple excision has been used successfully with different virus-infected hosts in obtaining virus-free cultures (Limasset and Cornuet, 1949; Morel and Martin, 1955a; Yora and Tsuchizaki, 1962). Tissue cultures have been used to produce virus-free clones from potato varieties originally infected with paracrinkle virus and potato virus S, but not with varieties infected with potato virus X (Kassanis, 1957). Poor survival of meristem cultures may drastically reduce the number of plantlets available for testing (Kassanis, 1957; Manzer, 1959; Nielsen, 1960; Yora and Tsuchizaki, 1962).

It is difficult to excise only meristematic tissue. Success or failure may depend on the size of the original tip used. Portions of sprouts or buds 2 mm. long did not result in potato plantlets free of virus X (Manzer, 1959). Apexes of 100 to 250 μ (Morel and Martin, 1955a; Kassanis, 1957) yielded virus-free tissue. On the other hand, Norris (1954) started with parent meristem initially 200 μ long, and only one of his subcultures proved to be virus-free.

Larger excisions, including apical meristems, have resulted in the elimination of some viruses. Clonal sweet potato plants derived from six of seven dissected meristems, which ranged from 0.4 to 1 mm., were free of the internal cork virus (Nielsen, 1960). When scions, 4 to 8 mm. in length (from the extreme tips of branches of Nightingale chrysanthemum), were grafted onto healthy plants of another variety, 13 of 244 scions of Good News chrysanthemum tested did not carry aspermy virus, but still contained a mosaic-type virus (Holmes, 1956). The success in eliminating bean yellow mosaic virus from white clover plants depended upon the conditions under which cuttings were grown. When terminal cuttings, 1 cm. long or less, were obtained from clones and held at 10°C. for rooting, about 35% of the cuttings from 45 clones proved virus-free. In contrast, only about 6% of field-grown cuttings from the same clones were virus-free (Baxter and McGlohon, 1959). It is not known if precautions were taken to prevent reinfection in the field; however, if the excised tissues in the aforementioned examples were originally virus-free, it follows that much more tissue than only meristem must have been free of virus.

Morel and Martin (1955b) explained their ability to free potatoes, dahlias, and carnations from virus thus, "the virus lags behind the tip of a rapidly growing shoot." Others have considered that the barriers may be more truly physiological than those physical ones of cytoplasmic streaming, plasmodesmata, or longitudinal displacement of the shoot apex (Solberg and Bald, 1963). They found that the invasiveness of stem apexes depended upon the virus-host combination. The U5 strain of TMV invaded the tissues of *N. glauca* more extensively than U1, and penetrated in detectable quantities closer to the stem apex. On the other hand, U1 in tobacco, its "natural" host, was the more invasive and virulent.

Young tissue cultures (which were considered largely meristematic) were more resistant to TMV infection and/or to subsequent multiplication than were older cultures composed largely of enlarging or senescent cells (Wu *et al.*, 1960). Chemicals which caused a slow progressive inhibition of growth favored increase of TMV. Wu *et al.*, (1960) suggested that active cell division accounted for low infectivity per unit fresh weight, and inferred that nucleoprotein synthesis required for cell division is competitive with TMV synthesis.

2. *Root Apex*

Roots have been divided into sections. Virus-free zones, when detected, appear in the region of the meristem. TMV was not detected in the tips of tomato roots 2 mm. long unless they were treated with EDTA (Crowley and Hanson, 1960). Brants *et al.* (1962) found a virus-free tip zone in roots divided into 0.5-mm. fragments even when the roots were treated with EDTA. The first two 200-μ sections of the roots of *Vicia faba* seedlings infected with clover yellow mosaic virus were virus-free within the limits of the assay in seven separate experiments (Smith and Schlegel, 1964b). These results appear to parallel previously described results with root cultures (Kassanis *et al.*, 1958; Bergmann, 1959). The remaining question is similar to that raised in regard to virus-free stem apexes. Is the virus-free zone due to the growth rate of the root tip being faster than virus movement, or is it due to the inability of many viruses to overcome the competition of metabolic activities of a meristematic cell?

3. *Injury to Meristematic Tissue*

Smith and McWhorter (1957) report that mitosis is inhibited in the apical meristems of both roots and stems of *Vicia faba* infected by the tomato ringspot virus. The cells become vacuolated and then necrotic. Alveolar inclusion bodies, similar to those of tobacco ringspot virus, develop in procambium and in the cells of the apical meristems, including the initials of the tunica and corpus. In the root tips, the virus inclusions were in all living cells of the stele and endodermis to within approximately 20 cells of the initials. These results contrast with those reported for other virus diseases in which inclusion bodies were rarely, if ever, observed in stem tips (Woods, 1933).

Wilkinson (1960) found that TMV has a definite effect on the mitotic divisions of some of the cells of infected plants, and he detected a postponement of the dispersal of nucleolar contents. He also reported nuclear abnormalities in the dividing cells of root tips from tomato plants infected with aspermy virus (Wilkinson, 1953). He theorized that there is competition between the virus particles and chromonematal material for the nucleoprotein contained in the nucleolus. Caldwell (1952, 1962) suggested that injury to meristematic cells by viruses is more common than studies to date would indicate.

D. *Seed Transmission*

Crowley (1957) summarized that of the several hundred plant virus diseases described, only forty-five are known to be seed transmitted, and in only four of these does the transmission exceed 50%. The percentage of seed infection with tobacco ringspot virus and tomato ringspot virus is consistently high with early inoculations (Kahn, 1956; Desjardins *et al.*, 1954), and 100% seed transmission of tobacco ringspot virus in soybean (bud blight disease) has been reported (Athow and Bancroft, 1959). Nevertheless, when seeds were rated which had been produced on TRSV-infected plants grown from infected seeds, the percentage of transmission averaged less than 100% (Athow and Laviolette, 1962). The general distribution of noninfected seeds over the entire plant suggested that the location of the seed-bearing pod does not greatly influence the failure of some seeds to become infected. Cadman (1963) has suggested that seed transmission probably plays an important part in the survival and spread of some kinds of virus diseases transmitted by nematodes.

Crowley (1957) analyzed four hypotheses which have been advanced to explain the rarity of seed transmission. Inasmuch as no one cause explains the rarity of seed transmission, the only explanation not at

variance with the available evidence, according to Crowley, and which might conceivably apply to large numbers of diseases in which no seed transmission occurs, is that of Bennett and Esau (1936). The hypothesis is that most viruses are unable to survive in the micro- or megaspore mother cells or embryo sacs; apparently all viruses are unable to infect developing embryos because of their lack of plasmodesmatal connections with the surrounding tissues. Caldwell (1962) suggested that the absence of a surplus of high-energy material in the developing embryo may account for the inability of virus to develop in the tissues.

1. *Timing of Infection*

A low percentage of seed transmission in infected plants, where seed transmission does occur, is very common. Reduction in percentage can result, in part, because of the timing of infection. Three seed-transmitted viruses were able to infect the embryos of their hosts both by infecting the gametes of their hosts and by infecting the embryos during the early stages of their development. However, none of the viruses was able to infect host embryos during the later stages of their development (Crowley, 1959). Lettuce plants, inoculated just before flowering, produced fewer virus-infected seed than those inoculated soon after planting. Plants that became infected after flowering had started did not transmit the virus through seed (Couch, 1955).

In contrast to the previous general relationship, seed transmission of barley stripe mosaic virus occurred in Compana barley even if the plants were inoculated when the seeds were in the "hard dough" stage, which suggests that this virus is able to infect both the gametes and developing embryos of its host (Eslick and Afanasiev, 1955). However, in four tolerant barley varieties, the age of the plant at inoculation influenced the amount of seed transmission (Singh *et al.*, 1960). These results agree with other reports that there appears to be no centralization of the mosaic in any particular part of the head (McKinney, 1954; McNeal and Afanasiev, 1956).

2. *Unfavorable Cell Environment*

Late infections will result in little or no seed transmission with seed-transmissible viruses, but in many cases the low percentage of seed transmission rather indicates the generally unfavorable environment of microspore or megaspore mother cells or pollen or embryo sac. Even when infected plants are grown from seeds that carry barley stripe mosaic virus, only about 36 and 53% transmission for the variety Onas and Rescue, respectively, resulted (McNeal and Afanasiev, 1956). Eslick and Afanasiev (1955) showed that when optimum timing of infection occurred, seed-transmission was about 64% in Compana and only about 4% in Titan. Both varieties appeared equally susceptible. Even when barley mosaic virus infected 100% of the developing embryos, only 50–90% seed transmission occurred (Gold *et al.*, 1954). This suggests inactivation of the virus during maturation and storage of the seed. Inactivation also occurs in the case of SBMV which is not seed transmitted. Approximately 100% of embryo infection occurred with SBMV if plants were inoculated at any time prior to flowering (Cheo, 1955; Crowley, 1959), but the virus was inactivated in bean embryos during their maturation. The virus was transmitted by pollen to seed on healthy plants, but the leaf and pod tissues were not infected (Crowley, 1959). A strain of SBMV was transmitted through 3–4% of seed produced by infected plants of cowpea (Shepherd and Fulton, 1962). Seed transmission of bean virus 1 and its N. Y. 15 strain occurred through either the pollen or ovules of infected plants; the absence of seed transmission of bean virus 2 correlated with its absence in pollen or embryos from infected plants (Medina and Grogan, 1961).

3. *Varietal Differences*

Individual lettuce plants of the variety Bibb varied considerably in their ability to transmit common lettuce mosaic virus through seed (Couch, 1955). The variety Cheshunt Early Giant has not been shown to transmit the virus through the seed. Blighting of floral shoots occurs, but new floral shoots are initiated which contain virus (Couch, 1955). Variety differences are indicated also in seed transmission of coffee ringspot, which was found in 7–10% of the seedlings arising from seed of diseased plants of the variety Excelsa, but none from the variety Arabica (Reyes, 1961).

4. *Pollen Transmission*

Pollen transmission can account for a low portion of seed transmission. Less than 0.15% of the plants were infected with lettuce mosaic virus by pollen infection and over 5% through the ovule (Ryder, 1964). With other diseases, a relatively high percentage of seed transmission may be accountable by pollen transmission. About 25% of the seeds resulting from fertilization of healthy ovules with pollen from diseased sour cherry trees were infected with necrotic ringspot or prune dwarf virus, or both (Gilmer and Way, 1960). Pollen usually transmitted bean virus 1 to a larger number of progeny plants than ovules (Medina and Grogan, 1961). Pollen transmission without seed transmission can apparently occur. Sowbane mosaic virus was recovered from pollen of *Atriplex coulteri* (Moq. D.), but no evidence of seed transmission was found in 200 seeds tested (Bennett and Costa, 1961).

5. *Seed Transmission on Recovered Limbs*

Wallace and Drake (1962) reported a high rate of seed transmission of avocado sun-blotch virus when seeds were taken from recovered limbs, which are symptomless carriers, whereas most seedlings from branches with characteristic symptoms were virus-free. A lower concentration of virus was present in the symptomless carrier plants, and it was suggested that high concentrations of virus might induce some injury to tissues through which the virus must pass to reach the embryo.

6. *Mutagenic Effect in Absence of Seed Transmission.*

Sprague *et al.*, (1963) recently reported that a strain of barley stripe mosaic virus, which is seed-borne in barley but not seed-borne in maize, appears to have a mutagenic effect on maize. This was indicated in the increased frequency of deficiencies of marker genes in F_1 seed when pollinations involved males from virus-infected plants.

In order to be transmitted by a seed, a virus must reach, infect, and survive in a haploid gametophytic cell and survive in the embryo during its development, maturation, storage, and germination (Crowley, 1957). Some viruses can do this in some hosts. However, the relatively low transmission rates in these cases, together with the absence of transmission for many other viruses which can infect portions of the seed, suggest that these regions generally provide a very unfavorable environment for viruses.

E. *Absence of Translocation in Some Necrotic-Responding Hosts*

The appearance of local lesions in hypersensitive hosts and the localization of a virus within the necrotic regions without long-distance spread is a general phenomenon occurring with many virus-host combinations. An early hypothesis to explain the localization of the virus is that the death of cells prevents continued movement of virus. Bawden (1964) points out several reasons why this explanation does not appear adequate. Localized infections can occur in the absence of necrosis, and systemic infections can occur in the presence of necrosis. A clear demonstration that a virus may not be permanently localized in a necrotic area is shown by TMV in *Nicotiana glutinosa.* Not only can the lesions slowly enlarge for a week after they form; but when plants with fully formed lesions are placed at over 30°C., the virus resumes its spread, and moves into new cells and into uninoculated leaves (Harrison, 1956b; Bawden, 1964).

Furthermore, restriction of lateral spread should not be equated with restriction of long-distance transport. Ross (1959) reported that resistance did not develop surrounding a TMV-induced lesion when leaves were also infected with potato virus X. The TMV-induced lesion continued to expand, indicating continued cell-to-cell spread; but Ross did not mention if TMV became systemic. Virus could theoretically move from one edge of a leaf to the other without ever moving into the stem or other leaves of the same plant. This has been considered in microscopic studies. Disorganizational changes in phloem cells were observed in the proximity of an encroaching lesion (Furumoto *in* Wildman, 1959), and it was suggested that impairment of the phloem may occur before the virus can be transported. It would be interesting to see whether the postulated impairment of phloem transport could be demonstrated with radioactive organic compounds that normally move along the same pathway. The location of some virus in relation to necrosis is suggested by the detection of TMV-inclusions in a narrow halo of living tissue surrounding the necrotic center, but not in healthy tissue between the lesions (Milicic, 1962). Not only virus but also cell derangement (Wildman, 1959), detectable differences from normal with ultraviolet microscopy (Worley, 1964b) and altered composition of cell walls (Weintraub and Ragetli, 1961) may be found beyond the margin of necrosis. The appearance of specific antigen in the lower epidermis of a bean leaf, hypersensitive to SBMV, which was inoculated over the upper epidermis, indicates the spread of virus into deeper tissues in a local-lesion host (Worley, 1964b).

If the translocating form of virus is also drastically reduced in a hypersensitive host, and if its survival is less secure in the altered environment, the number of particles that may be able to reach the transport system of the phloem may be significantly reduced. The zone surrounding a TMV-induced lesion becomes highly resistant to reinfection by TMV (Ross, 1959; Ross, 1961a; Yarwood, 1960b), and resistance can be detected also in uninoculated leaves (Ross, 1961b; Loebenstein, 1963). An antiviral factor was separated from leaves of *Nicotiana glutinosa* L. 2 days after inoculation with the common strain of TMV (Sela *et al.*, 1964). Diener (1963) has discussed the physiology of localized virus infections. More recent studies have also indicated various enzymic changes (Van Kammen and Brouwer, 1964; Solymosy and Farkas, 1963), including an early increase in total enzyme per cell as well as an over-all increase in respiration (Weintraub *et al.*, 1964).

Weintraub and Ragetli (1964) interpret the destructive results of infection as a violent reaction of the cell to limited viral synthesis. There is general agreement that the amount of TMV per cell is less in a local lesion-responding host than in a systemic host, but the estimates vary between 5×10^2 (Weintraub and Ragetli, 1964) and 10^4 to 5×10^4 particles per cell (Rappaport and Wildman, 1957).

When TMV is inoculated onto leaves of *Datura stramonium*, or when potato virus X is inoculated onto leaves of *Gomphrena globosa*, these viruses cannot be detected in the tissue beyond the margin of dead cells of the lesion (White, 1958). The same author observed a correlation between the rate of local lesion development and the subsequent systemic spread of the virus. When TMV was inoculated into *Datura stramonium* or *Nicotiana glutinosa*, the lesions appeared in these hosts in 3 and 4 days, respectively, and the virus did not become systemic; whereas when TMV was inoculated into *Nicotiana rustica*, the local necrotic lesions did not appear until the fifth day, and on this host TMV became systemic. A similar delay in the appearance of primary lesions with systemic infection was noted with the movement of TNV in soybeans. Preinoculation heating, which delayed the appearance of primary lesions one day longer than on unheated controls, also resulted in 100% systemic infection as compared with about 20% in the unheated controls (Resconich, 1963).

The possible relationship between the studied actions in local lesion hosts and the restriction of spread of viruses between cells or the prevention of long-distance transport remains to be assessed. Introduction of viruses into local-lesion hosts by graft union has been discussed in a previous section, and it indicates that long-distance transport of these viruses is possible in these hypersensitive hosts when virus particles reach appropriate cells of the stem in sufficient number.

VII. Summary

The primary infections initiated by viruses may originate in different regions and in different tissues of the plant. The translocation and distribution of a virus will be affected by the number and location of these primary infection sites.

The translocation of viruses within plants cannot be placed on a quantitative basis or studied directly and separately from other interrelated phases of virus infection and multiplication. The subsequent distribution of virus in a quantitative sense depends on a complex of interactions between virus and the different cells of the host, rather than solely on the number of virus particles and their rate of translocation. It is not surprising, then, that although many viruses are able to multiply in most of the living cells of a plant, they may not be uniformly distributed throughout.

Information has been gained regarding continuous cell-to-cell movement in parenchyma and the more rapid transport in the phloem (presumably in sieve tubes) and xylem (presumably in xylem elements), but much remains to be learned about these pathways and about the translocation of viruses in other specialized cells. Much research indicates that rapid transport of virus movement is correlated with carbohydrate transport in the phloem, but present data do not provide information concerning the direction of virus movement within specific regions during very short periods of time. Unfortunately, radioactive isotopes have not benefited virus translocation studies as they have benefited translocation studies with solutes (Crafts, 1961).

Knowledge concerning the distribution of virus within different tissues has been forwarded by careful studies of the development of primary characteristic symptoms. Translocation pathways have also been implicated in such investigations. Characteristic crystals, some shown to be almost wholly composed of virus, have aided in obtaining distribution profiles of virus. Staining methods have had little value in distribution studies in the past because of their lack of specificity (Lindner, 1961). More recently, the development of fluorescent antibody techniques useful with plant tissues has provided a more specific "reagent" which should increase our ability to determine the distribution of virus-antigen within plant tissues (Schramm and Röttger, 1959; Nagaraj and Black, 1961; Worley and Schneider, 1963). The ferritin-antibody technique (Singer, 1959) will possibly aid in detailing intracellular location of viruses (Shalla, 1964b).

Knowledge of intracellular movement and distribution of viruses increased considerably from studies not primarily concerned with movement but with virus infection and multiplication. Cinephotography of normal living cells (Honda *et al.*, 1963), as well as continuous observations by phase microscopy of living cells at early stages of infection (Wettstein and Zech, 1962; Bald and Solberg, 1961; Hirai and Wildman, 1963), have refocused attention on the rapid dynamic activities within a living cell. Awareness of motion, transfer between cytoplasm and organelles, the pleomorphism of various structures, and a more sequential picture of intracellular events after infection have resulted. Advances in electron microscopy of intracellular structures (Steere, 1957; Esau, 1963) and of viruses *in situ* have added to the intracellular view.

Although the severity of the effects of a virus may be a function of biological interaction between virus and cell, the severity of the effects on the plant may also depend on the rate of movement and subsequent distribution of virus within the plant. It is well known that certain varieties of plants will localize primary infections and prevent the subsequent spread of virus to uninoculated parts. Even though a drastic local reaction occurs in hypersensitive hosts, the damage to the crop can be limited because the virus does not usually spread. Some of the interest in virus translocation has been concerned with the limitation or prevention of translocation and the restriction of virus distribution. In spite of the general characterization of virus diseases as "systemic," important basic problems arise because of the localized invasion of certain hosts, the infrequency of seed transmission in many virus-host combinations, and the freedom from viruses of certain propagation sources under some conditions. This review has summarized some of the recent progress and indicated some of the questions that remain.

Acknowledgments

The author is grateful to the many persons who have helped him with this manuscript. Drs. R. L. Steere, T. O. Diener, H. A. Scott, and J. F. Worley were kind enough to read, criticize, and discuss the paper. The author is also indebted to Drs. J. F. Worley and H. Zech for making some information available prior to publication.

References

Athow, K. L., and Bancroft, J. B. (1959). *Phytopathology* 49, 697.
Athow, K. L., and Laviolette, F. A. (1962). *Phytopathology* 52, 714.
Babos, P., and Kassanis, B. (1962). *Virology* **18**, 206.
Babos, P., and Kassanis, B. (1963). *Virology* **20**, 498.
Bald, J. G. (1964). *Virology* **22**, 377.
Bald, J. G., and Solberg, R. A. (1961). *Nature* **190**, 651.
Bawden, F. C. (1964). "Plant Viruses and Virus Diseases," 4th ed. Ronald Press, New York.
Bawden, F. C., and Harrison, B. D. (1955). *J. Gen. Microbiol.* 13, 494.
Bawden, F. C., and Kleczkowski, A. (1960). *Virology* **10**, 163.
Bawden, F.C., Chaudhuri, R. P., and Kassanis, B. (1951). *Ann. Appl. Biol.* 38, 774.
Bawden, F. C., Hamlyn, B. M. G., and Watson, M., (1954). *Ann. Appl. Biol.* 41, 229.
Baxter, L. W., Jr., and McGlohon, N. E. (1959). *Phytopathology* 49, 810.
Beemster, A. B. R. (1958). "Translocation of Virus X in the Potato (*Solanum tuberosum* L.) in Primarily Infected Plants," pp. 1–98. Veenman and Zonen, Wageningen.
Beemster, A. B. R. (1959). *Proc. Intern. Congr. Crop Protect, 4th, Hamburg, 1957* Vol. 1, p. 297.
Benda, G. T. A. (1955). *Ann. Appl. Biol.* 43, 71.
Benda, G. T. A. (1956). *Virology* **2**, 820.
Benda, G. T. A. (1957). *Virology* **3**, 601.
Benda, G. T. A. (1958). *Virology* **6**, 718.
Benda, G. T. A. (1959). *Protoplasma* **50**, 410.
Benda, G. T. A. and Naylor, A. W. (1957). *Am. J. Botany* **44**, 443.
Bennett, C. W. (1934). *J. Agr. Res.* **48**, 665.
Bennett, C. W. (1953). *Advan. Virus res.* **1**, 39.
Bennett, C. W. (1956). *Ann. Rev. Plant Physiol.* **7**, 143.
Bennett, C. W. (1957). *Virology* **3**, 322.
Bennett, C. W. (1960). *U.S. Dept. Agr. Tech. Bull.* **1218**, 1–63.
Bennett, C. W., and Costa, A. S. (1961). *Phytopathology* **51**, 546.

Bennett, C. W., and Esau, K. (1936). *J. Agr. Res.* **53**, 595.
Bercks, R. (1952). *Zuechter* **22**, 85.
Bergmann, L. (1959). *Trans. N.Y. Acad. Sci.* **21**, 227.
Berkeley, G.H., and Weintraub, M. (1952). *Phytopathology* **42**, 258.
Best, R. J. (1956). *J. Australian Inst. Agr. Sci.* **23**, 174.
Bird, J., Cibes, H., and Tio, M. A. (1958). *Puerto Rico Univ. Agr. Expt. Sta. Tech. Paper* **27**, 5–17.
Black, L. M. (1954). *Exptl. Parasitol.* **3**, 72.
Boardman, N. K., and Zaitlin, M. (1958). *Virology* **6**, 758.
Borges, M. de L. V., and Ferreira, J. F. D. (1959). *Portugaliae Acta Biol.* **A6**, 18.
Boyle, L. M., and McKinney, H. H. (1938). *Phytopathology* **28**, 114.
Bradley, R. H. E. (1952). *Ann. Appl. Biol.* **39**, 78.
Bradley, R. H. E. (1956). *Can. J. Microbiol.* **2**, 539.
Bradley, R. H. E. (1962). *Virology* **16**, 366.
Brandes, J. (1956). *Phytopathol. Z.* **26**, 93.
Brants, D. H. (1961). *Acta Botan. Neerl.* **10**, 113.
Brants, D. H. (1963). *Virology* **20**, 388.
Brants, D. H., Graafland, W., and Kerling, L. C. P. (1962). *Tijdschr. Plantenziekten* **68**, 198.
Bridgmon, G. H., and Walker, J. C. (1951). *Phytopathology* **41**, 865.
Burdon, R. H., Billeter, M. A., Weissmann, C., Warner, R. C., Ochoa , S., and Knight, C. A. (1964). *Proc. Natl. Acad. Sci. U.S.* **52**, 768.
Buvat, A. M. (1957). *Compt. Rend. Acad. Sci.* **245**, 198.
Cadman, C. H. (1962). *Nature* **193**, 49.
Cadman, C. H. (1963) *Ann. Rev. Phytopathol.* **1**, 143.
Cadman, C. H., and Lister, R. M. (1961). *Proc. 4th Conf. Potato Virus Diseases, Braunschweig, 1960* pp. 17-21. Veeman and Zonen, Wageningen.
Caldwell, J. (1930). *Ann. Appl. Biol.* **17**, 429.
Caldwell, J. (1931). *Ann. Appl. Biol.* **18**, 279.
Caldwell, J. (1952). *Ann. Appl. Biol.* **39**, 98.
Caldwell, J. (1962). *Nature* **193**, 457.
Canny, M. J. (1963). *Ann. Botany (London)* **26**, 603.
Capoor, S. P. (1949). *Ann. Appl. Biol.* **36**, 307.
Cheo, P. C. (1955). *Phytopathology* **45**, 17.
Close, R. (1964). *Ann. Appl. Biol.* **53**, 151.
Cohen, M., Siegel, A., Zaitlin, M., Hudson, W. R., and Wildman, S. G. (1957). *Phytopathology* **47**, 694.
Costa, A. S., Darcy, M. de S., and Duffus, J.E. (1958). *Virology* **5**, 145.
Couch, H. B. (1955). *Phytopathology* **45**, 63.
Crafts, A. S. (1931). *Stain Technol.* **6**, 127.
Crafts, A. S. (1961). "Translocation in Plants," Holt, New York.
Crowley, N. C. (1957). *Australian J. Biol. Sci.* **10**, 449.
Crowley, N. C. (1959). *Virology*, **8**, 116.
Crowley, N. C., and Hanson, J. B. (1960). *Virology*, **12**, 603.
Das, C. R., and Milbrath, J. A. (1961). *Phytopathology* **51**, 489.
Desjardins, P. R., Latterell, R. L., and Mitchell, J. E. (1954). *Phytopathology* **44**, 86.
Diener, T. O. (1962). *Virology* **16**, 140.
Diener, T. O. (1963). *Ann. Rev. Phytopathol.* **1**, 197.

Dijkstra, J. (1962). *Virology* **18**, 142.
Engelbrecht, A. H. P., and Esau, K. (1963). *Virology* **21**, 43.
Engelbrecht, A. H. P., and Weier, T. E. (1963). *Am. J. Botany* **50**, 621. Abstr.
Erwin, D. C., and Meyer, R. (1961). *Phytopathology* **51**, 472.
Esau, K. (1941). *Hilgardia* **13**, 437.
Esau, K. (1956). *Am. J. Botany* **43**, 739.
Esau, K. (1960a). *Virology* **10**, 73.
Esau, K. (1960b). *Virology* **11**, 317.
Esau, K. (1961). "Plants, Viruses, and Insects" Harvard Univ. Press, Cambridge, Massachusetts.
Esau, K. (1963). *Am. J. Botany* **50**, 495.
Esau, K., Currier, H. B., and Cheadle, V. I. (1957). *Ann. Rev. Plant Physiol.* 349.
Esau, K., Namba, R., and Rasa, E. A. (1961). *Hilgardia* **30**, 517.
Eslick, R. F., and Afanasiev, M. M. (1955). *Plant Disease Reptr.* **39**, 722.
Ferreira, J. F. D., and Borges, M. de L. V. (1958). *Bol. Broteriana* **32**, 329.
Ford, R. E., and Ross, A. F. (1962). *Phytopathology* **52**, 226.
Fraenkel-Conrat, H., (1956). *J. Am. Chem. Soc.* **78**, 882.
Fry, P. R., and Matthews, R. E. F. (1963). *Virology* **19**, 461.
Fukushi, T., Shikata, E., and Kimura, I. (1962). *Virology* **18**, 192.
Fulton, R. W. (1962). *Virology* **18**, 477.
Furumoto, W. A., and Wildman, S. G. (1963). *Virology* **20**, 53.
George, J. A., and Davidson, T. R. (1963). *Can. J. Plant Sci.* **43**, 276.
Gierer, A., and Schramm, G. (1956). *Nature* **177**, 702.
Gilmer, R. M., and Way, R. D. (1960). *Phytopathology* **50**, 624.
Gold, A. H., Suneson, C. A., Houston, B. R., and Oswald, J. W. (1954). *Phytopathology* **44**, 115.
Gold'in, M. J. (1960). *Virology* **10**, 538.
Gold'in, M. J., and Vostrova, N. G., (1959). *Dokl.-Biol. Sci. Sect. (English Transl.)* **128**, 858.
Grogan, R. G., and Walker, J. C. (1948). *J. Agr. Res.* **77**, 315.
Hamers-Casterman, C., and Jeener, R. (1957). *Virology* **3**, 197.
Harrison, B. D. (1955). Ph. D. Thesis, London Univ. Cited in Nixon (1956).
Harrison, B. D. (1956a). *Ann. Appl. Biol.* **44**, 215.
Harrison, B. D. (1956b). *J. Gen. Microbiol.* **15**, 210.
Harrison, B. D. (1960). Advan. Virus Res. **7**, 131.
Harrison, B. D. (1961). *Proc. 4th Conf. Potato Virus Diseases, Braunschweig,* 1960 pp. 50–59, Veenman and Zonen, Wageningen.
Hayashi, Y. (1963). *Virology* **21**, 226.
Helms, K., and McIntyre, G. A. (1962). *Virology* **18**, 535.
Herold, F., and Weibel, J. (1962). *Virology* **18**, 302.
Herridge, E. A., and Schlegel, D. E. (1962). *Virology* **18**, 517.
Hildebrand, E. M. (1943). *In* "Micrurgical and Germ-Free Techniques" (J. A. Reyniers, ed.), pp. 72–92. Thomas, Springfield, Illinois.
Hirai, T., and Hirai, A. (1964). *Science* **145**, 589.
Hirai, T., and Wildman, S. G. (1963). *Plant Cell Physiol. Tokyo* **4**, 265.
Hirai, T., Suzuki, N., Kimura, I., Nakazawa, M., and Kashiwagi, Y. (1964). *Phytopathology* **54**, 367.
Hitchborn, J. H. (1958). *Ann. Appl. Biol.* **46**, 563.
Hobart, O. F. Jr. (1956). *Iowa State Coll. J. Sci.* **31**, 49.

Holmes, F. O. (1956). *Phytopathology* **46**, 599.
Honda, S. I., Hongladarom, T., and Wildman, S. G. (1963). *J. Cell Biol.* **19**, 77A.
Hooker, W. J., and Benson, A. P. (1960). *Virology* **10**, 245.
Hooker, W. J., and Summanwar, A. S. (1964). *Exptl. Cell Res.* **33**, 609.
Houston, B. R., Esau, K., and Hewitt, W. B. (1947). *Phytopathology* **37**, 247.
Hutton, E. M., and Bald, J. G. (1945). *J. Council Sci. Ind. Res.* **18**, 48.
Jedlinski, H. (1956). *Phytopathology* **46**, 673.
Jensen, S. G. (1962). Ph.D. Thesis, Univ. Nebraska.
Johnson, J. (1951). *Phytopathology* **41**, 78.
Kahn, R. P. (1956). *Phytopathology* **46**, 295.
Kassanis, B. (1957). *Ann. Appl. Biol.* **45**, 422.
Kassanis, B., and Nixon, H. L. (1961). *J. Gen. Microbiol.* **25**, 459.
Kassanis, B., Tinsley, T. W., and Quak, F. (1958). *Ann. Appl. Biol.* **46**, 11.
Kikumoto, T., and Matsui, C. (1961). *Virology* **13**, 294.
Kimura, I. (1962). *Ann. Phytopathol. Soc. Japan* **27**, 197.
Kleczkowski, A. (1950). *J. Gen. Microbiol.* **4**, 53.
Köhler, E. (1956). *Phytopathol. Z.* **26**, 147.
Kolehmainen, L., Zech, H., and Wettstein, D. von (1965). *J. Cell Biol.* In press.
Kontaxis, D. G. (1961). *Nature* **192**, 581.
Kontaxis, D. G., and Schlegel, D. E. (1962). *Virology* **16**, 244.
Koshimizu, Y., and Iizuka, N. (1957). *Protoplasma* **48**, 113.
Kunkel, L. O. (1939). *Phytopathology* **29**, 684.
Kursanov, A L. (1963). *Advan. Botan. Res.* **1**, 209.
Lambertz, P.(1954). *Planta* **44**, 147.
Lashbrook, R. V., and Loring, H. S. (1950). *Federation Proc.* **9**, 194.
Lauffer, M. A., and Price, W. C. (1945). Arch. Biochem. **8**, 449.
Levon, H. (1953). *Exptl. Cell Res.* **4**, 362.
Limasset, P., and Cornuet, P. (1949). *Compt. Rend. Acad. Sci.* **228**, 1971.
Lindner, R. C. (1961). *Botan. Rev.* **27**, 501.
Littau, V. C., and Black, L. M. (1952). *Am. J. Botany*, **39**, 87.
Livingston, L. G. (1964). *Am. J. Botany* **51**, 950.
Loebenstein, G. (1963). *Phytopathology* **53**, 306.
Loring, H. S. (1942). *J. Gen. Physiol.* **25**, 497.
McGuire, J. M. (1964). *Phytopathology* **54**, 799.
McKinney, H. H. (1954). *Plant Disease Reptr.* **38**, 152.
MacKinnon, J. P. (1960). *Can. J. Microbiol.* **6**, 679.
McLaren, A., and Takahashi, W. N. (1957). *Radiation Res.* **6**, 532.
McNeal, F. H., and Afanasiev, M. M.(1956). *Plant Disease Reptr.* **40**, 407.
McWhorter, F. P. (1941). *Stain Technol.* **16**, 143.
Manzer, F. E. (1959). *Am. Potato J.* **36**, 191.
Matsui, C. (1959). *Virology* **9**, 306.
Matsui, C., and Yamada, M. (1958). *Ann. Phytopathol. Soc. Japan* **23**, 76.
Matsui, C., and Yamaguchi, A. (1964). *Virology* **22**, 40.
Matthews, R. E. F. (1959). *Biochem. Biophys. Res. Commun.* **1**, 165.
Medina, A. C., and Grogan, R. G. (1961). *Phytopathology* **51**, 452.
Menon, K. P. V., and Shanta, P. (1962). *Current Sci.* **31**, 153.
Milbrath, J. A. (1963). *Phytopathology* **53**, 1106.
Milicic, D. (1962). *Phytopathol. Z.* **44**, 282.
Mitchell, J. W., and Worley, J. F. (1964). *Science* **145**, 409.

Mitchell, J. W., Preston, W. H., Jr., and Beal, J. M. (1956). *Phytopathology* **46**, 479.
Mitchell, J. W., Schneider, I. R., and Gauch, H. G. (1960). *Science* **131**, 1863.
Miyamoto, Y. (1959). *Virology* **7**, 250.
Morel, G., and Martin, C. (1955a). *Compt. Rend. Acad. Agr. France* **41**, 472.
Morel, G., and Martin C. (1955b). *Intern. Hort. Congr. 14th, The Hague-Scheveningen, 1955, Rept.* Vol. 1, p. 303, Veenman and Zonen, Wageningen.
Mundry, K. W. (1963). *Ann. Rev. Phytopathol.* **1**, 173.
Nagaraj, A. N. (1962). *Virology* **18**, 329.
Nagaraj, A. N., and Black, L. M. (1961). *Virology* **15**, 289.
Namba, R. (1962). *Virology* **16**, 267.
Nielsen, L. W. (1960). *Phytopathology* **50**, 840.
Nixon, H. L. (1956). *Virology* **2**, 126.
Norris, D. O. (1954). *Australian J. Agr. Res.* **5**, 658.
Ohmann-Kreutzberg, G., Pawlitschek, W., and Schmidt, H. B. (1960). *Phytopathol. Z.* **38**, 13.
Price, W. C. (1936). *Phytopathology* **26**, 503.
Rappaport, I., and Siegel, A. (1955). *J. Immunol.* **74**, 106.
Rappaport, I., and Wildman, S. G. (1957). *Virology* **4**, 265.
Rappaport, I., and Wildman, S. G. (1959). *Phytopathology* **49**, 231.
Rasa, E. A., and Esau, K. (1961). Hilgardia, **30**, 469.
Rawlins, T. E., Weierich, A. J., and Schlegel, D. E. (1956). *Virology* **2**, 308.
Reitberger, A. (1956). *Zuechter* **26**, 106.
Resconich, E. C. (1961). *Virology* **15**, 16.
Resconich, E. C. (1963). *Phytopathology* **53**, 913.
Reyes, T. T. (1961). *Plant Disease Reptr.* **45**, 185.
Robb, S. M. (1963). *Nature* **200**, 1339.
Robb, S. M. (1964). *Virology* **23**, 141.
Roberts, D. A. (1952). *Phytopathology* **42**, 381.
Roberts, F. M. (1940). *Ann. Appl. Biol.* **27**, 348.
Roberts, F. M. (1948). *Ann. Appl. Biol.* **35**, 266.
Roberts, F. M. (1950). *Ann. Appl. Biol.* **37**, 385.
Rochow, W. F., and Ross, A. F. (1955). *Virology* **1**, 10.
Roland, G. (1957). *Parasitica (Gembloux)* **13**, 31.
Ross, A. F. (1959). *Phytopathology* **49**, 549. Abstr.
Ross, A. F. (1961a). *Virology* **14**, 329.
Ross, A. F. (1961b). *Virology* **14**, 340.
Rubio, M. (1956). *Phytopathology* **46**, 553.
Rubio, M., and van Slogteren, D. H. M. (1956). *Phytopathology* **46**, 401.
Rubio-Huertos, M. (1962). *Virology* **18**, 337.
Rubio-Huertos, M., and Hidalgo, F. G. (1964). *Virology* **24**, 84.
Ryder, E. J. (1964). *Plant Disease Reptr.* **48**, 522.
Samuel, G. (1934). *Ann. Appl. Biol.* **21**, 90.
Schneider, I. R. (1961). Unpublished data.
Schneider, I. R. (1964). *Phytopathology* **54**, 701.
Schneider, I. R., and Worley, J. F. (1958). *Phytopathology* **48**, 244.
Schneider, I. R., and Worley, J. F. (1959a). *Virology* **8**, 243.
Schneider, I. R., and Worley, J. F. (1959b). *Virology* **8**, 230.
Schneider, I. R., and Worley, J. F. (1959c). *Proc. Intern. Botan. Congr., 9th* Vol. 2, p. 347.

Schneider, I. R., and Worley, J. F. (1960). Unpublished data.
Schramm, G., and Röttger, B. (1959). *Z. Naturforsch.* **14b**, 510.
Scott, F. M. (1949). *Botan. Gaz.* **110**, 492.
Scott, F. M. (1959). Personal communication.
Scott, F. M., Sjaholm, V., and Bowler, E. (1960). *Am. J. Botany* **47**, 162.
Sela, I., Harpaz, I., and Birk, Y. (1964). *Virology* **22**, 446.
Shalla, T. A. (1959). *Virology* **7**, 193.
Shalla, T. A. (1964a). *J. Cell Biol.* **21**, 253.
Shalla, T. A. (1964b). *Phytopathology* **54**, 907. Abstr.
Sheffield, F. M. L. (1931). *Ann. Appl. Biol.* **18**, 471.
Sheffield, F. M. L. (1936). *Ann. Appl. Biol.* **23**, 498
Sheffield, F. M. L. (1946). *J. Roy. Microscop. Soc.* **66**, 69.
Shepherd, R. J., and Fulton, R. W. (1962). *Phytopathology* **52**, 489.
Shikata, E., Orenski, S. W., Hirumi, H., Mitsuhashi, J., and Maramorosch, K. (1964). *Virology* **23**, 441.
Siegel, A. (1960). *Virology* **11**, 156.
Siegel, A., and Wildman, S. G. (1956). *Virology* **2**, 69.
Siegel, A., and Zaitlin, M. (1964). *Ann. Rev. Phytopathol.* **2**, 179.
Siegel, A., Ginoza, W., and Wildman, S. G. (1957). *Virology* **3**, 554.
Siegel, A., Zaitlin, M., and Sehgal, O. P. (1962). *Proc. Natl. Acad. Sci. U.S.* **48**, 1845.
Singer, S. J. (1959). *Nature* **183**, 1523.
Singh, G. P., Arny, D. C., and Pound, G. S. (1960). *Phytopathology* **50**, 290.
Smith, K. M. (1931). *Proc. Roy. Soc.* **109b**, 251.
Smith, K. M. (1951). "Recent Advances in the Study of Plant Viruses," 2nd ed. Churchill, London.
Smith K. M. (1956). *Virology* **2**, 706.
Smith, K. M. (1958). *In* "Protoplasmatologia" (L. V. Heilbrunn and F. Weber, eds), Vol. 4, Section 4a, 16 pp. Springer, Vienna.
Smith, F. H., and McWhorter, F. P. (1957). *Am. J. Botany* **44**, 470.
Smith, S. H., and Schlegel, D. E. (1964a). *Science* **145**, 1058.
Smith, S. H., and Schlegel, D. E. (1964b). *Phytopathology* **54**, 1273.
Solberg, R. A., and Bald, J. G. (1962). *Am. J. Botany* **49**, 149.
Solberg, R. A., and Bald, J. G. (1963). *Virology* **21**, 300.
Solymosy, F., and Farkas, G. L. (1963). *Virology* **21**, 210.
Sorokin, H. (1927). *Phytopathology* **17**, 363.
Sprague, G. F., McKinney, H. H., and Greeley, L. (1963). *Science* **141**, 1052.
Steere, R. L. (1955). *Phytopathology* **45**, 196.
Steere, R. L. (1957). *J. Biophys. Biochem. Cytol.* **3**, 45.
Steere, R. L., and Williams, R. C. (1953). *Am. J. Botany* **40**, 81.
Stouffer, R. F., and Ross, A. F. (1961). *Phytopathology* **51**, 5.
Struckmeyer, B. E. (1963). *Am. J. Botany* **50**, 959.
Strugger, S. (1957). *Protoplasma* **48**, 365.
Sturgess, O. W. (1962). *Bur. Sugar Expt. Sta. (Brisbane) Tech. Commun.* **1**, 1–10.
Takahashi, W. N. (1962). *Phytopathology* **52**, 29.
Takahashi, T., and Hirai, T. (1963). *Virology* **19**, 431.
Taniguchi, T. (1963). *Virology* **19**, 237.
Thaine, R. (1962). *J. Exptl. Botany* **13**, 152.
Van Kammen, A., and Brouwer, D. (1964). *Virology* **22**, 9.

Veldee, S., and Fraenkel-Conrat, H. (1962). *Virology* **18**, 56.
Walker, J. C. (1953). *Botan. Rev.* **19**, 606.
Wallace, J. M., and Drake, R. J. (1962). *Phytopathology* **52**, 237.
Weintraub, M., and Ragetli, H. W. J. (1961). *Phytopathology* **51**, 215.
Weintraub, M., and Ragetli, H. W. J. (1964). *Can. J. Botany* **42**, 533.
Weintraub, M., Kemp, W. G., and Ragetli, H. W. J. (1961). *Phytopathology* **51**, 290.
Weintraub, M., Kemp, W. G., and Ragetli, H. W. J. (1963). *Phytopathology* **53**, 618.
Weintraub, M., Ragetli, H. W. J., and Dwurazna, M. M. (1964). *Can. J. Botany* **42**, 541.
Welkie, G. W., and Pound, G. S. (1958). *Virology*, **5**, 362.
Wettstein, D. von, and Zech, H. (1962). *Z. Naturforsch.* **17b**, 376.
White, N. H. (1958). *J. Australian Inst. Agr. Sci.* **24**, 319.
White, P. R. (1934). *Phytopathology* **24**, 1003.
Wildman, S. G. (1959). *In* "The Viruses" (F. M. Burnet and W. M. Stanley, eds.), Vol 2, pp. 1–31. Academic Press, New York.
Wilkinson, J. (1953). *Ann. Botany (London)* **17**, 219.
Wilkinson, J. (1960). *Ann. Botany (London)* **24**, 516.
Woods, M. W. (1933). *Contrib. Boyce Thompson Inst.* **5**, 419.
Woods, M. W., and Eck, R. V. (1948). *Phytopathology* **38**, 852.
Worley, J. F. (1964a). Ph.D. Thesis, George Washington Univ.
Worley, J. F. (1964b). Unpublished data.
Worley, J. F. (1965). *Phytopathology*. In press.
Worley, J. F., and Schneider, I. R. (1963). *Phytopathology* **53**, 1255.
Wu, Jia-Hsi, and Hudson, W. (1963). *Virology* **20**, 158.
Wu, Jia-Hsi, Hildebrandt, A. C., and Riker, A. J. (1960). *Phytopathology* **50**, 587.
Yarwood, C. E. (1951). *Phytopathology* **41**, 39. Abstr.
Yarwood, C. E. (1952). *Am. J. Botany* **39**, 613.
Yarwood, C. E. (1957). *Advan. Virus Res.* **4**, 243.
Yarwood, C. E. (1958). *Phytopathology* **48**, 39.
Yarwood, C. E. (1959a). *Phytopathology* **49**, 220.
Yarwood, C. E. (1959b). *Plant Disease Reptr.* **43**, 390.
Yarwood, C. E. (1960a). *Phytopathology* **50**, 111.
Yarwood, C. E. (1960b). *Phytopathology* **50**, 741.
Yarwood, C. E. (1962). *Plant Disease Reptr.* **46**, 317.
Yora, K., and Tsuchizaki T. (1962). *Ann. Phytopathol. Soc. Japan* **27**, 219.
Zaitlin, M. (1959). *Nature* **184**, 1002.
Zaitlin, M. (1962). *Phytopathology* **52**, 1222.
Zaitlin, M., and Boardman, N. K. (1958). *Virology* **6**, 743.
Zech, H. (1952). *Planta* **40**, 461.
Zech, H. (1954). *Exptl. Cell Res.* **6**, 560.
Zech, H. (1960). *Virology* **11**, 499.
Zech, H. (1963). Cited in Mundry (1963).
Zech, H., and Vogt-Köhne, L. (1956). *Exptl. Cell Res.* **10**, 458.
Zimmermann, M. H. (1964). *Biorheology* **2**, 15.

THE ANTIGENIC STRUCTURE OF TOBACCO MOSAIC VIRUS

Irving Rappaport

Department of Microbiology, New York Medical College, New York, New York

I. Introduction 223
II. Morphology of TMV 224
III. TMV as an Antigen 227
A. Precipitation of Whole Virus 227
B. Precipitation of Depolymerized TMV 236
IV. TMV as an Infectious Particle 255
A. Inactivation with Antiserum 255
B. Analysis of Strain Relationships 262
C. Biological Significance of the Protein Coat 268
References 271

I. Introduction

Tobacco mosaic virus (TMV) is an example of a superb antigen, capable of stimulating the production of high-titer antisera without the necessity of adjuvants. Antisera with 3 or more milligrams of specific antibody protein per milliliter are commonly obtained following a course of immunization of no more than a month's duration. Although all investigators who have ever immunized an animal with TMV are aware of this fact, no one has offered a good explanation for the exceptional antigenicity of the virus.

Compared with other antigens, TMV is exceedingly large, yet not so large that the virus cannot be treated as if it were a soluble protein. The serological reactions of TMV are characteristic of those antigens whose size places them between the small protein molecules, on the one hand, and bacteria or cells, on the other. Thus, interactions of TMV with antibodies are somewhat unique and reveal a number of problems not apparent with either the smaller or larger antigens. For these reasons the TMV anti-TMV reaction has attracted the attention of immunologists as well as virologists. Depending on the approach, immunological problems, or problems concerned with virus structure and function may be explored. Both approaches are obviously interrelated and often inseparable. It will be the purpose of this review to emphasize the contribution each has made toward a better understanding of the other.

Before discussing the serological properties of TMV, a cursory description of the physical aspects of the virus will be presented to serve as a reference point for future comment.

II. Morphology of TMV

The morphological picture of TMV that has emerged over the past decade is the result of a combined effort in which X-ray diffraction,

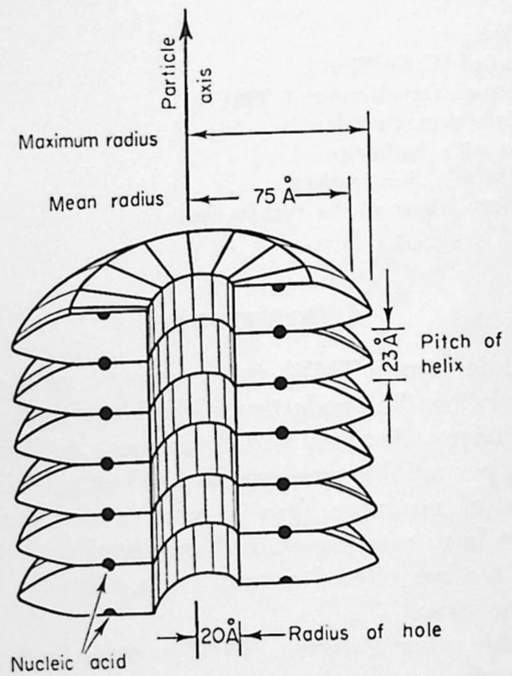


FIG. 1. Schematic representation of a short length of the virus particle cut in half along a plane through the particle axis, showing the helical arrangement of protein subunits (49 subunits on 3 turns of the helix), the helical groove and its accompanying helical ridge extending beyond the mean radius of the particle, and the hollow axial core. The serrated character of the helical ridge is not shown. The nucleic acid is shown at a radius of 40 Å. [From Franklin *et al.* (1957).]

electron microscopy, and chemical analysis have all contributed significantly. By 1957, the general picture of the rod shown in Fig. 1 (Franklin *et al.*, 1957), based primarily on X-ray diffraction, was accepted. Since then, additions to the model have been made, but these have been more in the nature of refinements than revisions.

Today, TMV is considered as the classic rod-shaped virus. It is 3000 Å long and has a maximum diameter of 180 Å. The rod is assembled from 2130 structural units each with a molecular weight of 17,500. In this case, the structural units are equivalent to the crystallographic and chemical subunits. The subunits are all identical, consisting of a single polypeptide chain containing 158 amino acids. The complete sequence of amino acids has been determined and appears in Fig. 2.

5 10 15 20
Acetyl-Ser-Tyr-Ser-Ileu-Thr-Thr-Pro-Ser-GluNH$_2$-Phe-Val-Phe-Leu-Ser-Ser-Ala-Try-Ala-Asp-Pro-Ileu-Glu-Leu-Ileu-

25 30 35 40 45
-AspNH$_2$-Leu-CysH-Thr-AspNH$_2$-Ala-Leu-Gly-AspNH$_2$-GluNH$_2$-Phe-GluNH$_2$-Thr-GluNH$_2$-GluNH$_2$-Ala-Arg-Thr-Val-GluNH$_2$-Val-

50 55 60 65
-Arg-GluNH$_2$-Phe-Ser-GluNH$_2$-Val-Try-Lys-Pro-Ser-Pro-GluNH$_2$-Val-Thr-Val-Arg-Phe-Pro-Asp-Ser-Asp-Phe-Lys-Val-

70 75 80 85 90
-Tyr-Arg-Tyr-AspNH$_2$-Ala-Val-Leu-Asp-Pro-Leu-Val-Thr-Ala-Leu-Leu-Gly-Ala-Phe-Asp-Thr-Arg-AspNH$_2$-Arg-Ileu-Ileu-

95 100 105 110 115
-GluNH$_2$-Val-GluNH$_2$-Asp-GluNH$_2$-Ala-AspNH$_2$-Pro-Thr-Thr-Ala-GluNH$_2$-Thr-Leu-Asp-Ala-Thr-Arg-Arg-Val-Asp-Asp-Ala-

120 125 130 135 140
-Thr-Val-Ala-Ileu-Arg-Ser-Ala-Asp-Ileu-AspNH$_2$-Leu-Ileu-Val-Glu-Leu-Ileu-Arg-Gly-Thr-Gly-Ser-Tyr-AspNH$_2$-Arg-

145 150 155
-Ser-Ser-Phe-Glu-Ser-Ser-Ser-Gly-Leu-Val-Try-Thr-Ser-Gly-Pro-Ala-Thr

FIG. 2. Amino acid sequence of the polypeptide chain in TMV. [From Anderer and Handschuh (1962).]

For some time, there had been difficulty in visualizing the true substructure of TMV with the electron microscope (Mattern, 1962). This was most frustrating, considering the wealth of information accumulated through X-ray diffraction and chemical studies. But with improved technique some very fine photographs have appeared (Nixon and Woods, 1960; Hart, 1963; Markham *et al.*, 1964). An excellent comparison of the X-ray model with some high-resolution electron micrographs can now be made (Finch, 1964). The photographs seen in Fig. 3 substantiate, in every way, what had previously been proposed as the virus structure. For additional information concerning the structure, assembly, and genetic material of TMV, the reader is referred to the following reviews by Anderer (1963c), Caspar (1963), Wittmann (1963), and Kleczkowski (1963).

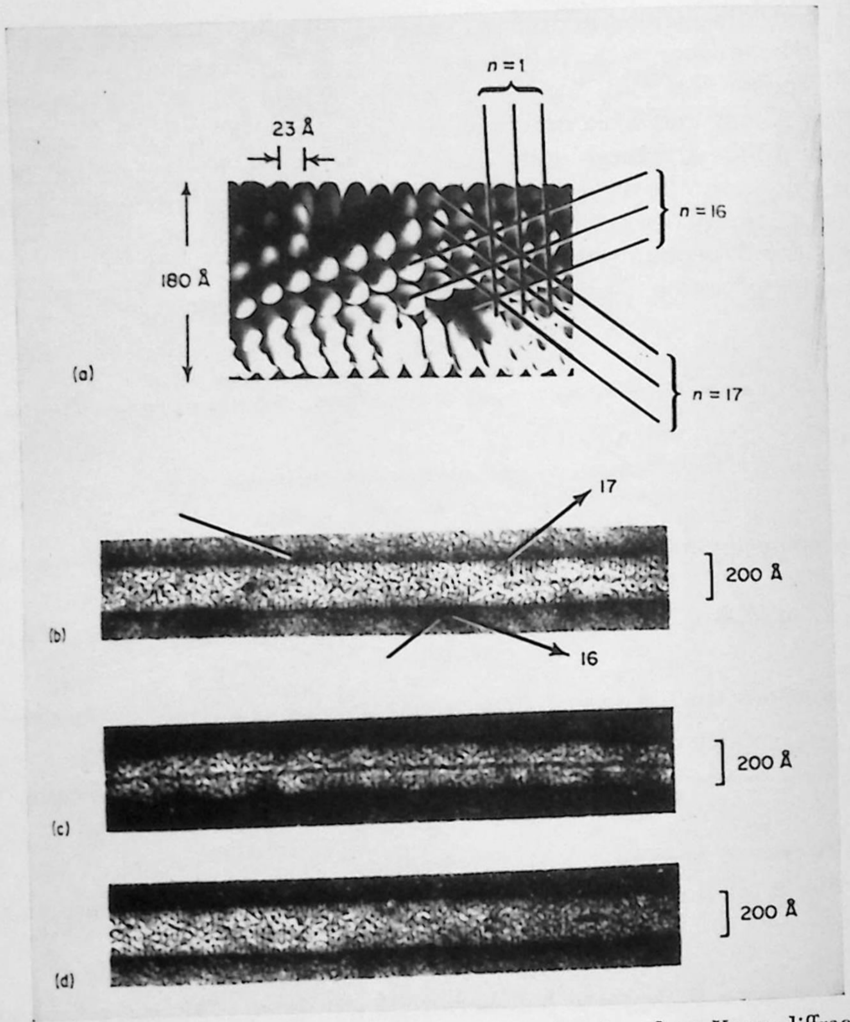


FIG. 3. Plate 1. (*a*) A model of tobacco mosaic virus based on X-ray diffraction studies (from Franklin *et al.*, 1959). About 1/10 specification of the length of the virus particle is shown. The directions of the 1- (arbitrarily chosen as right-handed), 16- and 17-fold helices are indicated. (*b*), (*c*), and (*d*) Electron micrographs of lengths of TMV particles negatively stained with uranyl formate, showing the 23 Å pitch of the basic ($n = 1$) helix. The particle in (*b*) appears to have a left-handed basic helix, and striations (arrowed) are visible in the directions of the corresponding 16- and 17-fold helices. The particle in (*c*) appears right-handed and that in (*d*) does not appear consistently left- or right-handed. [Finch (1964).]

III. TMV AS AN ANTIGEN

A. *Precipitation of Whole Virus*

1. *Quantitative Precipitin Curve*

a. Historical Perspective and Description of the Precipitin Curve. Much attention was initially paid to the quantitative precipitation curve of TMV with antiserum because it appeared odd compared with those described by Heidelberger and Kendall (1932, 1935). For one thing,

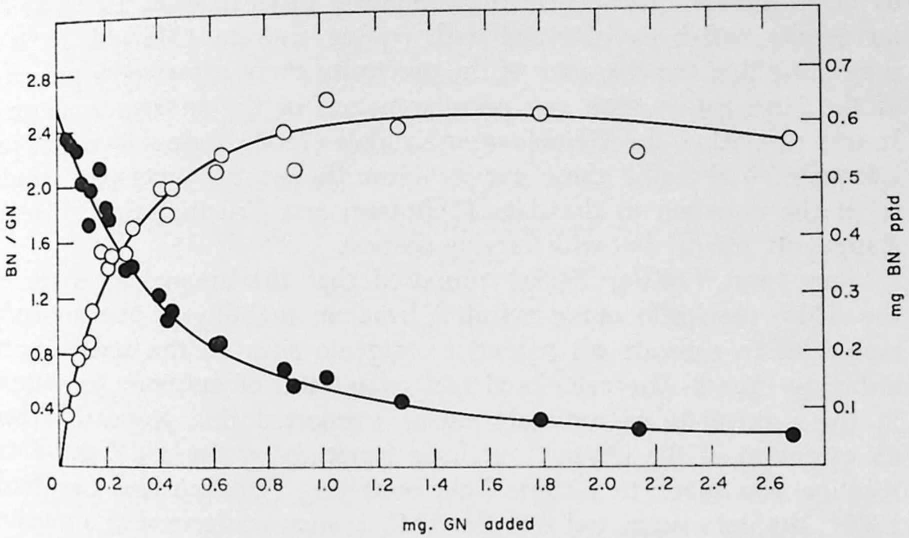


FIG. 4. Quantitative precipitin reaction. U2 vs. antisera 11 and 12. Data are for antibody N-precipitated in antibody excess to equivalence, and corrected to 1.0 ml. undiluted serum. Open circles: antibody N-precipitated: closed circles: ratio of antibody N to antigen N. [From Rappaport (1957a).]

the soluble protein and polysaccharide systems previously studied precipitated over a relatively narrow range of antigen concentration, whereas TMV precipitated over a very wide range of antigen concentration (Kleczkowski, 1941; Schramm and Friedrich-Freska, 1941; Beale and Lojkin, 1944; Malkiel and Stanley, 1947). Enormous quantities of antigen were required to effect solubilization of the precipitate in antigen excess (Kleczkowski, 1941). In addition, the antibody-antigen ratios in the region of antibody excess appeared anomalous. The frequently observed changes of ten-fold or more in the antibody-antigen ratio were 3 to 5 times that encountered in other precipitating systems. This last relationship is seen clearly in Fig. 4, where the quantity of antibody precipitated and the antibody-antigen ratios in antibody excess are plotted (Rappaport, 1957a).

The antibody nitrogen precipitated rises steeply with the first small additions of antigen. Further increments of TMV become less and less efficient as precipitinogens, until a constant maximum level of antibody is attained. The equivalence zone, where neither virus nor antibody can be detected in the supernatant, is uncommonly extensive with TMV, suggesting that a variety of antibody-antigen ratios may be equally efficient in precipitating the maximum amount of antibody from the serum. Large changes in the antibody-antigen ratio were also detected by electrophoretic analysis of the complexes (Kleczkowski, 1958). Similar results had been obtained with equine antisera (Malkeil, 1952a), suggesting that the character of the precipitin curve expressed a property of the virus rather than any peculiar nature of the antisera employed. It was clear that the Heidelberger-Kendall (1935) formulation was inadequate to describe these curves. Nevertheless, attempts were made to fit the equation to the data (Schramm and Friedrich-Freksa, 1941; Rappaport, 1957a) but with varying success.

Rappaport (1957a, 1959a) proposed that the anomalous shape of the TMV precipitin curve resulted from an inability of the antibody molecules to saturate all potential antigenic sites on the rod, even in antibody excess. The calculated molecular ratio of antibody to antigen in the precipitate in antibody excess supported this proposal. When an extension of the physical methods for studying the TMV anti-TMV reaction was made to include light scattering (Bradish and Crawford, 1960), the data suggested that the TMV system conformed in a number of ways to the general view of precipitation advanced earlier by Goldberg (1952, 1953) and Goldberg and Williams (1953).

b. Contribution of the Goldberg Theory of Precipitation. Goldberg assumed that antibody and antigen combined to produce noncyclical, branched-chain structures according to a pattern that would take on the most probable distribution. The molecular size of these polymers, he reasoned, would increase until the system reached a "critical point." At this point, the system would change from one composed primarily of small aggregates to one consisting of a few exceedingly large aggregates. This critical point is a function of the valence of the antigen and the composition of the system, and is the point at which precipitation begins. Whether or not the critical point can be attained in a given mixture of antigen and antibody depends on the ratio of total antigen to total antibody combining sites, and on $2AK$, the total concentration of antibody combining sites ($2A$) and the value of the intrinsic asso-

ciation constant (K) for the formation of antigen-antibody bonds. These relationships are graphically illustrated in Fig. 5.

Experimental evidence in support of Goldberg's theory for viral systems was obtained with bushy stunt virus (BSV) and TMV by Bradish and Crawford (1960), using the technique of light scattering. The theoretical distribution of complexes formed in the BSV system compared

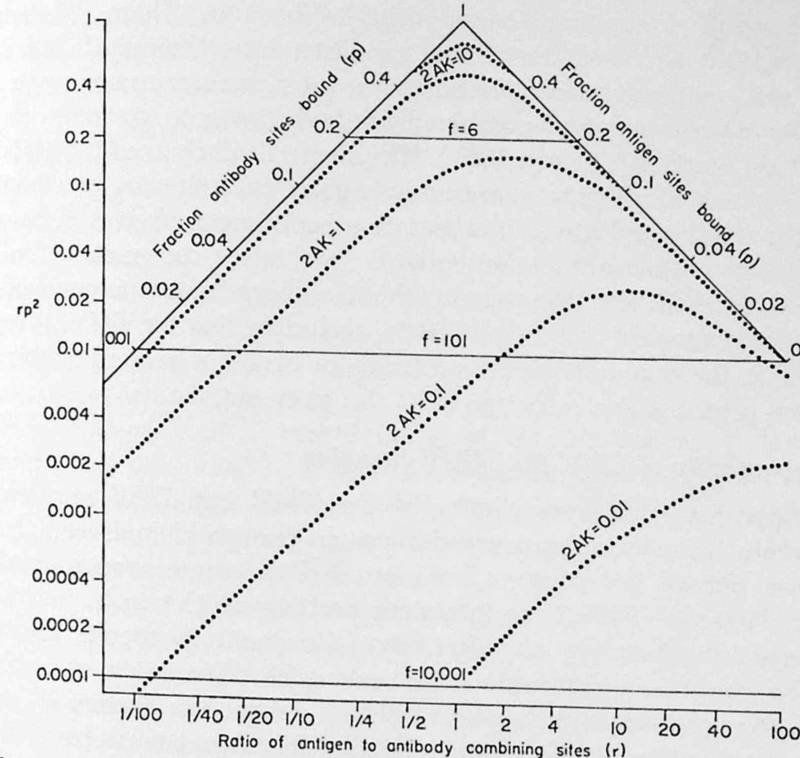


FIG. 5. Diagrammatic representation of the limiting conditions for attainment of the critical extent of reaction. [From Talmage and Cann (1961).]

favorably with those actually observed. No such calculations were attempted for TMV because of the great complexity introduced by its very large valence. In this regard, Goldberg correctly evaluated the contribution that the antigen valence made in establishing the limits of the visible reaction in precipitation. The extensive range or precipitation observed for an antigen such as TMV is a logical consequence of its very large valence. When the valence of the rod is changed by fracture or aggregation the range of the precipitation with the same antiserum is also altered. This dependence of precipitation on valence was most noticeable in the results of Bawden and Pirie (1945), who observed that following degradation the zone of precipitation narrowed, in accordance with Fig. 5, while aggregating the rods in excess of the normal 3000 Å widened the zone beyond that of the untreated TMV.

In contrast to the above, Malkiel (1947) reported results of quantitative analyses with sonically disrupted TMV, aggregated rods, and untreated virus, suggesting the opposite to be true. These differences, however, may reflect differences in conditions or technique. It is known that for quantitative precipitation, the problems concerned with the accurate measurement of combined antibody increase greatly with the size of the antigen (Kabat, 1961). This is particularly true for Malkiel's data in which a loss in total antibody is observed following precipitation with the aggregated virus. In absorption experiments, however, because the detection of residual antibody is qualitative, no such difficulties are encountered. On the whole, the Goldberg theory accommodates the greatest amount of precipitin data, including that for TMV. It would seem that the apparently unusual behavior of TMV in the precipitation reaction is primarily a reflection of its size as an antigen.

2. *Reversibility of TMV Anti-TMV Reaction*

The question of reversibility of the TMV anti-TMV reaction has undergone close scrutiny over the years. The problem is complicated because not all investigators have studied the same properties of the system. Because TMV is an infectious particle which can be inactivated by antibody, recovery of infectivity following a particular treatment can be used as a measure of the extent of dissociation of antibody. Discussion of this point, however, will be deferred to Section IV, which treats reactivation of infectivity in detail. More direct measures of bound and unbound antibody molecules without regard to infectivity may be employed but are much more difficult to quantitate. These different approaches to reversibility have led to considerable speculation concerning equilibria in viral systems (Tyrrell and Horsfall, 1953; Dulbecco *et al.*, 1956; Jerne and Avegno, 1956; Rappaport, 1959a; Bradish and Crawford, 1960; Mandel, 1960, 1961). However, there is no such doubt of the equilibrium attained with haptens (Karush, 1962) or small protein molecules (Singer and Campbell, 1955). With respect to the amount of antibody remaining uncombined with TMV in antibody excess, Rappaport (1959a) found that the Klotz (1953) equation for the reversible interaction of a multivalent protein and a small molecule was adequate for describing the data. Whether this implied a true state of equilibrium or not was questionable (Bradish and Crawford, 1960), for by light scattering no evidence of a true reverse reaction involving the reduction of large complexes to smaller complexes was observed.

3. *The Valence of TMV*

One parameter of the precipitin reaction is the relationship of R (the antibody N-antigen N ratio at equivalence) to the molecular weight of the antigen. In general, R decreases as the molecular weight of the antigen increases; for TMV this figure is usually less than 1. The maximum number of antigenic sites (or valence) may be obtained from the ratio data extrapolated into extreme antibody excess, where the largest number of antibody molecules are bound. The data already cited (Kleczkowski, 1941; Beale and Lojkin, 1944; Malkiel and Stanley, 1947; Rappaport, 1957a) suggest a maximum antibody antigen ratio of 2 to 3 in extreme antibody excess. On the average this represents about 625 antigenic combining sites per TMV rod. Other calculations (Rappaport, 1959a) have yielded figures as high as 950. Considering that the virus is constructed from 2130 identical subunits, each presumably possessing an antibody binding site, the low numbers of 625–950 require some explanation.

It is evident, from a consideration of the dimensions of both the antibody molecule ($240 \times 57 \times 19$ Å; Edelman and Gally, 1964) and TMV (even assuming a maximum cylinder of 180×3000 Å), only about half of the subunits can be combined with an antibody molecule. This, of course, assumes that all the antibody molecules are of the 6.6 S variety. If larger 19 S antibody molecules precipitated as well, the maximum number of antibody molecules capable of binding to a single rod would be reduced further. In actual practice, then, an investigator would ordinarily encounter TMV/antibody molecular ratios equal to less than half the number of subunits.

4. *Chemical Nature of the Antigenic Site*

Studies of the antigenic structure of globular proteins are fraught with difficulties not encountered with polysaccharide antigens, in which the primary structure alone may play the dominant role in determining specificity. Except for an extended protein such as silk fibroin (Cebra, 1961), which displays only a secondary helical structure, the chemical nature of an antigenic determinant on a native globular protein has not been determined.

With globular proteins, the folding of the polypeptide chains brings into apposition amino acids that may be considerably removed from each other in linear array. These convolutions in the polypeptide chain illustrating tertiary structure are represented in Fig. 6. The model is the result of the painstaking research on the structure of myoglobin by Kendrew *et al.* (1958). Because antibody specificity is directed toward a surface patch on whatever protein molecule is in question, the area encompassed may include specific amino acids from two or three different sequences along the chain. For example, in Scheme I, the cross-hatched area may represent a hypothetical antigenic site

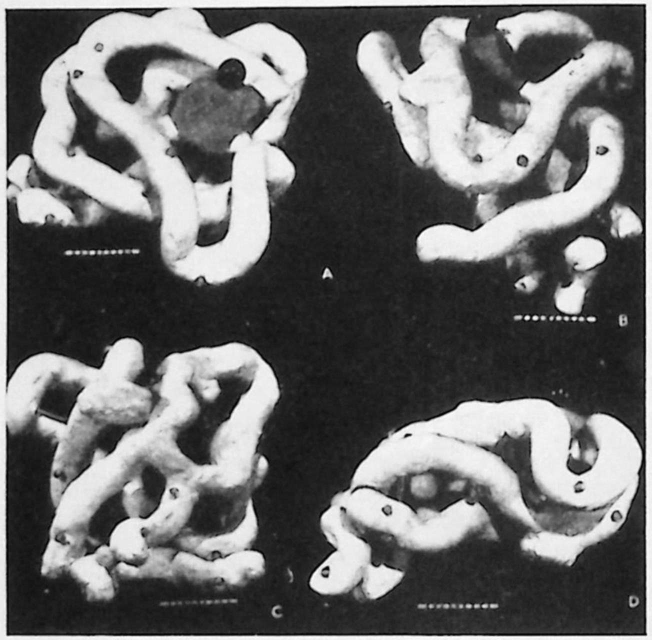
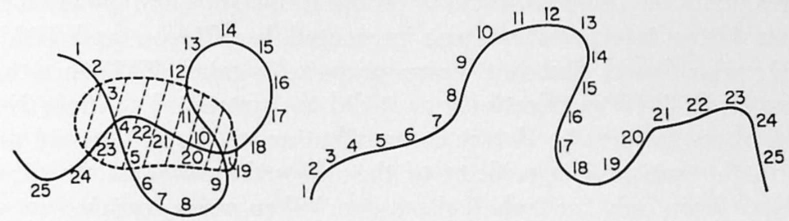


FIG. 6. Photographs of a model of the myoglobin molecule. Polypeptide chains are white; the gray disk is the heme group. The three spheres show positions at which heavy atoms were attached to the molecule (black: Hg of *p*-chloromercuribenzene sulfonate; dark gray: Hg of mercury diamine; light gray: Au of aurichloride). The marks on the scale are 1 Å apart. [Kendrew, *et al.* (1958).]

on the surface of a protein molecule. The site includes amino acids 3, 4, 5, 10, 11, 19, 20, 21, 22, and 23. In linear array, though, some of the amino acids are considerably remote from each other. The distance from the first amino acid involved to the last may be longer than the greatest dimension of the antibody combining site; thus, it would be impossible for the antibody to combine with all of the amino acids in the antigenic patch if the polypeptide chain is in an extended form. Furthermore, the introduction of new sequences of amino acids between the specific sequences would also contribute to an alteration in the shape of the original antigenic patch. Under the circumstances, the

probability of an isolated primary sequence of amino acids contributing significantly to the specificity of the antigenic patch is small. Notwithstanding this difficulty, efforts are being made to ascertain the chemical nature of the antigenic determinant on TMV, at least in part, and so far these efforts have met with a good measure of success.

Before proceeding, however, with a description of some of the experiments, it is well to emphasize the underlying assumption in these studies that only a single antigen is present on the surface of TMV. This assumption is founded on chemical, genetic, and structural evidence indicating that all of the subunits of a single TMV particle are the same. If they were not (Aach, 1960), one could not hope to succeed with such a project without first separating the chemically different components. Nevertheless, the only serological data supporting the assumption of



SCHEME I

a single surface antigen on TMV are those of Rappaport (1961b), which will be discussed in Section IV, B.

The first attempt to identify any of the amino acids involved in the antigenic patch took advantage of the restrictive action of carboxypeptidase on TMV. Harris and Knight (1952) reported that carboxypeptidase removed the C-terminal amino acid threonine from TMV protein. From the quantity removed and the remarkable constancy of only this amino acid being solubilized, a chemical estimate of the number of subunits per TMV rod was made, in good agreement with other data. No reduction in the infectivity of carboxypeptidase-treated TMV (CT-TMV) was noted. Removing threonine from the terminus, however, did alter the antigenic configuration sufficiently to be detected by antisera specific for CT-TMV (Harris and Knight, 1955). The antigenic differences between TMV and CT-TMV, perhaps not unexpected, do present a problem in interpretation. For example, addition of large excesses of threonine to precipitin tests performed with anti-TMV was unsuccessful in inhibiting the reaction. Furthermore, the entire C-terminal peptide, consisting of 17 amino acids, was likewise ineffectual as an inhibitor of precipitation (Knight, 1961). A similar negative result was obtained with a nitrous acid mutant isolated by Tsugita and Fraenkel-Conrat (1960, 1962). This mutant (mutant 171) differed from the parent strain by three amino acid replacements. Significantly, leucine replaced proline as the third amino acid in the C-terminal sequence. The elimination of proline from position 3 permitted further digestion by carboxypeptidase, so that the last three amino acids residues—leucine, alanine, and threonine—were removed. As expected, the antigenic specificity was changed, in agreement with previous studies, although qualitative precipitin studies were not sensitive enough to detect any difference between strain 171 with just threonine removed and preparations in which all three terminal amino acid residues were removed (Knight, 1961). On the whole, these studies suggest that threonine either is not included in the native antigenic patch or that its contribution to the specificity of the determinant group is insignificant. Some support for this latter interpretation was presented by Giveon and Wildman (1962), who found that antiserum prepared against TMV inactivated dethreonized TMV as effectively as it did the untreated controls. Nevertheless, these failures to detect a contribution of the C-terminal amino acids to the antigenic specificity of the exposed surface of the subunits may have been only for technical reasons. When more sensitive quantitative measures were employed by Anderer (1963a), the involvement of the C-terminal amino acids to the antigenic specificity of the virus was established.

Anderer (1963b) prepared twenty peptides from the TMV strain vulgare, and investigated the capacity of these to inhibit a TMV anti-TMV precipitating system. Considerable quantities of peptides were required in order to inhibit precipitation. Of all the peptides tested though, only four amino acid sequences were effective. The most efficient of these appear to be peptides in position 18–23 and those in position 123–134. Oddly enough, the terminal peptide (142–158) was not nearly as efficient (for the amino acid sequences, see Fig. 2). Whether peptides in positions 18–23 and 123–134 contribute to any part of the exposed surface of the polypeptide chain is not known. Considering their efficacy as inhibitors, it is likely, however, that they may.

The neutralizing activity of antisera prepared against the hexa-, penta-, tetra-, and terminal tripeptide was also investigated (Anderer and Schlumberger, 1965). Not only was inactivation of TMV infectivity obtained, but the data suggested that the efficiency of the antisera as inactivating agents improved with increasing length of the amino acid chain. The data, however, could be made more convincing if inactivation were carried out either at antiserum dilutions where the effects of normal serum were eliminated, or with purified antibody preparations.

The participation of the terminal hexapeptide (Thr-Ser-Gly-Pro-Ala-Thr) in the antigenic structure of the subunit is strongly suggested. Yet it is clear that the native surface patch or conformation of the polypeptide chain must involve other amino acids besides the terminal six, for antisera prepared against TMV do not precipitate with any of the artificial antigens.

Apropos of the above observations of Anderer and co-workers is the report of Benjamini *et al.* (1964) that peptide 8 (amino acid sequence 93–112) is involved in determining the antigenic specificity of depolymerized TMV. This tryptic peptide was the only one which inhibited the fixation of complement by an anti-TMV protein system. Considering that the antisera Benjamini *et al.* (1964) prepared was against depolymerized TMV, the lack of agreement between Anderer and Benjamini is highly significant. This difference suggests either that the anti-TMV protein antiserum was made against a "hidden" antigen, one not exposed on the surface of the intact rod, or that the configuration of the polypeptide chain at the antigenic site was altered following depolymerization. As a result of a possible change in configuration, a different portion of the chain may have become the major determinant of antigenic specificity. Because the antisera to TMV protein cross-reacted with the intact virus, it is more probable that the antibodies were directed against the outer surface antigen of TMV rather than to some inner "hidden" determinant. If so, we are left with the alternate assumption, that depolymerization affected the tertiary structure of the subunits to the extent that peptide 8 now exerted a greater influence on the antigenic specificity of the subunits than any other primary sequence of amino acids. The substitution of one dominant peptide for another in the antigenic site, as a result of polymerization, suggests that the quaternary structure of the virus is also an important factor in specifying the configuration of the polypeptide chain at the antigenic patch.

Anderer and Handschuh (1963), on the other hand, have suggested that the quaternary structure did not influence the antigenic configuration, because a positive precipitin reaction was still attainable with dinitrophenyl derivatives of TMV protein which were incapable of polymerizing into rods. This evidence is hardly sufficient to discount the contribution of the quaternary structure, considering the work of Benjamini *et al.* (1964) and the positive finding that both A- and X-proteins in the unpolymerized state react with anti-TMV, yet cannot completely absorb all antibody from the antiserum until they are properly polymerized. The influence of quaternary structure on the final conformation of the polypeptide chain is certainly implied by these findings.

B. *Precipitation of Depolymerized TMV*

1. *X-Protein*

a. Discovery and Characterization. Those subunits of TMV in the infected cell that do not polymerize with viral RNA (ribonucleic acid) may find themselves in a variety of aggregated states. The number of such states commonly found varies depending on the conditions of plant growth and extraction.

The first report of these anomalous proteins was made by Bawden and Pirie (1945), who succeeded in separating a noninfectious, low-molecular-weight nucleoprotein from TMV-infected leaves by differential centrifugation. These small pieces gave a typical "O"-type precipitin pattern which changed to a characteristic "H" following aggregation. Takahashi and Ishii (1952a,b) also reported an abnormal protein associated with TMV infection, which they characterized more fully and subsequently termed X-protein. The protein was soluble, of lower molecular weight than the virus, but would polymerize at pH 5.0 to form rods similar in length and width to TMV. Polymerization was readily reversed by raising the pH to 7.0 or by dialyzing a sample against distilled water. The polymerized particles, devoid of RNA, were noninfectious. They nevertheless cross-reacted with antisera prepared against the native virus (Takahashi and Ishii, 1952b) and elicited antibodies following immunization, which also reacted with the virus (Takahashii and Ishii, 1953). In subsequent studies two X-proteins (X-1 and X-2) were characterized (Takahashi, 1955).

A similar protein was described by Jeener and Lemoine (1953) as a crystallizable antigen devoid of RNA which was also immunologically similar to TMV. Jeener *et al.* (1954) extracted a protein from leaves systemically infected for over 2 months that revealed two distinct bands when examined in agar by the Oudin technique. Rapid movement in the gel suggested high diffusion coefficients for the two soluble antigens, indicating that they were associated with particles that were much smaller than the virus. Electrophoretic analysis of artificial mixtures of TMV and soluble antigen confirmed the serological data. Both components were resolved and possessed distinct mobilities which were less than that of the virus.

At about the same time as the Takahashi and Ishii (1952a) report of X-protein, Commoner *et al.* (1952) discovered a new electrophoretic component in TMV-infected sap which they called B. On further study, a number of discrete components in infected leaves were identified and characterized as A4, B3, and B6 (Commoner *et al.*, 1953b). These three components are known to aggregate, and so are often isolated in their polymerized form. Component B3 polymerizes, forming B8; B3 and B6 produce the copolymer B7, while A4 polymerizes with itself forming A8. Each component as well as its polymerized counterpart, was distinguishable electrophoretically (Commoner and Yamada, 1955). The relationship among the various soluble proteins isolated from infected leaves by different investigators is shown in Table I.

TABLE I
PROBABLE RELATIONSHIP OF THE SOLUBLE PROTEINS EXTRACTED FROM TOBACCO LEAVES SYSTEMICALLY INFECTED WITH TMV

Investigator	Soluble proteins	
Takahashi	X-1	X-2
Jeener	Soluble antigen 2	Soluble antigen 1
Commoner	B6	B8

Not all of the soluble proteins are found regularly in infected leaves. For example, X-2 was not detected in leaves systemically infected with Holmes' ribgrass (Takahashi, 1955). Nor was more than one abnormal protein detected by Delwiche *et al.* (1955) in TMV-infected leaves. In the latter case, though, Franklin and Commoner (1955) suggest that the procedure of Delwiche may have permitted B3–B6 copolymers to form, thus making it improbable that pure B8 and B6 could be extracted.

Bawden and Pirie (1956) insisted that the anomalous proteins extracted from TMV-infected plants were in reality nucleoproteins, since they always found RNA associated with them. However, this was undoubtedly the result of contamination, for it was shown by base analysis that the RNA associated with their X-protein was of host and not of viral origin (Dupont-Mairesse and Jeener, 1960).

b. Identity of X-protein and TMV Protein. There was considerable speculation in the past concerning the role of X-protein in TMV biosynthesis. However, once it was established that X-protein was in fact TMV protein much of the mystery surrounding X-protein disappeared. The data supporting this notion have been amassed from a variety of experiments. Some are biochemical, having to do with the synthesis of X-protein in infected leaves (Delwiche *et al.*, 1955; Commoner *et al.*, 1953a; Commoner and Yamada, 1955; van Rysselberge and Jeener, 1955, 1957). Part comes from electrophoretic studies of Schramm and Zillig (1955) in which it was determined that A-protein, which is depolymerized TMV, and X-protein migrate at the same rate, and further, that mixtures of A and X migrate as a single band. Much more evidence comes from a number of X-ray diffraction studies with A- and X-protein further demonstrating their identity (Franklin, 1955; Franklin and Commoner, 1955; Franklin *et al.*, 1959; Holmes and Franklin, 1958; Rich *et al.*, 1955). Chemical analyses, too, have contributed. The amino acid composition of B6 and X-protein was found to be the same as TMV by Newmark and Fraser (1956). Finally, reconstitution experiments with X-protein and TMV-RNA are significant. The first such attempt at copolymerization by Commoner *et al.* (1953b) with B3 and TMV-RNA was successful in producing a complex possessing the identical ultraviolet (UV) absorption spectrum as TMV but it was without infectivity. Successful reconstitution including recovery of infectivity was accomplished by Takahashi (1959a,b), indicating that X-protein can function in a biological fashion as well as TMV protein (Fraenkel-Conrat and Williams, 1955). There appears to be little doubt, then, that X-protein is TMV protein in the natural unpolymerized state. Nevertheless, X-protein and A-protein are treated separately in this review both for convenience and because of historical precedence.

c. Cross-Reaction between X-Protein and TMV. It was immediately ascertained by those investigating the antigenic specificity of these anomalous proteins that they were similar to the intact rod, for antisera prepared against the anomalous proteins cross-reacted with TMV, and vice versa. These cross-reactions, however, presented certain problems in interpretation (Takahashi and Gold, 1960).

Antisera prepared against TMV if absorbed with X-protein would still react with TMV. The same antiserum absorbed with polymerized X-protein could be entirely exhausted of its reactivity toward intact TMV. Although this was often the case, Bawden and Pirie (1956) were unable to detect any remaining antibodies to TMV after absorption of anti-TMV with X-protein. However, Bawden and Pirie's technique of performing serological reactions at 50°C. may be responsible for their failure to detect any residual antibody. It is known that the state of polymerization of TMV subunits is highly dependent on temperature. Therefore, it appears probable that during incubation at 50°C. polymerization of X-protein occurred to a sufficient extent as to create the new antigenic specificity associated with the native surface. These TMV antigens then effectively precipitated all activity from the antiserum.

The seemingly odd behavior of X-protein in absorption is also reflected in the quantitative precipitin curves (Figs. 7 and 8) obtained

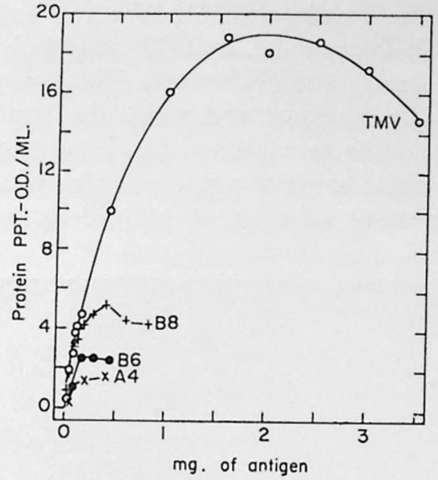


FIG. 7. Precipitin curves for the reaction of anti-TMV rabbit serum with TMV, B6, B8, and A4. Optical densities (at 750 mμ) are for Folin color developed by precipitates obtained with 0.05 ml. of serum and various amounts of antigen. [From Commoner and Rodenberg (1955).]

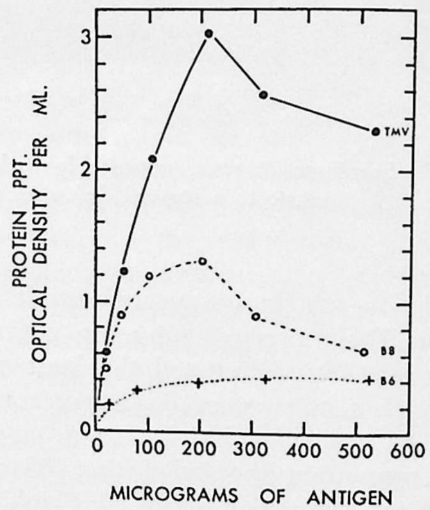


FIG. 8. Precipitin curves for the reaction of anti-B8 serum with TMV, B6, and B8. Optical densities (at 750 mμ) are for Folin color developed by precipitates obtained with 0.01 ml. of serum and various amounts of antigen. [From Commoner and Rodenberg (1955).]

by Commoner and Rodenberg (1955). The relationship of B8 (polymerized form of B3), B6, and A4, to TMV as revealed by an anti-TMV antiserum (Fig. 7) seems straightforward. The homologous reaction of TMV anti-TMV is the strongest and yields the largest amount of precipitate. The total protein precipitated by the anomalous proteins falls off in the order of their average aggregate size where B8 > B6 > A4. The converse experiment of reacting anti-B8 against TMV, B8, and

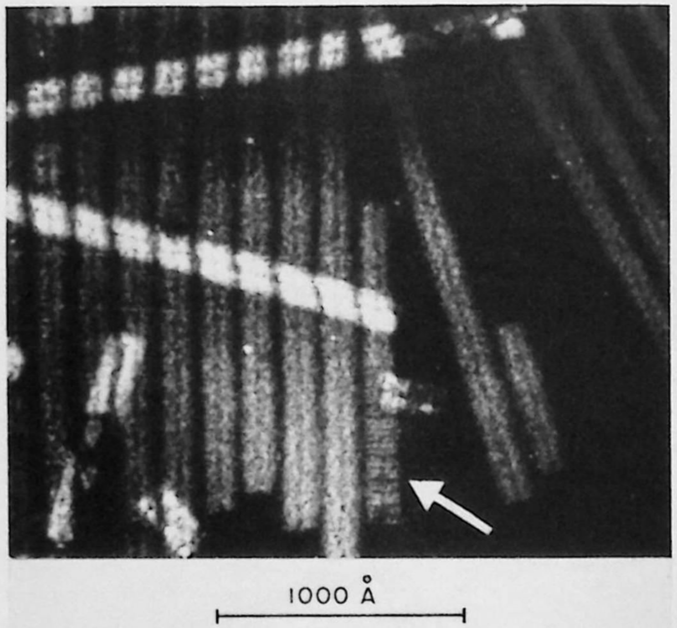


FIG. 9. A particle of tobacco mosaic virus, presumably nucleic acid-free, showing the transition from a helical structure to a stacked disk structure. [From Markham *et al.* (1963).]

B6 (Fig. 8) revealed a wholly unexpected result. The heterologous antigen, in this case TMV, precipitated more antibody than did the homologous antigen B8. B6 precipitated the least amount of protein. Evidently, B8, although a polymer of B3, is not antigenically identical to the native rod.

Other evidence supporting the belief that B8 was different, was obtained from an X-ray diffraction study of Franklin and Commoner (1955), who concluded that B8 possessed a variant stacked-disk structure rather than a helical form. A side-by-side comparison of these two distinct structures can be made in the remarkable electron micrograph seen in Fig. 9, published by Markham *et al.* (1963). The packing

relations of the subunits are similar in the stacked disk and helical structure, but "it is unlikely that the conformations of the polypeptide chains are exactly identical in both cases" (Caspar, 1963). Thus, B8 fails to precipitate as much antibody as native TMV because the antigenic configuration of the exposed surface is probably sufficiently different from that of the original to preclude complete precipitation. The greater reactivity of the anti-B8 antiserum toward TMV compared with its activity against the homologous B8 antigen may be explained by assuming that some helical structures were present in the B8 preparation. This is likely, considering that the stacked disks and the helical structures are in equilibrium (Franklin *et al.*, 1959). As a result, the more antigenic determinants on the helical structures could have stimulated a disproportionate amount of antibody, for it is known that the antigenicity of the whole virus is far better than X-protein.

The suggestion that more antibody capable of precipitating TMV was produced in response to B8 was also borne out by Oudin tests performed by Commoner and Rodenberg (1955). In these tests, antigen was incorporated in the gel and antibody in saline was overlayed. The rate of migration of the advancing precipitin band was measured and found to be as expected for anti-TMV antisera diffusing into TMV or B8 antigen. The homologous band moved more rapidly. Migration of anti-B8 in TMV, however, reflected the same reverse character noted with the quantitative precipitin tests. The heterologous band of anti-B8 in TMV moved more rapidly than the anti-B8 band in B8.

The rate of migration of the antibody molecules into the gel follows Fick's law $[ds/dt = AD(dc/dx)]$ closely. Because the antibodies involved are probably drawn from the same population, the diffusion coefficient D is likely to be the same for all. Thus, the one variable influencing the rate at which antibody molecules are moving across a particular cross-sectional area, A, is the concentration gradient, dc/dx, which is a function of the initial concentration of antibody molecules. Thus, with all other factors being equal, the greater the antibody concentration the more rapidly will the band move. The greater rate of movement, then, of the TMV anti-B8 band is clearly correlated with the greater amount of antibody precipitated from the anti-B8 antiserum by TMV.

d. Cross-Reaction between X-Protein and A-Protein. Kleczkowski (1957) reported the occurrence of multiple precipitating components in a TMV preparation which was purposely prepared without ultracentrifugation. When the antibody-antigen reactions were studied in Ouchterlony plates, two lines formed between the antigen preparation and the antiserum well in advance of the obvious virus antibody band. Similar multiple bands were reported by van Slogteren and van Slogteren

(1957). Such evident heterogeneity of both hyperimmune sera and TMV preparations had also been observed by Sang and Sobey (1954). The heterogeneity was not apparent, though, when more specific antisera produced after a single injection of virus were used.

An antigenic comparison was made between A-protein, X-protein, and TMV (Kleczkowski, 1957). For these tests, hyperimmune sera were used. A summary of the data appears in Figs. 10 and 11. In one Ouchterlony plate in which the wells were charged with anti-TMV antiserum and X-protein and TMV, as antigens (Fig. 10), the two forward lines, previously noted in advance of the TMV preparation, appeared and fused with two indistinct lines emanating from the X-protein well. Thus, X-protein and the two minor components from the TMV well appear

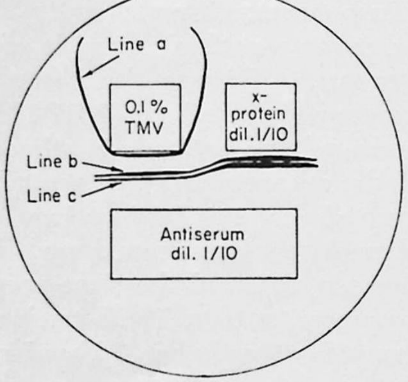


FIG. 10. A comparison of X-protein with a TMV preparation. [From Kleczkowski (1957).]

to be identical. A-protein tested in a similar system with anti-TMV revealed at least seven precipitin lines (Fig. 11). Two of these A-protein lines showed patterns of identity with the two minor components diffusing from the TMV well. Therefore, A-protein contains at least two antigenically distinct sets of proteins. One set appears to be identical to X-protein, while the other is not.

Kleczkowski (1957) proposes to name as X-protein the protein that is easily released from TMV by such mild treatment as placing the preparation in borate buffer at pH 8.7. He suggests that the protein released at higher pH should be called Y-protein. A-protein (Schramm *et al.*, 1955a) is by this definition a mixture of X and Y. The multiplicity of lines displayed by the Y-protein in the Ouchterlony tests is interpreted on the basis of TMV being constructed from several antigenically different subunits, a situation which appears to Kleczkowski to be similar to that of human serum albumin (HSA).

Lapresle *et al.* (1959), established that HSA forms as many as three bands, detectable by immunoelectrophoresis. Because it is not all clear whether HSA is a single polypeptide chain (Reithel, 1963), or composed of more than one kind of subunit, the comparison of HSA with A-protein is hardly valid. In cases where the protein molecules are known to be composed of two or more different subunits, as, for example, γ-globulin (Edelman and Gally, 1964) or the enzyme lactate dehydrogenase (Markert and Apella, 1963), more than one precipitin band in Ouchterlony plates may develop following a separation of the polypeptide chains. The behavior of TMV appears mysterious, because the evidence is excellent that the virus is assembled from only one kind of polypeptide

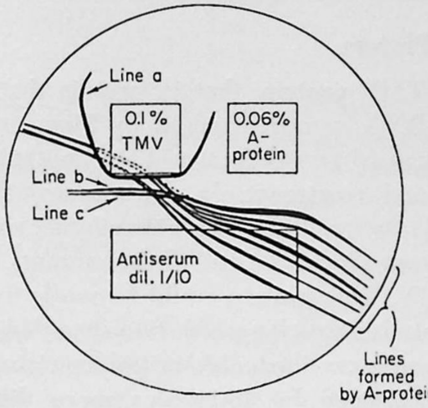


FIG. 11. A comparison of A-protein with a TMV preparation. [From Kleczkowski (1957).]

chain. We must seek then to explain this heterogeneous precipitin behavior without recourse to any structural heterogeneity.

2. *Comparison of X-Protein with Top Component of TYMV*

The discovery of X-protein led naturally to a comparison of X-protein with "top component" (TC), found in Chinese cabbage plants infected with turnip yellow mosaic virus (TYMV) (Markham *et al.*, 1948, Markham and Smith, 1949). TC is a less dense, non-RNA-containing and thus noninfectious component which has the same appearance under the electron microscope as the infectious TYMV particles. The general impression was that both TC and X-protein were expressions of incomplete viral synthesis.

A number of differences between these two and their infective counterparts were apparent. For one, the ratio of TYMV to TC particles in Chinese cabbage is remarkably constant, remaining close to 2:1 under a wide range of growing conditions and time after infection (Matthews, 1958). Compare this to the content of X-protein in TMV-infected tobacco, which is apparently quite sensitive to growing conditions, and can be extracted in quantity only from long-term, systemically infected leaves. Further, TC cannot be distinguished antigenically from TYMV, a condition very unlike that of A- and X-protein, which are easily recognized as antigenically distinct from TMV. Although TC is a poor antigen with respect to its capacity to stimulate antibody production, it nevertheless combines with as much antibody as does the nucleoprotein. Also in contrast to A- and X-protein, TC behaves in quantitative precipitin tests as if it were immunologically homogeneous, and forms only a single precipitin band in Ouchterlony plates with homologous antisera.

3. *Defective TMV Protein*

Truly defective TMV protein, that is, protein that does not normally copolymerize with RNA, was discovered by Siegel *et al.* (1962). They described the isolation of two defective TMV mutants (PM1 and PM2) following nitrous acid treatment. In both cases, the infectious units were strikingly similar to free RNA rather than intact virus.

A soluble protein produced by one of the mutants, PM2, cross-reacted with antisera to TMV. This protein could be made to aggregate (Zaitlin and Ferris, 1964) into highly elongated, two-stranded, open helical structures, which, however, were incapable of polymerizing with the RNA.

The protein synthesized by PM1 was more difficult to detect. It went unrecognized at first because the protein could not be polymerized, and would not react with anti-TMV. PM1 does react, though, with anti-PM2 and anti-U1 A-protein. This reaction was detected by measuring the decrease in the inactivating capacity of the antisera following absorption with PM1-infected leaf extracts. The data are in Table II. Thus, a truly anomalous soluble protein is only seen following infection with a virus bearing a genetic lesion in its cistron coding for coat protein. The result is the production of defective subunits that are incapable of forming the proper bonds to copolymerize with the infectious RNA.

The striking observation in this experiment is the positive reaction of anti-A-protein with PM1 antigen at the same time that anti-TMV failed to react. In considering possible explanations for this phenomenon, two immediately come to mind. The first would suggest that PM1 and A-protein share an antigen in common that is not the dominant surface antigen. This may well be a "hidden" antigen on TMV, but exposed on both A-protein and PM1 protein. The other suggests that the surface antigen on A-protein may be altered somewhat from that found on

TABLE II
DETECTION OF VIRUS-RELATED ANTIGEN IN PLANTS INFECTED WITH DEFECTIVE TMV MUTANTS

Rabbit antiserum ($\frac{1}{1000}$)	Absorbed with plant extract	Survival (%) of TMV when reacted with absorbed serum
Anti-TMV protein	Healthy	8.6
	PM1	24
	PM2	100
Anti-PM2 protein	Healthy	21
	PM1	100
	PM2	100
Anti-TMV	Healthy	3.4
	PM1	2.7
	PM2	100

NOTE: Antisera prepared against disaggregated TMV protein, disaggregated PM2 protein, and TMV were mixed with the indicated plant extracts. The mixtures were incubated at 37°C. for 90 minutes and then at 2°C. for 24 hours, followed by centrifugation at 105,000 *g* for 30 minutes. The supernatant solutions were mixed with equal volumes of TMV (1 µg./ml.), and the mixtures were assayed for infectivity. (Rappaport, Siegel, and Zaitlin (1964) unpublished data.)

the rod. The population of antibodies produced in response to A-protein antigen could then have a somewhat different range of specificity than anti-TMV antibodies, a range that may encompass the antigen on PM1. The entire question of the possible influence of polymerization on the configuration of the surface antigenic patch will be examined in detail following the section on A-protein.

4. *A-Protein*

a. Definition. The term A-protein was suggested by Schramm *et al.* (1955a) for TMV protein that had been produced by subjecting the virus to alkali degradation at pH 10.3. Much of this protein referred to had a molecular weight of about 90,000, but not all. In a loose sense, many of the other sized polymers of TMV subunits have also been regarded as A-protein. In fact, it is safe to say that probably all preparations of A-protein contain aggregates of different sizes in equilibrium with each other.

The particular state of A-protein aggregation is highly dependent on the protein concentration, ionic strength, and pH of the medium (Schramm and Zillig, 1955). Even some of the larger products of alkaline degradation result from a reaggregation of smaller polymers (Harrington and Schachman, 1956), contrary to what was originally proposed by

Schramm (1947). Temperature too, plays an important role, for A-protein prepared at pH 10.3 and brought to pH 6.5 in 0.1 μ buffer polymerizes at room temperature and completely depolymerizes at 0°C. to the monomeric form (Lauffer *et al.*, 1958; Lauffer, 1961; Ansevin and Lauffer, 1959, 1963).

A-protein is usually considered a 5 or 6 subunit structure (Klug and Franklin, 1957). However, Caspar (1963) has reexamined some of the data upon which this proposal has been based and concludes instead that A-protein is a trimer. The one bit of direct experimental evidence which lends support to Caspar's proposal is that obtained by Sarkar (1960).

Sarkar prepared A-protein from TMV strains vulgare, flavum, and dahlemense. When the different proteins were brought together they formed mixed polymers. Because the A-protein from each strain had a distinguishable electrophoretic mobility, the mixed polymers were expected to acquire some intermediate mobility, depending on the ratio of the two different subunits in the aggregate. This they did, but the variety of intermediate forms expected if A-protein were a hexamer was not observed. Only two intermediate peaks were detected, which Caspar interpreted as 1:2 and 2:1 mixed polymers, supporting his conclusion that A-protein was a trimer.

b. Initial Serological Observations. Studies on the serological properties of TMV protein were initiated in 1946 by Friedrich-Freksa *et al.* TMV was degraded with alkali at pH 9 to a molecular weight of approximately 360,000. These rather large split products, either with or without RNA, still reacted with antiserum to TMV, although the character of the precipitin curve was different from that observed with intact virus. Absorption of TMV antiserum with the RNA-free protein left behind some residual antibody to whole virus and to the RNA-containing split products. From these and other experiments it was quickly ascertained that the serological behavior of A-protein depends on its state of aggregation. In the fully aggregated state, A-protein cannot be distinguished serologically from TMV. When depolymerized, however, it can be. These observations raised two questions, and set the stage for all subsequent immunochemical work with A-protein.

The first question concerned the reason for the quantitative difference in the precipitation of A-protein and antiserum as compared to the intact virus. This is now of minor concern, for the precipitation behavior of A-protein can be accommodated within the Goldberg formulation (see Fig. 5). The second question revolved around the inability of the A-protein to absorb all antibody from anti-TMV. This observation is of major concern, for it suggests that a qualitatively different antigenic

TABLE III
AMOUNTS OF PRECIPITATES FORMED BY TMV AND BY A-PROTEIN WITH DIFFERENT ANTISERA AND TESTS OF THE SUPERNATANT FLUIDS FOR ANTIGEN AND ANTIBODY[a,b]

	Composition and pH of mixtures							Tests of supernatant fluids with		
		TMV (mg.)		A-protein (mg.)		Amount of				
Test No.	Antiserum (ml.)	pH 7.5	pH 5.6	pH 7.5	pH 5.6	precipitate (mg.)	*R*	Antiserum	TMV	A-protein
	Antiserum No. 1 to TMV (4.0 mg. antibody/ml.)									
1	0.5	8.0	—	—	—	10.0	0.25	—	—	—
2	0.5	1.0	—	—	—	2.9	1.9	—	+++	+++
3	1.0	0.4	—	—	—	1.2	2.0	—	+++	+++
4	1.0	0.2	—	—	—	0.6	2.0	—	+++	+++
5	0.5	—	8.0	—	—	10.1	0.26	—	—	—
6	0.5	—	1.0	—	—	2.8	1.8	—	+++	+++
7	0.1	—	—	5.0	—	0.4	?	+++	—	—
8	0.5	—	—	1.6	—	3.1	1.0–1.9	—	+	—
9	0.5	—	—	0.2	—	1.0	4.0	—	+++	+++
10	1.0	—	—	0.25	—	1.5	5.0	—	+++	+++
11	1.0	—	—	0.135	—	0.9	6.2	—	+++	+++
12	0.5	—	—	—	6.0	7.8	0.3	—	—	—
13	0.5	—	—	—	1.1	2.5	1.3	—	++	++
14	0.5	—	—	—	0.15	0.6	3.0	—	+++	+++
	Antiserum No. 2 to TMV (6.7 mg. antibody/ml.)									
15	0.025	—	—	5.0	—	0.45	?	+++	—	—
16	0.1	—	—	1.0	—	1.3	?	+	+++	+++
17	0.1	—	—	—	2.7	3.4	0.26	—	—	—

(*Continued*)

ESCUELA DE MEDICINA
UNIVERSIDAD DE CHILE
Valparaíso

TABLE III (*Continued*)

Test No.	Composition and pH of mixtures: Antiserum (ml.)	TMV (mg.) pH 7.5	TMV (mg.) pH 5.6	A-protein (mg.) pH 7.5	A-protein (mg.) pH 5.6	Amount of precipitate (mg.)	*R*	Tests of supernatant fluids with: Antiserum	TMV	A-protein
	Antiserum to A-protein (1.0 mg. antibody/ml.)									
18	1.0	4.0	—	—	—	5.0	0.25	—	—	—
19	4.0	0.4	—	—	—	1.0	1.5	—	+++	+++
20	1.0	—	—	0.8	—	1.75	1.2(?)	—	—	—
21	4.0	—	—	0.25	—	1.25	4.0	—	+++	+++

[a] From Kleczkowski (1961).

[b] The total volumes of the mixtures were made to be 5 ml. by suitably diluting antisera and antigens in buffers of the required pH values before mixing. The mixtures were incubated overnight at 2°. The precipitates were centrifuged down, washed three times in the buffer and their nitrogen contents, estimated by the micro-Kjeldahl method, were translated into amounts of precipitate by multiplying by 6.4.

The supernatant fluids were tested for antigen by adding 1 ml. of antiserum (diluted 1/25–1/100) to 1 ml. of a supernatant fluid diluted 1/10, 1/40 and 1/160, and for antibody by adding 1 ml. buffer containing 0.06 and 0.015 mg. A-protein or 0.01 and 0.0025 mg. TMV to 1 ml. of the supernatant fluid diluted 1/10 (except in tests Nos. 7 and 15 where it was diluted 1/2).

structure was present on the intact virus which was not present or had been modified on the A-protein molecules. This aspect of the serological behavior of A-protein will be examined in more detail.

c. Precipitation in Liquid Medium. The precipitation of A-protein with antiserum in liquid medium is as might be expected. It precipitates as a dense "O" type aggregate characteristic of globular proteins, compared to the "H" type for TMV (Bawden and Pirie, 1957). In addition, A-protein, because of its small number of valences, precipitates over a much more limited range of antibody-antigen ratio, as predicted by the Goldberg (1952) equations.

Kleczkowski (1961) performed a number of quantitative precipitin analyses with A-protein and TMV, using antisera produced against both antigens. The data appear in Table III. It can be seen that anti-TMV absorbed with A-protein (Test No. 8) leaves some residual activity for TMV, whereas absorption of the same antiserum with aggregated A-protein at pH 5.6 (Test No. 12) completely removes all antibody reactive for TMV.

The ratio data (R) also reflect the change in the precipitation behavior of A-protein following aggregation at pH 5.6. The values for R drop with aggregation and approach those for TMV. These same data are also useful in calculating the valence of A-protein.

In extreme antibody excess the antibody N/antigen N ratio for the A-protein system may reach between 6 and 7. Assuming that A-protein is a trimer of 52,500 mol. wt. (Caspar, 1963) the maximum number of antibody molecules bound per trimer is approximately 2. This is a remarkably small number for an antigen with the surface area of 3 subunits. The surface area of a molecule of 52,500 mol. wt. is large enough so that if all of the exposed surface were capable of binding antibody, certainly 5 or 6 antibody molecules bound would not appear too much. On the other hand, if only the original outer surface were binding antibody molecules, then the data are rational. One could expect no more than three antibody molecules bound, one for each subunit, and probably less considering the steric hindrance that may be involved in preventing more than two antibody molecules from binding at the normally exposed surfaces.[c]

[c] Kleczkowski (1961) calculated the number of antibody molecules bound per subunit at equivalence and concluded that ". . . one or two particles of A-protein combined with one antibody molecule." This statement is probably a typographical inversion. Kleczkowski assumed the rabbit antibody molecule to be 160,000 mol. wt.; antibody valence = 2 and A-protein as 90,000 mol. wt. *R* at equivalence, as defined by Kendall (1942), is mol. wt. antibody/antibody valence ÷ mol. wt. antigen/antigen valence. Thus, taking *R* at equivalence to be between 1 and 2

d. Precipitation in Gels. The presence of new antigenic sites on A-protein that were previously concealed within the intact rod has already been alluded to. The most compelling evidence favoring this exposure of new and different antigenic groupings comes from serological tests performed in agar wherein multiple bands or lines have been observed.

In Fig. 11, for example, a number of precipitin lines appear between the A-protein well and the anti-TMV well. A similar finding was reported a second time by Kleczkowski (1961). The significant point in all of these tests is not so much the multiplicity of the precipitin bands, which at first is striking, but that the lines all fuse with the single TMV band. This pattern of identity with the TMV precipitin line suggests that there are no antibodies directed against the components of A-protein that are not also directed toward the whole virus. Here we have a telling point in the argument for the absence of antibodies specific for undisclosed antigens of the virus. If antibody molecules in the anti-A-protein antiserum were directed toward the hidden antigens on the A-protein they should not combine with the undegraded virus. These antibody molecules should remain unabsorbed and react only with A-protein. It is clear that this does not occur. Thus, in this antiserum, which is capable of interacting with A-protein to produce as many as seven distinct bands, there are no antibody molecules that cannot be absorbed by the surface antigen of TMV. This fact is demonstrated again (Kleczkowski, 1961) in the absorption of the anti-A-protein antiserum with whole TMV (Test No. 18 in Table III). The absorption removed all activity for the A-protein.

Mention has already been made of the potential contribution of the quaternary structure of TMV to the antigenic specificity of the virus. In addition to the possibility that the quaternary structure influences the conformation of the individual polypeptide chains, Aach (1959) and Knight (1961) consider that new antigenic sites may arise at the juncture of two subunits. However, there is no experimental evidence to support this idea. Aach (1959) also suggested a mirrorlike right-left symmetry in the association of the subunits as a possibility for introducing new antigenic specificity. This symmetry has been excluded, however, by the X-ray diffraction data which do not support

(Table III), the antigen valence can be calculated:

$$\text{A-protein valence} = \frac{(1 \text{ to } 2)\ (90{,}000)}{(80{,}000)} = 1 \text{ to } 2$$

Therefore, 1 molecule of A-protein binds between 1 and 2 molecules of antibody at equivalence.

such a notion. The participation of RNA in influencing the final surface configuration of the rod was invoked by Starlinger (1955), but discounted by Kleczkowski (1959) as being insignificant. Anderer and Handschuh concluded (1963) that only the tertiary structure contributed to the antigenic specificity. But this was based on qualitative data intended to detect only gross changes in antigenic structure. Subtle changes would have escaped notice. Significant in this regard is the study of Rappaport *et al.* (1965), who showed that the variety of antigenic types found in A-protein preparations varied with the temperature.

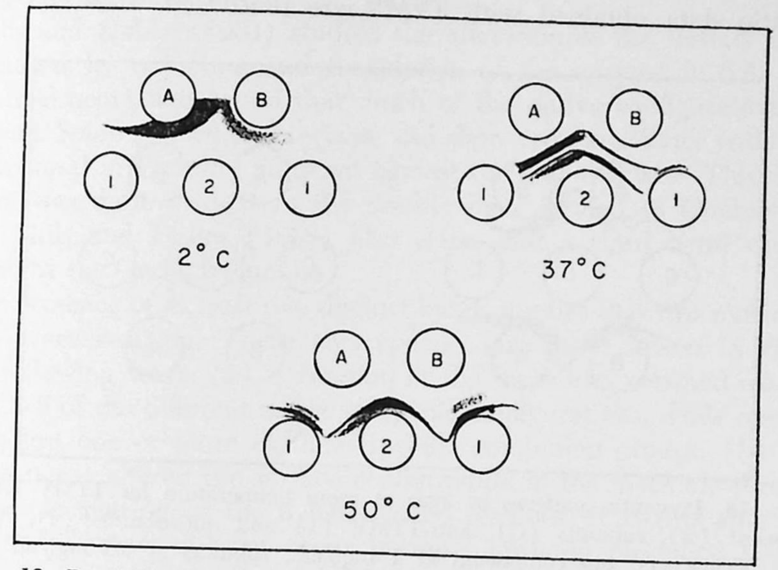


FIG. 12. Precipitin patterns in agar at different temperatures for TMV (1), A-protein (2), anti-TMV (B), and anti-A-protein (A). Antisera at 1/10, TMV at 1 mg./ml. and A-protein at 0.5 mg./ml. [From Rappaport *et al.* (1965).]

A-protein was equilibrated at different temperatures and then permitted to react with antiserum in Ouchterlony plates. The patterns which developed appear in Fig. 12. The data reveal an interesting relationship between temperature and the number and position of discrete precipitin lines. More lines appear at 37°C. than at 0° or 50°C. This is in keeping with the known behavior of A-protein with respect to temperature. The number of stable aggregate states and their sizes are a function of temperature. At low temperature, the least polymerized forms of TMV protein are prevalent. With increasing temperature, aggregation proceeds, and a number of intermediate-sized aggregates predominate. At high temperature polymerization continues to the point where short rods and helical structures characteristic of the virus rod are most common. The Ouchterlony data reveal a different set of precipitin lines for each of the temperatures investigated, and therefore suggest a strong correlation between the size of the aggregate and its antigenic specificity. Rappaport *et al.* (1965) suggested that slight conformational changes in the polypeptide chain accompanying polymerization induced an altered configuration of the exposed antigen. At one extreme the individual monomers may show little if any antigenic relationship to the intact virus, while at the other, antisera to whole virus may not react with the subunits. A case in point is presented in Fig. 13, where the precipitin data obtained with TYMV are illustrated. Here, no cross-

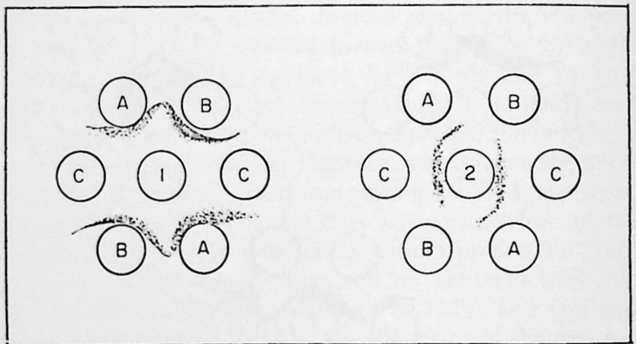


FIG. 13. Precipitin patterns in agar at room temperature for TYMV (A), top component (B), subunits (C), anti-TYMV (1) and antisubunits (2). Antisera at 1/10, TYMV and top component at 1 mg./ml., subunits at 0.5 mg./ml. [From Rappaport *et al.* (1965).]

reaction between TYMV subunits and complete virus was detected. This finding was astonishing, but paralleled precisely that reported for poliovirus (Scharff *et al.*, 1964) in which antisera to subunit antigens and the D antigen also failed to cross-react.

5. *A Hypothesis to Account for the Heterogeneous Serological Behavior of TMV protein*

In this section, an attempt will be made to explain the serological data obtained with TMV protein (X and A) in terms of a single population of antibodies reacting with chemically identical subunits. In essence, the problem is twofold. The first concerns the contribution of tertiary and quaternary structure to the conformation of the antigenic patch; the second involves the number of different antigenic sites present on

a single TMV subunit. Because these serological problems are common to other small globular proteins, a brief examination of two other systems, ribonuclease and bacteriophage lysozyme, at this point can be profitable.

Ribonuclease (RNase) is a small protein molecule with a molecular weight of 12,700, consisting of a single chain of amino acids. It is known to possess three (Cinader and Pearce, 1956) and perhaps as many as five antigenic combining sites (Mills and Haber, 1963). The tertiary configuration of the molecule (its enzymic and antigenic activity) is determined by the proper pairing of four disulfide bonds (Anfinsen *et al.*, 1961; Smyth *et al.*, 1963).

Mills and Haber (1963) studied the alteration of the tertiary structure caused by reduction and reoxidation of the enzyme in 8 *M* urea (urox treatment) and found that much of the native configuration was destroyed. Some molecules, however, did show cross-reactivity with antisera prepared in Freund's adjuvant against the native RNase. This cross-reaction was most evident in the double lines formed in Ouchterlony plates. Mills and Haber (1963) also claim that a third band can be detected by the Oudin technique.

The presence of at least two distinct bands implies that two molecular species were reacting. These two species may have arisen in either of the following ways. (*a*) A fraction of the molecules retained essentially all 3–5 of the different native antigenic configurations, while another fraction lost one or more of these specific combining groups. (*b*) The urox treatment altered the surface configuration of the molecule through an improper pairing of the 8 half-cystine residues. It is probable that the first alternative did not occur because antibody produced against alum-precipitated, native RNase failed to react with the urox material. This failure suggested that none of the native antigens remained on the RNase after urox treatment. It is more likely that the second alternative is correct and that the urox treatment gave rise to related antigenic patches. These related antigens were cross-reactive, but only with antiserum with broad specificity prepared against RNase in complete Freund's adjuvant. Thus, it appears that an alteration of tertiary structure alone may have been sufficient to produce an entirely new antigen.

Bacteriophage lysozyme is another small protein molecule similar in molecular weight to a TMV subunit. The lysozyme molecule consists of a single polypeptide chain of 16,000 molecular weight. It is highly helical and maintains its tertiary structure without disulfide links. In this respect, it is more like TMV than is RNase. Lysozyme unfolds readily in 8 *M* urea, but refolds when the urea is removed, regaining the tertiary structure, enzymic, and antigenic activity it had lost. The molecule will partially unfold in 4 M urea, retaining a portion of its antigenic activity (Merigan and Dreyer, 1963). Thus, with lysozyme a reversible change in tertiary structure can produce an alteration of antigenic activity without the intervention of any disulfide bonds.

As mentioned above, the TMV subunit is similar to the phage lysozyme in that it, too, possesses no disulfide bridges. This lack may well permit a certain degree of flexibility in the TMV polypeptide chain not possible in a molecule like RNase. Assuming such slight flexibility of the TMV polypeptide chain to exist, increased stabilization may occur as a result of polymerization. The noncovalent bonds formed during assembly of the rod may exert a noticeable effect upon the tertiary structure of the subunits, to the extent that the exposed surfaces now take on a new configuration. The very act of polymerization may impose the final restriction on the conformation of the polypeptide chain. Granting this hypothesis, the precise quaternary structure of the virus becomes important. Mere aggregation of subunits is insufficient to guarantee the original surface antigen of the virus. The stacked disk structure, even though rodlike, does not display the same antigenic configuration as the proper helix (see Section III,B,4).

The second point that needs to be considered is the number of potential antigenic sites on a TMV subunit. *A priori,* one may argue, that a protein molecule consisting of a single polypeptide chain in which there is no repetition of the amino acid sequence would probably have more than one different surface patch of antigenic specificity. Considering the view of the myoglobin molecule seen in Fig. 6 as representative of the tertiary configuration of a protein molecule, it is highly unlikely that any two surface areas would have the same configuration. The number of such combining sites would be limited by the size and shape of the molecule. By analogy to molecules of similar size (RNase and lysozyme) it is reasonable to assume that a TMV subunit may possess between three and five antigenic sites. Because each antigenic site is a patch on the surface of a three-dimensional molecule it is probable that each antigenic site on the same TMV subunit would be different. Obviously, at least one of these assumed multiple antigenic sites is located on the exposed viral surface.

One important prediction of this multiantigenic site hypothesis is that absorption of an antisubunit antiserum containing antibodies to all three to five potential antigens with TMV should leave behind antibodies specific for the subunits. As already mentioned with respect to Kleckowski's data (1961), this has not been observed, although this is no guarantee that it will not be in the future. To some extent Aach (1959) found that a partial absorption of anti-A-protein antiserum with TMV did leave behind more reactivity for A-protein than it did for the intact TMV. The data suggested that a new antigenic site may have been present on the A-protein and was making itself known in this way. Had the results of a complete absorption been reported, a more positive statement could be made. The data of Rappaport *et al.* (1965) and those data presented in Table II also suggest that some antibody specific to A-protein may be present in these antisera but it was not definitely shown. Benjamini *et al.* (1964) also intimate that their antisera to TMV protein contain antibodies specific to the subunits, but they present no supporting data.

It seems reasonable to expect, nevertheless, that in further studies antisera will be produced against TMV subunits with activity directed toward previously unexposed antigens. At such time, reagents specific for TMV protein can be prepared and investigated. None as yet has been described with any conviction. What studies have been reported so far, particularly those with TMV protein in gels, can be reconciled to the assumption that a single surface antigen stimulated the production of a population of antibodies with broad specificity. Different portions of this antibody population may then be capable of combining with this same antigenic site even after that site has undergone some conformational change resulting from polymerization.

IV. TMV AS AN INFECTIOUS PARTICLE

A. *Inactivation with Antiserum*

1. *Static Curves*

Early investigation demonstrated that normal (Mulvania, 1926) and immune sera (Purdy, 1929) had inactivating effects on TMV. These studies were enlarged by Chester (1934, 1936), who established many of the basic relationships for the neutralization of TMV. Although some reservations concerning the efficacy of specific antiserum for inactivating TMV were raised (Kassanis, 1943), these were soon dispelled. With good antisera specific inactivation can be easily separated from the normal serum effects simply by dilution (Rappaport and Siegel, 1955). An example of this is shown in Fig. 14.

The effect of normal serum does not extend beyond a $1/_{1000}$ serum dilution and usually no further than $1/_{500}$. Partial purification of the antiserum will also eliminate the normal serum effect (Chester, 1936).

An important fact revealed in the antibody inactivation experiments is that viral infectivity survives even in the presence of excess antibody. This is established by data such as those shown in Table IV. One

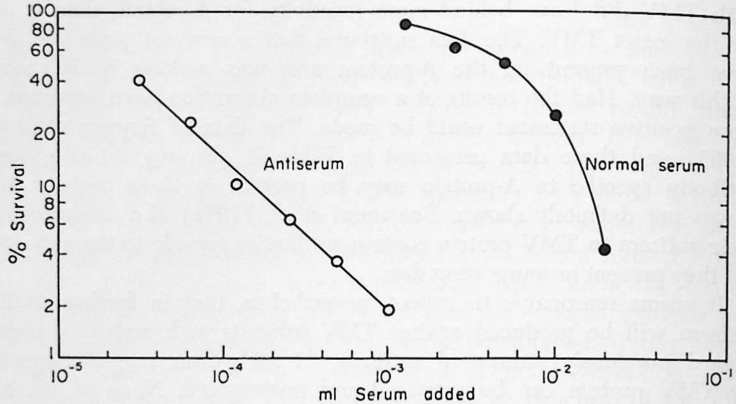


FIG. 14. Quantitative inactivation of U1 with normal serum and with antiserum FY_2. [From Rappaport and Siegel (1955).]

TABLE IV

INACTIVATION OF TOBACCO MOSAIC VIRUS (TMV) WITH UNABSORBED AND ABSORBED ANTISERUM[a]

Final antiserum concentration (ml./ml.)	Final virus concentration (mg./ml.)	Virus surviving (%)	Supernatant condition ascertained from precipitin curve	Virus surviving (%) after addition of fresh virus at 5×10^{-4} mg./ml. (final conc.) to supernatant solution
2.5×10^{-4}	5×10^{-4}	3.6	Antibody excess	28.0
1.25×10^{-4}	5×10^{-4}	5.7	Antibody excess	66.0
6.25×10^{-5}	5×10^{-4}	11.2	Antibody excess	83.0
3.13×10^{-5}	5×10^{-4}	15.3	Equivalence	100
1.57×10^{-5}	5×10^{-4}	24.1	Antigen excess	100
7.85×10^{-6}	5×10^{-4}	51.8	Antigen excess	100
3.98×10^{-6}	5×10^{-4}	75.2	Antigen excess	100

[a] From Rappaport (1957a).

cannot charge this survival in antibody excess to the presence of nonavid antibody, for antibody remaining uncombined is as efficient in inactivating fresh virus as is whole antiserum. Virus heterogeneity also plays no role in these observations. Single lesion isolates and subcultures of the surviving infectious units were made. The new preparation displayed the same survival curves as the parent population (Rappaport, 1959a).

2. *Kinetic Curves*

Kinetic studies of inactivation are rarely performed with TMV—and not because the data are unrevealing. Rather, this approach presents certain technical difficulties not inherent in bacteriophage or animal virus systems that have undoubtedly discouraged many investigators. One significant difference in the TMV system is the high virus concentration required. Because of the relatively poor assay, approximately 10^{10} TMV particles per milliliter are necessary in the initial serum-virus

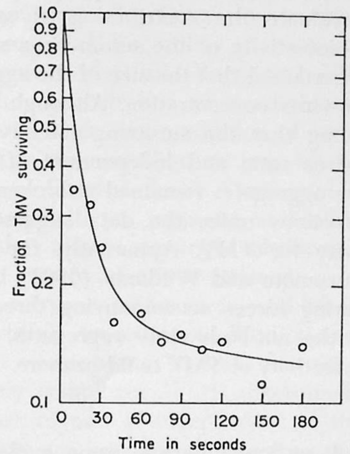


FIG. 15. Kinetics of virus inactivation. Time course of inactivation of 10^{-3} mg./ml. U2 with equal volume of 5.0×10^{-4} ml./ml. antiserum 11 and 12. [From Rappaport (1957a).]

mixture (as contrasted to 10^6 or less with phage) to obtain meaningful survival data. Under these circumstances, even though antibody is in excess, it is not so great that it is not measurably diminished by its reaction with virus. This relationship is also revealed in Table IV. A typical kinetic curve obtained with this antiserum appears in Fig. 15. It may be observed from the figure that the survivors fall rapidly and without delay during the first few seconds. At first, the decline in infectivity appears logarithmic. Thereafter, the curve takes on a more gentle slope, and even though antibody is in excess, the curve reaches an asymptote within 30 minutes.

The initial rate of inactivation was studied as a function of the serum and the virus concentration (Rappaport, 1957b). The kinetics obtained appeared to be first-order, so that inactivation of TMV could be treated as a pseudomonomolecular reaction. In this regard, all assays of surviving infectivity following antibody treatment revealed the immediacy of inactivation. No lag period was detected in any of the kinetic experiments, suggesting that a single event was sufficient to inactivate the virus.

3. *Aggregation and Specific Infectivity*

Aggregation also occurs during inactivation, introducing an additional complexity usually avoided in animal and phage systems. It was therefore necessary to evaluate the contribution of aggregate formation toward lowering the infectivity of the serum-virus mixtures. Rappaport and Siegel (1955) ascertained that the size of the aggregate was directly related to the serum-virus concentration. Although this result was not unexpected, the finding that the surviving infectivity was a function only of the serum-virus ratio and independent of the aggregate size was surprising. If the aggregates remained unbroken during inoculation and behaved as infectious units, the data suggested an exceedingly low specific infectivity for TMV. Apparently, the aggregates do not remain intact, for Furumoto and Wildman (1963) have recently established that the shearing forces accompanying direct inoculation were sufficient to disrupt the antibody-virus aggregates. Their data in fact elevate the specific infectivity of TMV to 0.1 or more.

4. *Reversibility*

In Section III,A,2, on precipitation, some evidence of reversibility of the TMV anti-TMV reaction was presented. Additional pertinent data will be discussed in this section.

Given a measure of reversibility, one may expect that reactivation of infectivity should accompany dissociation of the antibody-virus complexes. At neutral pH, however, almost no reactivation is detected either by large dilution (Rappaport, 1959a) or by the addition of excess UV-inactivated virus (Rappaport, 1959b). Only at pH 2.5 is antibody completely dissociated and all infectivity recovered (Rappaport, 1961a). The parallel between the TMV system and poliovirus (Mandel, 1960, 1961) is particularly striking in this respect, for precisely the same results were obtained, even to the extraction of infectious RNA, with phenol or detergent, from antibody inactivated TMV and poliovirus (Rappaport, 1959b; Mandel, 1964).

a. Danysz Phenomenon. The Danysz phenomenon is the classic example of irreversibility of the antibody-antigen reaction. Originally, it described the reaction between diphtheria toxin and antitoxin. By titration, the amount of toxin necessary to just neutralize a given amount of antitoxin could be determined. If instead of a single addition of toxin to antitoxin, a number of aliquots of toxin were added to the antitoxin, the resulting mixture was toxic. This phenomenon results primarily from a combination of antibody and antigen in multiple proportions, and from an extremely rapid forward reaction compared to the reverse. Thus, small additions of antigen to excess amount of antibody bind more antibody molecules per unit weight of antigen added than a single addition of the same amount of antigen. This is graphically illustrated by the relationship BN/GN vs. GN seen in Fig. 4.

Attempts to demonstrate the Danysz phenomenon with TMV and antiserum were unsuccessful. Regardless of the manner of antigen addition, the end results were identical (Rappaport, 1959a). The final level of survivors and the concentration of uncombined antibody was the same as that measured after single addition of virus to antiserum. The data suggested that a new equilibrium was established rapidly after each antigen addition. Fazekas de St. Groth and Webster (1963) reported similar findings with an influenza anti-influenza system, where they too observed that aliquot or single additions of virus to antisera did not alter the final level of infectivity attained.

Whether in fact a true equilibrium is established in the TMV anti-TMV system is doubtful. According to Bradish and Crawford (1960), the data for tobacco mosaic virus "indicate that an established distribution of virus-antibody complexes . . . is determined by the A/G ratio for the reaction mixture and is independent of the order or manner in which the reactants are introduced. The true reverse reaction, however, involving the reduction of an established distribution of complexes to one of smaller complexes, has not been observed."

With reference to this reduction in the established distribution, the following experiment, in which the fraction of viral activity remaining after different combinations of active and inactive TMV were mixed with antiserum, is significant. In the first series, UV-inactivated TMV was incubated with antiserum, and at different intervals of time viable virus was added. In the second series, viable TMV was incubated with antiserum and UV-inactived virus added afterward. The final concentration of all reactants was the same. The data appearing in Fig. 16 contrast sharply with those in which only viable virus was used (Rappaport, 1959a). Although the concentration of all reactants was the same, the final survival levels reached were very different and entirely dependent on the order and manner of mixing. These data suggest the lack of a true equilibrium and lend support to the conclusions reached by Bradish and Crawford (1960).

What makes interpretations particularly difficult in these experiments is the relationship of inactivation and reactivation to association and dissociation of the antibody molecules. Although it is generally assumed that the infectivity assays and the antibody determinations relate to the same properties of the system, this may in fact be erroneous. Association and dissociation are gross phenomena concerned with average conditions in and around the complexes. Inactivation, on the other hand, measures the loss of infectivity resulting from the specific interaction

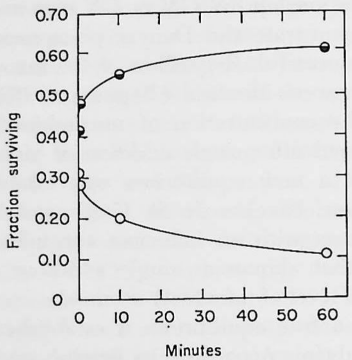


FIG. 16. Fraction of TMV surviving following addition of viable or UV-killed virus to the original antiserum-virus mixtures at different intervals of time. O–O, 5.0 ml. antiserum at 2×10^{-4} ml./ml. added to infectious U1 at 2×10^{-3} mg./ml.; at time intervals indicated, 10.0 ml. UV-killed U1 at 10^{-2} mg./ml. was added. ◒–◒, 5.0 ml. antiserum at 2×10^{-4} ml./ml. added to UV-killed U1 at 2×10^{-2} mg./ml.; at time intervals indicated, 10.0 ml. live U1 at 10^{-3} mg./ml. was added. Incubation at room temperature continued for 1 hour before survivors were assayed. [Rappaport (1959) unpublished data.]

of an antibody molecule at a critical site. Conditions at this particular point may not be average. The concept of a stabilization of the antibody virus union, as proposed by Lafferty (1963a,b) for influenza, or Mandel (1962) for poliovirus, cannot be discounted in the TMV system. If such stabilizations were to occur with TMV as well, they could easily account for any of the apparent contradictions between measurements of reversibility based strictly on the antigen-antibody interactions and those concerned with inactivation and reactivation of biological activity.

5. *Critical Sites*

TMV is an antigen with a large number of valences, many of which may be combined with antibody without loss of infectivity. Indeed, the virus may survive in the presence of excess antibody with hundreds of antibody molecules bound to its surface. These survivors are no different from the rest of the virus population. They do not constitute an antibody-resistant fraction, nor do they survive because of any deficiency in the character of the excess uncombined antibody (Rappaport, 1959a). They survive because some critical site has remained free of antibody. Thus, association of antibody molecules with most sites is uneventful with respect to infectivity. If inactivation occurs when an antibody molecule combines with a critical site on the rod, then we must distinguish between two kinds of antigenic sites, those that are critical and those that are not. An estimate of the number of critical antigenic sites can be made from a knowledge of the milligrams of antibody in the antiserum and the amount of antiserum required to reduce the infectivity of a known number of infectious particles to 0.37 (Rappaport, 1959a). These estimates all suggest that approximately 4% of the antigenic sites are critical. Estimates made at a lower survival level suggest fewer critical sites per rod. This is a consequence of the log-log relationship seen in serum-survival curves. Either the number of critical sites is a variable, or the efficiency of the antiserum for inactivating the virus decreases with increasing serum concentration. Rappaport (1959a) suggested that reduced efficiency was at fault in this case, and that the reduction resulted from increased steric hindrance as more antibody was added to the rods (Rappaport, 1957a). Because all of the antigenic sites cannot be occupied by antibody molecules at any one time, a number of different antibody-virus combinational patterns are possible. These result in a heterogeneous population in which the survivors comprise those particles having none of their critical sites covered but do have a variable number of their critical sites protected from further combination with antibody. In this model, even in extreme antibody excess a small viable fraction of virus may remain in equilibrium with the vast majority of inactivated virus particles.

This argument advances the concept of one or more unique antigenic sites on the surface of the virus, i.e., sites that are intimately involved with the biological activity of the virus. They must remain free of antibody in order for the virus to function. Because there is no chemical evidence suggesting odd subunits, does the critical site have a morphological basis, or must the critical site be considered only an operational definition? Certainly, with respect to enzymes, critical sites at least do have a defined position. They refer to those antigenic determinants situated close enough to the catalytic site for a combined antibody molecule to interfere with the proper approach of the substrate molecule (Cinader and Lafferty, 1963). With the tailed bacteriophages, interference occurring on a specific adsorptive organ is also rational (Lanni and Lanni, 1953), for the tail is a morphological and functionally distinct part of the phage. No such statement can be made for TMV. It is therefore necessary to consider the possibility that there are no critical sites on the virus. Instead there may only be critical antibody combinations in which an antibody molecule is bound with both of its combining sites to the same virus particle (Lafferty, 1963a,b; Lafferty and Oertelis, 1963; Mandel, 1962). This configuration may present a formidable obstacle to an uncoating enzyme or may even prevent nonenzymic depolymerization of the rod.

B. *Analysis of Strain Relationships*

1. *Introduction*

The most utilitarian aspect of serology, namely, its ability to recognize fine distinctions in structure, has been a boon to the taxonomist. Historically, serological cross-reaction has always ranked high, compared with other single tests as a criterion for suggesting or establishing kinship among microorganisms. Only recently have more fundamental aspects of structure (Lwoff *et al.*, 1962) and nucleic acid composition challenged its position (Marmur *et al.*, 1963). Our interests, though, will not be with classification of TMV per se, which has been thoroughly reviewed by Hitchborn and Thomson (1960); Markham (1959); Knight (1959), and Matthews (1957). An excellent compilation of the older data is given by Bawden and Pirie (1938). Rather, our interest in strains is motivated by our concern over the general problem of antigenic structure and chemical composition.

2. *Antigenic Studies*

a. Complement Fixation. Complement fixation is just one of a number of techniques available to the viral taxonomist. Although in the case of TMV most workers have employed the precipitin technique, Tall *et al.* (1949), Wright (1963), Weaver and Price (1962), and Price (1954), examined a number of TMV strains and found them to be distinct by complement fixation tests. The technique as it applies to viruses has been recently reviewed by Fulton (1958), and so will not be considered further at this time.

b. Inactivation. The antibody neutralization of infectivity test, which is most commonly employed for determining relationships among the animal and bacterial viruses, has not been the method of choice among plant virologists. That the technique of inactivation is perfectly capable of uncovering strain relationships was shown by Rappaport *et al.* (1957).

In fact, with one antiserum pool, Holmes' masked strain could be distinguished from the common strain of TMV (Rappaport *et al.*, 1957). This was a remarkable find considering that the two strains have the identical amino acid composition (Tsugita, 1962), and were not distinguished by any other serological or physicochemical test (Siegel and Wildman, 1954; Ginoza and Atkinson, 1955). Although quantitative inactivation appears in this case to be the most sensitive procedure for revealing differences, it is not known whether this would hold in all cases, because too few strains have thus far been investigated.

c. Precipitation. There is, of course, no reason why other immunological techniques could not be employed, but for technical reasons the precipitin tests are preferable. Milligram quantities of TMV may be prepared with only a routine investment of time and effort. When the ease of obtaining large amounts of highly purified TMV is contrasted with some of the heroic efforts required to prepare comparable amounts of animal or bacterial viruses, it is clear why quantitative immunochemical tests were used early with TMV.

Most commonly the cross-absorption technique is employed, where an antiserum is repeatedly absorbed with a heterologous strain, until no further precipitation is detected. A test with the homologous virus strain at this point will reveal whether or not any antibody specific for the immunizing virus has remained. If so, a specific reagent has been prepared which is capable of distinguishing the two strains. This procedure can be multiplied any number of times with as many virus preparations as desired. The result is often the production of a whole series of reagents with which serological relationships may be explored. Matthews (1957) gives a lucid and detailed description of the method.

Besides being simple to perform, the cross-absorption test is far more sensitive to subtle differences in structure than is the quantitative precipitin analysis. When a direct comparison of the two methods was made, Malkiel (1948) found that TMV strain, J14D1, and the green aucuba (GA) strains were indistinguishable by quantitative precipitin analysis, but both TMV and J14D1 could be distinguished from GA following the appropriate cross-absorptions. In a more recent paper, Aach (1957) analyzed 7 different TMV strains, 5 in the vulgare group and 2 in the dahlemense group. By quantitative precipitin analysis the two groups were discerned easily but the individual members within each were not. On the other hand, cross-absorption revealed the individuality of each of the five members of the vulgare group.

The prime motivation in much of this research is to correlate if possible chemical composition, antigenic structure, and biological activity. Before it was appreciated that the nucleic acid alone was responsible for the infectivity of the virus (Gierer and Schramm, 1956) and probably of all the symptoms as well, it was assumed that a knowledge of the chemical composition of the different strains would provide a clue to their biological behavior. Although there is no question of the influence of chemical composition of the protein subunits on the antigenic specificity of the virus, there is considerable doubt that the protein plays any role in the development of symptoms. This was made perfectly clear with mixedly reconstituted virus (Fraenkel-Conrat and Singer, 1957; Fraenkel-Conrat *et al.*, 1957), in which, for example, artificial hybrids of TMV and Holmes' ribgrass were produced. The symptoms were those expressed by the nucleic acid, while the antigenic properties before replication were those of the protein, even to the extent of antibody inactivation of the mixed reconstituted virus. The RNA alone was responsible for both symptom expression and production of specific coat protein. Nevertheless, the correlation between chemical composition and serological properties of strains has occupied a good deal of attention and is in itself a challenging study. An interesting case is that of the cucumber viruses CV3 and CV4 and their relationship to TMV.

Bawden and Pirie (1937) demonstrated by cross-precipitin tests that CV3 and CV4 were antigenically related to TMV even though they had no common host. Knight (1955a), in analyzing the products of carboxypeptidase treatment on a number of TMV strains, came to the conclusion that the polypeptides of all strains of TMV were characterized by threonine as their C-terminal amino acid residue. With this in mind, he reexamined the relationship between CV3, CV4, and TMV (Knight, 1955b). If one used a number of criteria to establish strain relationships in addition to serological ones, then the cucumber viruses did not appear related to TMV. Knight felt that the chemical evidence was most compelling. Primarily, CV3 and CV4 contain no cysteine. The C-terminal amino acid of CV3 is serine and that of CV4 is alanine. The base composition of CV and TMV-RNA differs. Yet, Holmes and Franklin (1958) examined a number of TMV strains, including CV4, by X-ray diffraction, and concluded that CV4 showed the same general structural features of TMV, although it may contain 2 less subunits per 3000 Å length than TMV (Klug and Caspar, 1960). In general, however, there appeared to be no greater departure in the patterns of CV4 from the common strain of TMV than from any of the other strains analyzed. This particular situation became even more confused with the discovery of two new TMV strains (Knight *et al.*, 1962).

A strain of TMV originally obtained from diseased tomato plants, was called "tomato atypical virus" (TAMV). After propagating the virus in tobacco, Knight *et al.* (1962) then isolated two strains, one of which was named yellow or Y-TAMV and the other a green mottling variety was called G-TAMV. These two are closely related and obviously TMV strains by a variety of criteria. Nevertheless, the C-terminal amino acid residue of Y-TAMV was found to be serine rather than threonine. This, of course, only points out once more the hazards of according too much significance to a single criterion for determining strain relationships. Further, because cross-reactions between TMV and particularly between G-TAMV and Y-TAMV are strong, the minor influence of the single C-terminal amino acid in determining the antigenic specificity of the subunit is underscored again (see Section III,A,4).

It happens that of all the strains examined serologically by Knight *et al.* (1962), Y-TAMV and G-TAMV were most closely related to Holmes' ribrgass. The polypeptides of these three strains contain the amino acid methionine in common, a distinction not shared by other TMV strains except for dahlemense. Whether or not methionine is directly involved in the antigenic structure, however, is not known. The efforts of Anderer to elucidate the exact chemical composition of the surface antigenic site have already been cited.

As far as quantitative immunochemical analysis is concerned, only the cross reactions between two strains of TMV, the common strain (U1) and a mild strain (U2) have been examined in this fashion (Rappaport, 1961b).

TMV strains U1 and U2 are known to differ in a number of ways (Siegel and Wildman, 1954; Ginoza and Atkinson, 1955). Evidence that the protein-nucleic acid bonds differ has been obtained from inactivation studies with heat (Fassell and Wildman, 1963) and ultraviolet light (Siegel and Norman, 1958). Differences in surface structure were also reflected in X-ray diffraction diagrams (Franklin and Klug, 1956). The X-ray data suggested that the helical groove on U2 was more highly developed and that the packing radius of the subunits for U2 was slightly smaller than U1. Unfortunately, an amino acid analysis of U2 protein has not been made, so no chemical comparison is available. However, from the published characteristics of this strain it may belong with other mild strains in the dahlemense group.

The cross-reaction between rabbit anti-U2 and U1 was studied in detail. A number of absorptions were performed with the object in mind of fractionating the antiserum into its component parts. If each specific antiserum fraction were assigned a letter (a, b, c, . . . , etc.), and this letter corresponded, in fact, to a discrete antigenic group on the virus particle (A, B, C, . . . , etc.), then by quantitative analysis of the precipitin reactions with these reagents, the fraction of antigenic sites belonging to each particular group could be counted. In other words, the experiments were designed to reveal the number of U2 surface antigens that were specific to U2 as well as the number shared in common with U1. This would be revealed most simply by a reduction in the maximum antibody-antigen ratio as a consequence of only a portion of the antigenic sites reacting with any one specific reagent. The data, as seen in Fig. 17, indicate that no fractionation was accomplished. There appear to be no antigenic sites on U2 specific to

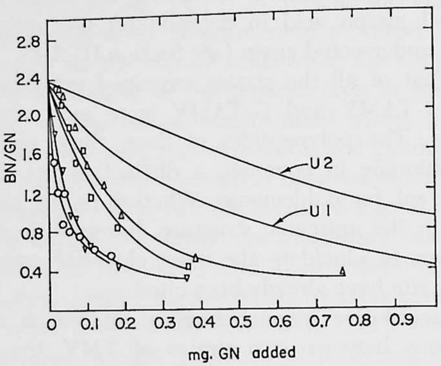


FIG. 17. Quantitative precipitation reaction. Ratios of antibody N to antigen N (BN/GN) revealed after different absorptions of anti-U2 antiserum pool 17–18. Lines U2 and U1 are reference lines of the homologous and heterologous reaction. The following data are uncorrected for the different dilutions encountered. △, after complete heterologous absorption of the antiserum with U1 the remaining antibody was precipitated with U2; ▽, reaction with homologous U2 virus of antibody recovered after acid dissociation from U1 anti-U2 precipitate; ○, after partial absorption of antiserum with homologous virus, the remaining unabsorbed antibody was treated again with U2; □, the precipitate from the partial homologous absorption was dissolved in acid and antibody recovered. This antibody was reacted with U2. [From Rappaport (1961b).]

U2, nor are there antigenic sites shared in common. The data suggest, instead, that there are equal numbers of similar combining sites on both kinds of virus particles. This is seen most clearly in one of the absorption experiments.

U1 was precipitated maximally with anti-U2. The precipitate was washed thoroughly, resuspended in saline, and the complex was dissociated by adjusting the pH of the medium to 2.5. The antibody fraction prepared in this way by dissociation of the heterologous precipitate was presumably directed only toward the common antigens on U1 and U2. The remaining unabsorbed antibody by definition was specific for the homologous sites on U2. As the results in Fig. 17 show, both anti-

serum reagents combined with the same number of antigenic sites when they were retested quantitatively on U2. The antiserum was fractionated, but into two populations, one of which showed broad reactivity encompassing related structures, the other displaying a narrower specificity; but no evidence was obtained suggesting that these serum fractions were correlated with particular antigen fractions.

3. *Conclusions*

In considering the protein structure of TMV our ideas have progressed in the space of a few short years from that of a single molecule with a staggering molecular weight of 38×10^6 and enormous complexity, to one which conceives of the virus as composed of over 2000 small polypeptide chains, each with the same amino acid composition (Crick and Watson, 1957). Once the problem was reduced in size to the analysis of a 17,500 mol. wt. subunit constructed from 158 amino acids, rapid progress was made. The work of Tübingen and Berkeley elucidating the exact amino acid sequence of approximately 200 TMV strains leaves no doubt that TMV is assembled from small identical building blocks. What can then be said of the serological data collected from strain analysis suggesting a heterogeneous antigenic surface for the virus? It has already been suggested (Rappaport, 1961b) that these data reflected the heterogeneity of the antisera rather than a mosaic of antigenic determinants on the rod. All the data accumulated since then lend added support to the single determinant hypothesis presented. When one considers the complexity of the antibody response to proteins (Fahey and Goodman, 1964) and even to chemically defined haptens (Onoue *et al.*, 1964; Landsteiner, 1947), it is clear that the letters A, B, C, D, . . . that were previously assigned to represent distinct antigens on the surface of the virus in truth describe portions of the original antibody population that were fractionated on the basis of variations in specificity. Geneticists, facing the identical problem of relating multiplicity of reagents to genetically determined antigenic structure (Stormont, 1955; Owen, 1959) have reached the same conclusion. For this reason, the serological studies of TMV strain relationships need to be reinterpreted in keeping with our current appreciation of the complexity of the antibody response and our knowledge of the viral subunit structure. Because we are, in all probability, dealing with single surface antigens, the cross-reactions represent configurational similarities of the exposed polypeptide chains. The common practice of describing relationships among TMV strains in terms of common and specific antigens is thus no longer valid.

The serological techniques are without doubt extremely sensitive and, for this reason, a note of caution will be sounded before leaving this section. The methods are in fact so sensitive that the investigator is always plagued with the problem of making antibodies to minute contaminants which may go undetected by any other means. Because of this uncertainty, it is essential to examine the serological data carefully. Preparative procedures should come under close scrutiny and the participation of the host components in the antigenic specificity evaluated (Malkiel, 1947).

It was shown by Ginoza *et al.* (1954), for example, that the common strain of TMV normally absorbs a host nucleoprotein when prepared in cacodylate buffer but does not if prepared in phosphate buffer. This was in contrast to other strains which did not bind nucleoprotein under either circumstance. Whether this nucleoprotein contributed to the serological properties of the amber-colored TMV was not determined.

In the animal virus field, influenza has been a classic example of the virus commonly prepared with a host-associated antigen (Knight, 1946). This is reasonable considering that myxoviruses assemble at the cell membranes. The true significance of this fact, however, awaits further investigation. It is known, though, that with some effort of purification influenza virus can be freed of host antigen (Kroeger, 1962). With plant viruses, the culprit may be "fraction 1" (Wildman and Bonner, 1947). This normal plant protein appears to be ubiquitous among green plants and antigenically similar wherever found (Dorner *et al.*, 1958). Unless care is exercised in the preparation of virus material, fraction 1 protein may be a very likely contaminant. Recently van Regenmortel (1963) criticized MacLeod and Markham (1963) for their report of an antigenic similarity between turnip yellow mosaic virus and wild cucumber virus because they failed to consider the possible contamination of their material with fraction 1. The presence of trace amounts of fraction 1 in a TMV preparation may also explain the antigenic relationship between fraction 1 and TMV suggested in a footnote by Wildman *et al.* (1949).

C. *Biological Significance of the Protein Coat*

1. *Introduction*

There is no longer any doubt that TMV may dispense with its protein entirely either as a result of chemical manipulation or mutation and still retain its infectivity. But when protein is combined with the nucleic acid, it apparently plays some role in establishing the infection. For the present, that role is unknown.

As far as inactivation with antiserum is concerned, it is known, however, that antibody to the coat protein is without effect on the nucleic acid. Even after the whole virus is inactivated by antibody, the RNA within the rod retains its infectivity, for it can be extracted with phenol from the inactive precipitate with its original capacity for infection undiminished (Rappaport, 1959b). Furthermore, if the antibody is dissociated from the virus at pH 2.5, full activity is also restored (Rappaport, 1961a). In addition, papain digestion of the bound antibody releases some infectivity as well (Malkiel, 1950). The antibody then must function in a very special way to prevent infection, for its effect is entirely reversible (Rappaport, 1961a). This is quite different from the action of certain chemical agents (Malkiel, 1952b; Hart, 1955b; Schramm *et al.*, 1955a; Knight, 1961; Price, 1963) on TMV which alter the protein structure but do not necessarily destroy viral infectivity. One such agent has already been mentioned in Section III,A,4, namely, carboxypeptidase. What was most astonishing in these experiments in which the C-terminal amino acid threonine was removed was the complete absence of any noticeable effect of the enzyme treatment on the infectivity of the preparations. Even mutant 171 (Tsugita and Fraenkel-Conrat, 1960, 1962). from which three terminal amino acids were removed, showed no reduction in infectivity. Consequently, the configurational changes imposed on the subunits by the removal of up to three amino acid residues must be quite subtle and biologically trivial. The three terminal amino acids then may play no role in whatever initial act of infection the protein is involved.

A more drastic alteration in the virus structure was caused by alkali treatment (Schramm *et al.*, 1955a,b). This treatment produced besides free RNA and A-protein, an infectious nucleoprotein which had the appearance under the electron microscope of rods with exposed sections of RNA much like those described by Hart (1955a).

Under controlled conditions the anionic detergent Duponol C (sodium lauryl sulfate) was shown by Hart (1955a,b) to partially degrade TMV. An examination of this degraded material under the electron microscope revealed a variety of figures with portions of the RNA moiety of the TMV particles exposed. Similar figures were also observed following phenol treatment (Corbett, 1964). Although considerable loss in infectivity attended degradation, some biological activity remained. The residual infectivity was sensitive to RNase, even though it was still associated with protein. Because of this unique situation, it was possible that these infectious units were able to bypass the early steps in infection and behave in a fashion comparable to free RNA. If so, the Duponol-treated virus may be unaffected by antibody. On the contrary, it was shown by Rappaport *et al.* (1964) that these same RNase-sensitive infectious units could be inactivated as efficiently as the untreated controls by specifically purified antibody devoid of any RNase activity. This was substantial evidence that the RNase-sensitive infectious units were functionally associated with protein and not free RNA, for it was known that the RNA was unaffected by antibody to the virus (Gierer and Schramm, 1956; Starlinger, 1955; Rappaport, 1959b). The remaining protein still served to imprison the RNA.

2. Studies on Interference

Evidence pertinent to the role of the protein in infection has been amassed from studies of interference. The data suggest that the protein coat may play an entirely passive role, in contrast to the participation of a specific absorptive organ like the tail of a T-even bacteriophage during infection. Neither UV-inactivated TMV nor A-protein interferes with active virus in initiating an infection, unless inordinately high concentrations of each are employed (Wu and Rappaport, 1961; Rappaport and Wu, 1962; Santilli *et al.*, 1961; Wu *et al.*, 1962; Bawden, 1954; Bawden and Pirie, 1957). But then, these very high concentrations of inactive TMV or proteins may be functioning in an unspecific fashion, making interpretation of the results ambiguous.

Only live virus or infectious RNA can compete successfully for infectible sites. Furthermore, competition between infectious RNA and TMV is very much a one-sided affair (Wu and Rappaport, 1962). When a mixture of virus and RNA was inoculated on *Nicotiana glutinosa* leaves, the number of lesions established by the RNA was only slightly affected by concentrations of competing virus that would have markedly reduced the number of lesions if whole virus had been used. These results are in harmony with the data of Siegel *et al.* (1957), who showed that infectious RNA underwent intracellular changes in UV sensitivity, sooner than the intact virus.

The initial event in establishing an infection must be the adsorption or engulfment of the infectious unit. If the infectious unit is whole virus, the protein must be removed, perhaps by some uncoating enzyme, before the nucleic acid can act as messenger. The possibility should also be entertained that this second step or release of the RNA may be nonenzymic. After all, chemically and enzymically altered TMV particles retain their infectivity, indicating that the hypothetical uncoating enzyme can either tolerate considerable structural alteration in the exposed surface of the subunits and still function, or that no uncoating enzyme for TMV exists. The ease with which TMV structural units polymerize and depolymerize *in vitro* without enzymic intervention may

also hint at the nature of the *in vivo* event. At any rate, it is presumably this uncoating step with which the antibody molecules interfere.

These considerations of the many problems involved suggest that competition in the TMV system is entirely restricted to infectious RNA strands; that UV-inactivated virus fails to compete because of a UV lesion in the nucleic acid and not because of any difficulty in the removal or unraveling of the subunits. On the other hand, antibody-inactivated virus contains infectious RNA which cannot be released and so cannot function. Here we see that as long as protein remains associated with the nucleic acid, antibody is capable of neutralizing the infectivity. One may add that the protein coat serves not only to protect the virus from the vicissitudes of extracellular life but also functions in an unknown way during some unclear primary act of infection.

ACKNOWLEDGMENT

I wish to thank Drs. S. G. Wildman, W. Furumoto, and E. D. DeLamater for their critical reading of the manuscript. The preparation of this review was supported in part by Public Health Service Research Grant AI-06 114-02.

REFERENCES

Aach, H. G. (1957). *Z. Naturforsch*, **12b**, 614.
Aach, H. G. (1959). *Biochim. Biophys. Acta* **32**, 140.
Aach, H. G. (1960). *Nature* **187**, 75.
Anderer, F. A. (1963a). *Biochim. Biophys. Acta* **71**, 246.
Anderer, F. A. (1963b). *Z. Naturforsch.* **18b**, 1010.
Anderer, F. A. (1963c). *Advan. Protein Chem.* **18**, 1.
Anderer, F. A., and Handschuh, D. (1962). *Z. Naturforsch.* **17b**, 536.
Anderer, F. A., and Handschuh, D. (1963). *Z. Naturforsch.* **18b**, 1015.
Anderer, F. A., and Schlumberger, H. D. (1965). *Biochim. Biophys. Acta* **97**, 503.
Anfinsen, C. B., Haber, E., Sela, M., and White, F. H., Jr. (1961). *Proc. Natl. Acad. Sci. U.S.* **47**, 1307.
Ansevin, A. T., and Lauffer, M. A. (1959). *Nature* **183**, 1601.
Ansevin, A. T., and Lauffer, M. A. (1963). *Biophys. J.* **3**, 239.
Bawden, F. C. (1954). *Advan. Virus Res.* **2**, 31.
Bawden, F. C., and Pirie, N. W. (1937). *Brit. J. Exptl. Pathol.* **18**, 275.
Bawden, F. C., and Pirie, N. W. (1938). *In* "Tabulae Biologicae" (W. Junk, C. Oppenheimer, and W. Weisbach, eds.), Vol. XVI, pp. 355–371, Drukkerij, Bosch and Zoon, Holland.
Bawden, F. C., and Pirie, N. W. (1945). *Brit. J. Exptl. Pathol.* **26**, 275.
Bawden, F. C., and Pirie, N. W. (1956). *J. Gen. Microbiol.* **14**, 460.
Bawden, F. C., and Pirie, N. W. (1957). *J. Gen. Microbiol.* **17**, 80.
Beale, H. P., and Lojkin, M. E. (1944). *Contrib. Boyce Thompson Inst.* **13**, 385.
Benjamini, E., Young, J. D., Shimizu, M., and Leung, C. Y. (1964). *Biochemistry* **3**, 1115.
Bradish, C. J., and Crawford, L. V. (1960). *Virology* **11**, 48.
Caspar, D. L. D. (1963). *Advan. Protein Chem.* **18**, 37.

Cebra, J. J. (1961). *J. Immunol.* **86**, 205.
Chester, K. S. (1934). *Phytopathology* **24**, 1180.
Chester, K. S. (1936). *Phytopathology* **26**, 949.
Cinader, B., and Lafferty, K. J. (1963). *Ann. N.Y. Acad. Sci.* **103**, 653.
Cinader, B., and Pearce, J. H. (1956). *Brit. J. Exptl. Pathol.* **37**, 541.
Commoner, B., and Rodenberg, S. D. (1955). *J. Gen. Physiol.* **38**, 475.
Commoner, B., and Yamada, M. (1955). *J. Gen. Physiol.* **38**, 459.
Commoner, B., Newmark, P., and Rodenberg, S. (1952). *Arch. Biochem. Biophys.* **37**, 15.
Commoner, B., Schieber, D. L., and Dietz, P. M. (1953a). *J. Gen. Physiol.* **36**, 807.
Commoner, B., Yamada, M., Rodenberg, S. D., Wang, T. Y., and Basler, E. J. (1953b). *Science* **118**, 529.
Corbett, M. K. (1964). *Virology* **22**, 539.
Crick, F. H. C., and Watson, J. D. (1957). *Ciba Found. Symp. Nature Viruses*, p. 5.
Delwiche, C. C., Newmark, P., Takahashi, W. N., and Ng, J. M. (1955). *Biochim. Biophys. Acta* **16**, 127.
Dorner, R. W., Kahn, A., and Wildman, S. G. (1958). *Biochim. Biophys. Acta* **29**, 240.
Dulbecco, R., Vogt, M., and Strickland, A. G. R. (1956). *Virology* **2**, 162.
Dupont-Mairesse, M., and Jeener, R. (1960). *Biochim. Biophys. Acta* **41**, 373.
Edelman, G. M., and Gally, J. A. (1964). *Proc. Natl. Acad. Sci. U.S.* **51**, 847.
Fahey, J. L., and Goodman, H. (1964). *Science* **143**, 588.
Fassell, G. M., and Wildman, S. G. (1963). *Biochim. Biophys. Acta* **72**, 230.
Fazekas de St. Groth, S., and Webster, R. C. (1963). *J. Immunol.* **90**, 140.
Finch, J. T. (1964). *J. Mol. Biol.* **8**, 872.
Fraenkel-Conrat, H., and Singer, B. (1957). *Biochim. Biophys. Acta* **24**, 540.
Fraenkel-Conrat, H., and Williams, R. C. (1955). *Proc. Natl. Acad. Sci. U.S.* **41**, 690.
Fraenkel-Conrat, H., Singer, B., and Williams, R. C. (1957). *Symp. Chem. Basis Heredity Johns Hopkins Univ. McCollum-Pratt Inst. Contrib.* **153**, 501.
Franklin, R. E. (1955). *Biochim. Biophys. Acta* **18**, 313.
Franklin, R. E., and Commoner, B. (1955). *Nature* **175**, 1676.
Franklin, R. E., and Klug, A. (1956). *Biochim. Biophys. Acta* **19**, 403.
Franklin, R. E., Klug, A., and Holmes, K. C. (1957). *Ciba Found. Symp. Nature Viruses*, p. 39.
Franklin, R. E., Caspar, D. L. D., and Klug, A. (1959). *In* "Plant Pathology Problems and Progress, 1908–1958" (C. S. Holton, ed.), p. 447. Univ. of Wisconsin Press, Madison, Wisconsin.
Friedrich-Freksa, H., Melchers, G., and Schramm, G. (1946). *Biol. Zentr.* **65**, 187.
Fulton, F. (1958). *Advan. Virus Res.* **5**, 247.
Furumoto, W., and Wildman, S. G. (1963). *Virology* **20**, 53.
Gierer, A., and Schramm, G. (1956). *Nature* **177**, 702.
Ginoza, W., and Atkinson, D. E. (1955). *Virology* **1**, 253.
Ginoza, W., Atkinson, D. E., and Wildman, S. G. (1954). *Science* **119**, 269.
Giveon, T., and Wildman, S. G. (1962). *Biochim. Biophys. Acta* **55**, 988.
Goldberg, R. J. (1952). *J. Am. Chem. Soc.* **74**, 5715.
Goldberg, R. J. (1953). *J. Am. Chem. Soc.* **75**, 3127.
Goldberg, R. J., and Williams, J. W. (1953). *Discussions Faraday Soc.* **13**, 224.
Harrington, W. F., and Schachman, H. K. (1956). *Arch. Biochem. Biophys.* **65**, 278.

Harris, J. I., and Knight, C. A. (1952). *Nature* **170**, 613.
Harris, J. I., and Knight, C. A. (1955). *J. Biol. Chem.* **214**, 215.
Hart, R. G. (1955a). *Proc. Natl. Acad. Sci. U.S.* **41**, 261.
Hart, R. G. (1955b). *Virology* **1**, 402.
Hart, R. G. (1963). *Virology* **20**, 636.
Heidelberger, M., and Kendall, F. E. (1932). *J. Exptl. Med.* **55**, 555.
Heidelberger, M., and Kendall, F. E. (1935). *J. Exptl. Med.* **61**, 563.
Hitchborn, J. H., and Thomson, A. D. (1960). *Advan. Virus Res.* **7**, 163.
Holmes, K. C., and Franklin, R. E. (1958). *Virology* **6**, 328.
Jeener, R., and Lemoine, P. (1953). *Nature* **171**, 935.
Jeener, R., Lemoine, P., and Lavand'homme, C. (1954). *Biochim. Biophys. Acta* **14**, 321.
Jerne, N. K., and Avegno, P. (1956). *J. Immunol.* **76**, 200.
Kabat, E. A. (1961). "Experimental Immunochemistry," 2nd ed. Thomas, Springfield, Illinois.
Karush, F. (1962). *Advan. Immunol.* **2**, 2.
Kassanis, B. (1943). *Brit. J. Exptl. Pathol.* **24**, 152.
Kendall, F. E. (1942). *Ann. N.Y. Acad. Sci.* **43**, 85.
Kendrew, J. C., Bodo, G., Dintzis, H. M., Parrish, R. G., Wyckoff, H., and Phillips, D. C. (1958). *Nature* **181**, 662.
Kleczkowski, A. (1941). *Brit. J. Exptl. Pathol.* **22**, 44.
Kleczkowski, A. (1957). *J. Gen. Microbiol.* **16**, 405.
Kleczkowski, A. (1958). *Immunology* **1**, 36.
Kleczkowski, A. (1959). *Virology* **7**, 385.
Kleczkowski, A. (1961). *Immunology* **4**, 130.
Kleczkowski, A. (1963). *Biol. Rev. Cambridge Phil. Soc.* **38**, 364.
Klotz, I. M. (1953). *In* "The Proteins" (H. Neurath and K. Bailey, eds.), Vol. 1, Part B, p. 727. Academic Press, New York.
Klug, A., and Caspar, D. L. D. (1960). *Advan. Virus Res.* **7**, 225.
Klug, A., and Franklin, R. E. (1957). *Biochim. Biophys. Acta* **23**, 199.
Knight, C. A. (1946). *J. Exptl. Med.* **83**, 281.
Knight, C. A. (1955a). *J. Biol. Chem.* **214**, 231.
Knight, C. A. (1955b). *Virology* **1**, 261.
Knight, C. A. (1959). *In* "The Viruses" (F. M. Burnet and W. M. Stanley, eds.), Vol. 2, p. 127. Academic Press, New York.
Knight, C. A. (1961). *In* "Immunochemical Approaches to Problems in Microbiology" (M. Heidelberger and O. J. Plescia, eds.), p. 402. Rutgers Univ. Press, New Brunswick, New Jersey.
Knight, C. A., Silva, D. M., Dahl, D., and Tsugita, A. (1962). *Virology* **16**, 236.
Kroeger, A. V. (1962). *J. Immunol.* **89**, 136.
Lafferty, K. J. (1963a). *Virology* **21**, 61.
Lafferty, K. J. (1963b). *Virology* **21**, 76.
Lafferty, K. J., and Oertelis, S. (1963). *Virology* **21**, 91.
Landsteiner, K. (1947). "The Specificity of Serological Reactions" Harvard Univ. Press, Cambridge, Massachusetts.
Lanni, F., and Lanni, Y. T. (1953). *Cold Spring Harbor Symp. Quant. Biol.* **18**, 159.
Lapresle, C., Kaminski, M., and Tanner, C. E. (1959). *J. Immunol.* **82**, 94.
Lauffer, M. A. (1961). *In* "The Molecular Basis of Neoplasia" (15th Annual Symposium Fundamental Cancer Research, M.D. Anderson Hospital and Tumor Institute), p. 614. Univ. of Texas Press, Austin, Texas.

Lauffer, M. A., Ansevin, A. T., Cartwright, T. E., and Brinton, C. C., Jr. (1958). *Nature* **181**, 1338.
Lwoff, A., Horne, R., and Tournier, P. (1962). *Cold Spring Harbor Symp. Quant. Biol.* **27**, 51.
MacLeod, R., and Markham, R. (1963). *Virology* **19**, 190.
Malkiel, S. (1947). *J. Immunol.* **57**, 43.
Malkiel, S. (1948). *J. Immunol.* **60**, 255.
Malkiel, S. (1950). *J. Immunol.* **64**, 197.
Malkiel, S. (1952a). *J. Immunol.* **69**, 525.
Malkiel, S. (1952b). *J. Immunol.* **69**, 533.
Malkiel, S., and Stanley, W. M. (1947). *J. Immunol.* **57**, 31.
Mandel, B. (1960). *Ann. N.Y. Acad. Sci.* **83**, 515.
Mandel, B. (1961). *Virology* **14**, 316.
Mandel, B. (1962). *Cold Spring Harbor Symp. Quant. Biol.* **27**, 123.
Mandel, B. (1964). *Virology* **22**, 360.
Markert, C. L., and Apella, E. 1963). *Ann. N.Y. Acad. Sci.* **103**, 915.
Markham, R. (1959). *In* "The Viruses" (F. M. Burnet and W. M. Stanley, eds.), Vol. 2, p. 33. Academic Press, New York.
Markham, R., and Smith, K. M. (1949). *Parasitology* **39**, 330.
Markham, R., Matthews, R., and Smith, K. M. (1948). *Nature* **162**, 88.
Markham, R., Frey, S., and Hills, G. J. (1963). *Virology* **20**, 88.
Markham, R., Hitchborn, J. H., Hills, G. J., and Frey, S. (1964). *Virology* **22**, 342.
Marmur, J., Falkow, S., and Mandel, M. (1963). *Ann. Rev. Microbiol.* **17**, 329.
Mattern, C. F. T. (1962). *Virology* **17**, 76.
Matthews, R. E. F. (1957). "Plant Virus Serology." Cambridge Univ. Press, London and New York.
Matthews, R. E. F. (1958). *Virology* **5**, 192.
Merigan, T. C., and Dreyer, W. J. (1963). *Ann. N.Y. Acad. Sci.* **103**, 765.
Mills, J. A., and Haber, E. (1963). *J. Immunol.* **91**, 536.
Mulvania, M. (1926). *Phytopathology* **16**, 853.
Newmark, P., and Fraser, D. (1956). *J. Am. Chem. Soc.* **78**, 1588.
Nixon, H. L., and Woods, R. D. (1960). *Virology* **10**, 157.
Onoue, K., Yagi, Y., and Pressman, D. (1964). *J. Immunol.* **92**, 173.
Owen, R. D. (1959). *Proc. Intern. Congr. Genet., 10th, Montreal, 1958* Vol. 1, p. 364. Univ. of Toronto Press, Toronto.
Price, W. C. (1954). *Trans. N.Y. Acad. Sci.* **16**, 196.
Price, W. C. (1963). *Advan. Virus Res.* **10**, 171.
Purdy, H. A. (1929). *J. Exptl. Med.* **49**, 919.
Rappaport, I. (1957a). *J. Immunol.* **78**, 246.
Rappaport, I. (1957b). *J. Immunol.* **78**, 256.
Rappaport, I. (1959a). *J. Immunol.* **82**, 526.
Rappaport, I. (1959b). *Nature* **184**, 1732.
Rappaport, I. (1961a). *Biochim. Biophys. Acta* **47**, 206.
Rappaport, I. (1961b). *Nature* **189**, 986.
Rappaport, I., and Siegel, A. (1955). *J. Immunol.* **74**, 106.
Rappaport, I., and Wu, J. H. (1962). *Virology* **17**, 411.
Rappaport, I., Siegel, A., Owen, R. D., and Wildman, S. G. (1957). *J. Immunol.* **78**, 259.
Rappaport, I., Wildman, S. G., and Furumoto, W. (1964). *Virology* **23**, 389.
Rappaport, I., Siegel, A., and Haselkorn, R. (1965). *Virology* **25**, 325.
Reithel, F. J. (1963). *Advan. Protein Chem.* **18**, 123.

Rich, A., Dunitz, J. D., and Newmark, P. (1955). *Nature* **175**, 1074.
Sang, J. H., and Sobey, W. R. (1954). *J. Immunol.* **72**, 52.
Santilli, V., Piacitelli, J., and Wu, J. H. (1961). *Virology* **14**, 109.
Sarkar, S. (1960). *Z. Naturforsch.* **15b**, 778.
Scharff, M. D., Maizell, J. V., and Levintow, L. (1964). *Proc. Natl. Acad. Sci. U.S.* **51**, 329.
Schramm, G. (1947). *Z. Naturforsch.* **2b**, 112.
Schramm, G., and Friedrich-Freksa, H. (1941). *Z. Physiol. Chem.* **270**, 233.
Schramm, G., and Zillig, W. (1955). *Z. Naturforsch.* **10b**, 493.
Schramm, G., Schumacher, G., and Zillig, W. (1955a). *Z. Naturforsch.* **10b**, 481.
Schramm, G., Schumacher, G., and Zillig, W. (1955b). *Nature* **175**, 549.
Siegel, A., and Norman, A. (1958). *Virology* **6**, 725.
Siegel, A., and Wildman, S. G. (1954). *Phytopathology* **44**, 277.
Siegel, A., Ginoza, W., and Wildman, S. G. (1957). *Virology* **3**, 554.
Siegel, A., Zaitlin, M., and Sehgal, O. P. (1962). *Proc. Natl. Acad. Sci. U.S.* **48**, 1845.
Singer, S. J., and Campbell, D. H. (1955). *J. Am. Chem. Soc.* **77**, 3499.
Smyth, D. G., Stein, W. H., and Moore, S. (1963). *J. Biol. Chem.* **238**, 227.
Starlinger, P. (1955). *Z. Naturforsch.* **10b**, 339.
Stormont, C. (1955). *Am. Naturalist* **89**, 105.
Takahashi, W. N. (1955). *Virology* **1**, 393.
Takahashi, W. N. (1959a). *Virology* **9**, 437.
Takahashi, W. N. (1959b). *In* "Plant Pathology Problems and Progress, 1908–1958" (C. S. Holton, ed.), p. 493. Univ. of Wisconsin Press, Madison, Wisconsin.
Takahashi, W. N., and Gold, A. H. (1960). *Virology* **10**, 449.
Takahashi, W. N., and Ishii, M. (1952a). *Nature* **169**, 419.
Takahashi, W. N., and Ishii, M. (1952b). *Phytopathology* **42**, 690.
Takahashi, W. N., and Ishii, M. (1953). *Am. J. Bacteriol.* **40**, 85.
Tall, M. G., Price, W. C., and Wartman, K. (1949). *Phytopathology* **39**, 288.
Talmage, D. W., and Cann, J. R. (1961). "The Chemistry of Immunity in Health and Disease." Thomas, Springfield, Illinois.
Tsugita, A. (1962). *J. Mol. Biol.* **5**, 293.
Tsugita, A., and Fraenkel-Conrat, H. (1960). *Proc. Natl. Acad. Sci. U.S.* **46**, 636.
Tsugita, A., and Fraenkel-Conrat, H. (1962). *J. Mol. Biol.* **4**, 73.
Tyrrell, D. A., and Horsfall, F. L. (1953). *J. Exptl. Med.* **97**, 845.
van Regenmortel, M. H. V. (1963). *Virology* **21**, 657.
van Rysselberge, C., and Jeener, R. (1955). *Biochim. Biophys. Acta* **17**, 158.
van Rysselberge, C., and Jeener, R. (1957). *Biochim. Biophys. Acta* **23**, 18.
van Slogteren, E., and van Slogteren, D. H. M. (1957). *Ann. Rev. Microbiol.* **2**, 149.
Weaver, E. P., and Price, W. C. (1952). *Proc. Soc. Exptl. Biol. Med.* **79**, 125.
Wildman, S. G., and Bonner, J. (1947). *Arch. Biochem.* **14**, 381.
Wildman, S. G., Bonner, J., and Cheo, C. (1949). *J. Biol. Chem.* **180**, 985.
Wittmann, H. G. (1963). *In* "Informational Macromolecules" (H. J. Vogel, V. Bryson, and J. O. Lampen, eds.), p. 177. Academic Press, New York.
Wright, N. S. (1963). *Virology* **20**, 131.
Wu, J. H., and Rappaport, I. (1961). *Virology* **14**, 259.
Wu, J. H., and Rappaport, I. (1962). *Nature* **193**, 908.
Wu, J. H., Hudson, W., and Wildman, S. G. (1962). *Phytopathology* **52**, 1264.
Zaitlin, M., and Ferris, W. R. (1964). *Science* **143**, 1451.

ARTHROPOD TRANSMISSION OF ANIMAL VIRUSES

Herbert S. Hurlbut* †

Naval Medical Research Institute, Bethesda, Maryland

I. Introduction 277
II. Arthropod Infection 278
A. Portal of Entry 278
B. Sequence of Tissues Infected 280
C. Exit Portals 281
III. Vertebrate Infection 282
A. Primary Site of Infection 282
B. Viremia 283
IV. Effects of Alternation of Hosts 284
A. General Considerations 284
B. Temperature Relationships 285
C. Central Nervous System Infections 285
D. Host Range Potential 285
V. Interhost and Environmental Relationships 285
A. General Considerations 285
B. Primary Transmission Cycles 286
C. Epidemic and Epizootic Cycles 286
D. Anopheline-Transmitted Viruses 287
E. Birds and Their Ectoparasites as Agents of Dispersal 287
F. Infection Rates and Transmission Rates in Arthropods 288
G. Feeding Habits and Winter Survival of Mosquito Vectors 288
References 289

I. Introduction

This review is limited to the arboviruses with particular attention being given to arthropod infection and those aspects of vertebrate infection closely related to transmission by arthropods. The reader seeking more complete treatment will find a comprehensive review of the mechanisms of transmission of viruses by arthropods (Day, 1955); a more recent review of this subject limited to mosquitoes (Chamberlain and Sudia, 1961); a general treatment of the arboviruses (Kissling, 1960); the epidemiology and ecology of the arthropod-borne encephali-

* The opinions or assertions contained herein are the private ones of the author and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large.

† From the Bureau of Medicine and Surgery, Navy Department, Research Task MR 005.09-1200.01.

tides (Miles, 1960, 1964); a symposium on the arboviruses (Darlington *et al.*, 1960); a symposium on the biological transmission of disease agents (Maramorosch, 1962); a symposium on the biology of the tick-borne encephalitis complex (Libíková, 1962); a consideration of arthropod vectors as reservoirs of microbial disease agents (Philip and Burgdorfer, 1961); the arthropod transmission of plant viruses (Maramorosch, 1963); a seminar on arboviruses (Bruno-Lobo and Shope, 1963); and the behavior of viruses in their arthropod hosts (Smith, 1964).

II. Arthropod Infection

A. *Portal of Entry*

1. *Feeding Process*

The structure of the blood-sucking apparatus of the female mosquito is described by Christophers (1960) and Clements (1963). Griffiths and Gordon (1952) observed the feeding process. The cutting stylets penetrate about 2 mm. into the skin and after penetration are capable of exploratory movements by the action of the cutting elements. When a capillary is found, blood is obtained either by inserting the parts into a capillary or by pool feeding after the capillary wall is cut. The food channel is about 0.03 mm. in diameter, and 2–4 $mm.^3$ of blood are taken in about 2 minutes.

The structure of the feeding apparatus of the ticks has been described in recent years by Arthur (1952) and Gregson (1960). Descriptions of the feeding process have been presented by Lavoipierre and Riek (1955) and Gregson (1960). Because of the size and structure of the mouth parts, there is more extensive laceration and hemorrhage than with the mosquito. Blood collects in the lacerated area beneath the surface and is sucked up. Argasid ticks complete their feeding in from 20 minutes to 3 hours, depending upon the species. Ixodid ticks remain firmly attached and feed at a slower rate for several days. The quantity of blood taken is many times the body weight, which varies greatly according to the stage of development of the tick.

The feeding processes of *Phlebotomus* and *Culicoides* apparently have not been observed, although accounts for the triatomid bugs and the flea are available (Lavoipierre *et al.*, 1959; Lavoipierre and Hamachi, 1961).

2. *Digestion*

Digestion in the mosquito proceeds rapidly and is virtually completed in 48 hours at 25°C. (Christophers, 1960). Some of the fluid is absorbed

during and directly after feeding and may be seen as clear droplets passed anally at these times. Boorman (1960a) did not detect Semliki Forest (SF) virus in the clear material but recovered virus in some samples that were red from blood cells being passed through the gut of *Aedes aegypti* after infective feedings. Of particular interest is the observation that virus was present in the hemolymph 10 minutes after blood feeding (Boorman, 1960b). The author suggested that this may be the source of subsequent tissue infection. The stomach contents during digestion exhibit stratification indicating that digestion takes place at the stomach wall surface. At 24 hours there is a central mass of unmodified blood, at 37 hours blood cells can no longer be detected (Christophers, 1960). Tests of susceptibility to virus and digestion of blood by feeding artificial mixtures through membranes should be interpreted with caution because of the possibility of altering the natural process significantly. Bertram and Bird (1961) have investigated the fine structure of the midgut epithelium. They report no zonal segregation of cells and consider a single cell type probable. The peritrophic membrane is formed *in situ* by the cells of the posterior midgut epithelium, being at first an amorphous matrix which consolidates during the next 24 hours after the blood meal. These authors call attention to the complexity of cell structure and activity during digestion. They detected no differences when SF virus was present.

Digestion in the tick proceeds more slowly. It is of the primitive intracellular type that is accompanied by rapid proliferation of digestive cells that protrude into the lumen of the gut. The lumen of the gut may be partly filled with such cells in unfed ticks, and when blood is taken these cells slough off. In argasids the epithelium is rapidly re-formed at this time (Tatchell, 1964); in ixodids sloughing of some and flattening of the remaining cells takes place as the gut distends, followed by rapid proliferation of epithelial cells (Hughes, 1954). Hemolysis of the blood cells is seen and digestion follows. In argasids, digestion is at first relatively rapid, lasting 1–2 weeks; then a slow phase begins, the rate remaining constant until the next blood meal. This provides a food reserve in argasids in the absence of a fat body (Tatchell, 1964). Coxal fluid is produced during and soon after detachment in argasids to eliminate excess water, but in ixodids coxal glands are absent and fluid is eliminated through the integument and in lesser quantities with the feces during feeding (Edney, 1957).

Balashov (1957) emphasizes the marked cyclicity in the functioning of the digestive epithelium of ticks associated with blood intake. This cyclicity extends in sequence to other tissues and organ systems in relation to growth and egg development. In view of the great proliferation of cells that takes place, arbovirus infections may be influenced by these events.

B. Sequence of Tissues Infected

1. Mosquito

LaMotte (1960) fed *Culex pipiens* on viremic chicks and followed the sequence of tissues infected with Japanese encephalitis (JE) virus. Titers were at the maximum in the midgut wall between the first and third weeks and declined between the third and fourth weeks (27°C.). During the second week virus appeared in the hemolymph, reaching maximum levels between the second and fourth weeks and declining during the fourth week. General tissue invasion took place during the viremic period. The ovaries contained virus at the end of the second week, reached a maximum titer during the third week, and then declined. Virus appeared in the salivary glands and cephalic and thoracic ganglia during the second week, with maximum levels developing in the third week. It persisted in these sites in high concentrations throughout the remaining life of the insect. In each of the tissues mentioned, maximum virus content ranged from 10^4 to 10^5 mouse LD_{50}. No cell damage could be found by the usual histological methods, and the length of life of the insect was apparently not affected. It would be of interest to follow the virus in those sites of persistent infection by the techniques now available, such as fluorescent antibody, and also search other tissues for concentrations of particles noninfectious in the usual test systems.

2. Tick

Pavlovskii and Solovyev (1940) recovered the virus of tick-borne encephalitis (TBE) from the intestinal tract of *Ixodes persulcatus* in the highest concentration (10^6 mouse LD_{50}) of all tissues tested during 25 days following the infectious feeding. The next highest concentrations were in the salivary glands and ovaries and small amounts in the Malpighian tubules and the central nervous system. No virus was found in the fat body. This interesting early study apparently has not been followed up to determine more precisely tissue invasion sequences and virus concentrations under varying conditions. Transtadial passage as well as the long duration of infections in ticks have been reported by numerous authors: recently for TBE virus (Benda, 1958); Kyasanur forest disease (KFD) virus (Varma *et al.*, 1960; Singh *et al.*, 1963); Colorado tick fever (CTF) virus (Burgdorfer, 1959). Benda (1958) observed that the amount of virus in the tick tended to decrease transtadially unless the tick was fed upon a virus-susceptible animal. Apparently the prolonged feeding period of ixodid ticks permits the vertebrate to become viremic and feed back virus to the vector tick. The great proliferation of tick gut cells during feeding and digestion, and the importance of the gut as a site of virus infection have been pointed out. Further detailed study of these processes should yield important information in the mechanisms involved.

C. Exit Portals

1. Oral

The salivary glands of *A. aegypti* show evidence of cyclic change correlated with blood-feeding activity and subsequent digestion and absorption (Orr, *et al.*, 1961). Discharge of the salivary secretion of this mosquito during feeding has been described by Griffiths and Gordon (1952). The fluid can be seen being ejected from the end of the hypopharynx at short intervals during the exploratory movements after the stylets have penetrated the epidermis and before a capillary is pierced. Volumes of saliva have not been measured nor have virus concentrations been precisely determined. LaMotte (1960) found that the salivary glands of laboratory-infected *C. pipiens* contained 10,000 to 100,000 mouse LD_{50} of JE virus, and Collins (1963), using a membrane-feeding technique, found that *Anopheles albimanus* expelled from 1,500–10,000 mouse LD_{50} of SF virus. Thomas (1963) estimated that the salivary glands of *Culex tarsalis* weigh about 0.003 mg., and that glands infected with Western equine encephalitis (WEE) virus contained from 1000 to 2500 suckling mouse LD_{50}. Cell penetration at the site of injection would be favored by such a high concentration of virus even in the presence of antibody. Results obtained from infections established artificially by intracutaneous injection with a conventional needle should be interpreted with caution.

A systematic study of the salivary secretions of mosquitoes in relation to arbovirus infections might yield important information. Such determinations as the amount of virus per unit volume and factors governing the amount of saliva expelled would be useful. The effect of salivas of different species upon arboviruses would also be of interest since these differ in the presence or absence of anticoagulins and agglutinins, and therefore, perhaps, in other respects (Clements, 1963).

Lavoipierre and Riek (1955) and Gregson (1960) observed the expulsion of salivary fluid of ticks during feeding. It is seen as a puff of clear fluid being expelled into the lacerated area below the skin surface as alternate blood-sucking and salivation proceed. The concentration of virus in the saliva has not been measured. Transmission of virus during tick bite has been reported by numerous authors. A recent report is given by Benda (1958) of TBE virus transmission by *Ixodes ricinus*, by Varma *et al.* (1960) for KFD virus, and by Burgdorfer (1959) for CTF virus. The sequence of events leading to salivary gland infection have not been recorded nor have the effects of cyclic glandular activity on virus concentration been investigated.

2. *Anal*

The feces of *I. ricinus*, experimentally infected with the virus of TBE, contain virus (Benda, 1958). Danes *et al.* (1962) and Benda *et al.* (1962) infected experimental animals with aerosols derived from TBE virus-infected mouse brain, but similar tests with tick feces have not been reported. The excreted virus may also enter the host through the lesion made by the biting tick.

3. *Osmoregulatory Portals*

Coxal fluid secreted by *Ornithodoros moubata*, experimentally infected with West Nile (WN) virus, has been shown to contain virus (Whitman and Aitken, 1960).

4. *Genital Portals*

The offspring of female ticks infected with TBE virus are infected in varying proportions (Benda, 1958). The factors influencing transovarial transmissions are not understood (Blaskovic and Rehacek, 1962), although high titer with a suitable strain of virus at the time the eggs are formed seems to be important. Eklund *et al.* (1962) failed to find evidence of transovarial transmission of CTF virus in field and laboratory studies. Pavlovskii and Solovyev (1941) record the isolation of TBE virus from Gene's organ the secretion of which coats the eggs at the time they are deposited. Virus in the seminal fluid has not been reported. Transovarial transmission in the mosquito appears to be a rare event. St. Louis encephalitis (SLE) virus has been recovered from eggs deposited by infected *Culex quinquefasciatus*, but rarely from the resulting larvae and never from the adult offspring (Chamberlain *et al.*, 1964). However, JE virus may be present in high concentration in the ovaries of experimentally infected *C. quinquefasciatus* (LaMotte, 1960).

III. Vertebrate Infection

A. Primary Site of Infection

The primary site of infection is not known. However, after intraplantar injection, Malkova and Frankova (1959) found TBE virus concentrated first in the lymph nodes of infected mice followed by a rise in titer in the blood. Further studies in mice demonstrated the initial virus concentration to be in the lymph nodes associated with the site of inoculation and, in immunized animals, arrest of spread of virus beyond this point was observed (Malkova and Kolman, 1964). Han and Pogodina (1964), using fluorescent antibody technique, saw evidence of multiplication at the site of inoculation when mice, hamsters, and pigs were injected subcutaneously with TBE virus. Kundin *et al.* (1963) studied the distribution of WN virus in experimentally infected mice and chicks by means of fluorescent antibody. Antigen was seen in the muscles of veins and arteries and in other tissues. The mice were injected intraperitoneally and intracerebrally and the chicks subcutaneously. Huang and Wong (1963) distinguished a visceral stage of infection with JE virus in peripherally injected mice preceding the nervous system invasion, which they suggest is by way of capillary endothelium. Hurlbut (1964) detected low level viremia in swine infected with JE virus by mosquito bite, even in the presence of appreciable amounts of circulating antibody, and suggested that establishment of infection at the site of inoculation would be favored by the high concentration of virus in the mosquito saliva and the way of introduction of the saliva into the tissues. For valid observations on the natural sequence of events in the vertebrate infection, it may be necessary to start with the bite of the infected arthropod. Serial boosting of immunity in nature by yearly reinfections may also be influenced by these circumstances.

B. Viremia

1. *Acute Infection*

The duration and level of viremia during the acute infection of the vertebrate host must be related to arthropod susceptibility when evaluating vector efficiency (Chamberlain and Sudia, 1961). In cases of high level viremia mechanical transmission of virus from the contaminated mouth parts of mosquitoes has been observed under experimental conditions with Eastern equine encephalitis (EEE) virus by Chamberlain and Sudia (1961), WEE virus by Barnett (1956), and SF virus by Woodall and Bertram (1959). It seems to be related to the high concentration of virus encountered with certain group A viruses. Failures are reported in experiments with JE virus and Murray Valley encephalitis (MVE) virus (Gould *et al.*, 1962; Altman, 1963). However, mechanical transmission of TBE virus by mosquitoes and ticks has been reported (Zoltowski and Wroblewska-Mularczykowa, 1961; Blaskovic and Rehacek, 1962). At times, when mosquitoes or other blood-sucking

Diptera are extremely numerous in nature, mechanical transmission might contribute to explosive dissemination of a virus. In restricted environments, such as bird and rodent nests where fleas and mites become numerous, this method of virus transfer might occur.

2. Latent Infections

Experimental latent infections with recurrent viremia have been observed in bats (LaMotte, 1958; Nosek *et al.*, 1961); in hedgehogs and dormice (Kozuch *et al.*, 1963); and in reptiles (Thomas *et al.*, 1958; Thomas and Eklund, 1960, 1962; Gebhardt and Hill, 1960; Gebhardt *et al.*, 1964; Hayes *et al.*, 1964). The recurrent viremias, including infections with JE, TBE, WEE, and EEE viruses, were associated with emergence from hibernation and in some cases were of a level that permitted infection of blood-sucking arthropods. Gebhardt *et al.* (1964) recovered WEE virus from naturally infected snakes under circumstances that indicate overwintering of the virus in that host. Sulkin *et al.* (1963) observed experimental infections with SLE and JE viruses in bats, characterized by viremias of 15–30 days' duration and high concentrations of virus in the central nervous system without evidence of cell damage or encephalitis. Reeves *et al.* (1958a) recovered WEE virus from the tissues of eight species of wild birds 1 to 10 months after inoculation. Thus, evidence is accumulating that vertebrates experience latent infections with these viruses, associated in many cases with reduced body temperature.

IV. Effects of Alternation of Hosts

A. General Considerations

The transmission of arboviruses by arthropods to mammals and birds and back to arthropods brings about a cycle of alternation of temperature. It also alternates parenteral and enteric portals of entry. The humoral environments are also very different both chemically and probably as to defense mechanisms. Day (1957) pointed out that the regular alternation of dissimilar hosts must exercise a more stringent selection than a simple host system. Thus, a greater stability may be imposed upon populations of these viruses by the greater complexity of the environment. A highly variable strain perhaps would not be at a particular disadvantage, but variants would be established with greater difficulty. Measurement of the dynamic tensions due to mutation rates within such systems would be of interest. The taxonomic structure of the arbovirus group as it is now emerging, with distinguishable primary and secondary subgroupings, may be related to the greater stability enforced by the alternation of hosts.

B. Temperature Relationships

Smith (1960) discusses the effects of temperature and mentions the observation of Reeves *et al.* (1958b) that WEE virus isolated from wintering *C. tarsalis* appears to be attenuated. Smith suggests that low temperature in the mosquito may select a less pathogenic strain.

The transmission of tick-borne viruses introduces a different pattern of events in the alternation of hosts. For example, body heat of mammalian and avian hosts is transmitted to the ixodid tick during the several days of its attachment, providing an arthropod temperature above the ambient conditions. Moreover, the prolonged period of blood-sucking permits a feedback of virus in cases where a viremia develops, thus renewing the arthropod infection (Benda, 1958). The argasid ticks present a still different pattern since their feeding time on the host is very short, except the larvae which remain attached for several days.

C. Central Nervous System Infections

Central nervous system concentrations of virus in experimental arthropod infections have been reported as follows: TBE virus in a tick (Pavlovskii and Solovyev, 1941); JE virus in a mosquito (LaMotte, 1960); WEE virus in a grasshopper; SF virus in a housefly; WN virus in a beetle larva; and SLE virus in a blowfly (Hurlbut and Thomas, 1960). Comparative studies to determine whether central nervous system invasion is a part of the natural infection of vectors would be of interest.

D. Host Range Potential

Certain arboviruses when introduced parenterally will propagate in a wide variety of arthropods; others are very restricted in this capacity and some are intermediate (Hurlbut and Thomas, 1960). The Group B viruses, in particular, exhibit such a gradient and there is some indication that a similar characteristic exists in their capacity to infect vertebrates. For example, JE and SLE viruses have a broader range than the dengue viruses. The factors governing host range are not understood at the present time.

V. Interhost and Environmental Relationships

A. General Considerations

The literature on the field ecology of arboviruses has been reviewed in the papers cited in the introduction of this review. Here, attention will be given only to more recent work. It is convenient to divide it under several headings according to aspects that have received particular attention.

Arboviruses are usually isolated during their epidemic or epizootic manifestations. These manifestations are relatively brief and the question of how the virus is maintained at other times is left unanswered. This question is most vexing in temperate climates where winter interrupts the activity of arthropod vectors. Winter survival of virus in tick vectors occurs, but mosquito vectors, short-lived and wintering in relatively small numbers, are less plausible agents of survival. Latent infection in a vertebrate is an alternate answer. In the tropics, continuous cycles of mosquito-borne virus probably occur where favorable conditions of rainfall and host availability exist.

Primary cycles of transmission may be defined as those in which the element of continuity predominates in a stable environment. Epidemic and epizootic cycles may be thought of as being derived from primary cycles and having the predominant element of explosive dissemination of the virus.

B. Primary Transmission Cycles

Burgdorfer and Eklund (1959) studied the ecology of CTF virus in rodents and the ticks that infest them. The golden mantled ground squirrel was most commonly infected and was a favored host of the tick vector, *Dermacentor andersoni*, from which the virus was also isolated. The distribution of cases of CTF paralleled the distribution of this rodent. Downs *et al.* (1962), Aitken *et al.* (1963), and Worth (1963) and Causey (1963) have studied virus isolations and interhost relationships under tropical conditions where virus circulates in apparently continuous cycles between mosquitoes and rodents. This was accomplished by the capture, release, and recapture of wild rodents, and by the use of sentinel animals and the capture of mosquitoes that fed upon them. Blaskovic (1963) has reviewed recent work on natural cycles of TBE virus.

C. Epidemic and Epizootic Cycles

A series of papers by Scherer *et al.* (1959) on ecological studies of JE virus in Japan presents evidence of cycles in wild birds and domestic pigs associated with occurrence of the human disease. These authors distinguish a pre-emergence period, April–June, when the virus is not ordinarily detected. This period terminates when virus is found in the vector mosquito in late June, when vector populations reach maximum levels, and a period of virus dissemination through the wild bird, pig, and human populations follows. A period of decline begins in late September with the disappearance of the vector. While pigs could be immunized, as a control measure, it was thought that this would be ineffective since birds would remain as a major source of virus during the epidemic season. It was concluded that man can be protected most effectively by immunization if a satisfactory vaccine can be developed. Grayston *et al.* (1962) published a series of papers giving detailed information on the ecology of this virus in Taiwan. Swine are commonly infected, as in Japan, but the extent of avian involvement is not so well defined. Virus isolations were made from *Culex tritaeniorhynchus* most commonly in June and July. Confirmed human cases of encephalitis followed 1 to 2 weeks later. Sun (1964) reported that the vector population in Taiwan exhibits a yearly midsummer peak that is part of a seasonal succession of mosquito species. Hurlbut (1964) isolated JE virus from naturally infected domestic pigs in Taiwan, determined the concentration of virus present in the circulating blood and the percentage of mosquitoes becoming infected that fed upon them. He considered pigs to be the most important source of virus during the summer epidemics. According to laboratory experiments, a log neutralization index between 1.0 and 1.9 in pigs would keep the viremia below a significant mosquito-infecting level.

Records of overt disease in wild vertebrates caused by arboviruses have been summarized by Work (1963). These have, on occasion, directed attention to silent natural cycles as well as to unusual manifestations of infection.

D. Anopheline-Transmitted Viruses

The recent recognition of anopheline mosquitoes as vectors of arboviruses is an important new development. O'nyong-nyong fever virus caused an extensive epidemic in East Africa in 1959. Isolations of virus were made from *Anopheles funestus* and *A. gambiae*, the probable vectors (Haddow *et al.*, 1960). Tensaw virus, a new agent of the Bunyamwera group, was isolated from *A. crucians* in 1962, and experimental transmission tests indicate that this virus is readily transmitted by an anopheline mosquito. Rabbits and dogs are probable hosts; birds are refractory (Chamberlain, 1963). Chamberlain points out that a number of viruses of the Bunyamwera group have been isolated from, and may prove to be transmitted by, anopheline mosquitoes.

E. Birds and Their Ectoparasites as Agents of Dispersal

Work on water birds infected with tick-borne encephalitis virus has been summarized by Ernek (1963). Federov (1958) examined 39 species of wild birds and isolated 6 strains of tick-borne encephalitis virus from the birds and 3 from ticks (*Ixodes persulcatus*) removed from them. Hoogstraal *et al.* (1961, 1963, 1964) made extensive surveys in Egypt of ticks on migrating birds from Africa, Europe, and Asia. Ticks and infested birds were identified and rates of infestation recorded together with detailed accounts of known ranges of the birds and ticks. The thrush nightingale (*Luscinia luscinia*) was commonly infested with *I. ricinus*. This bird breeds from Eastern Europe to Central Asia and winters in East Africa. Stamm and Newman (1963) reported viruses of EEE and WEE viruses in 9% of southward migrating birds caught in southern Alabama between September 16 and October 2, 1960, and considered the probability of virus transport by viremic birds to Central America. It was concluded that this occurs although the frequency of the occurrence cannot be estimated at this time.

F. Infection Rates and Transmission Rates in Arthropods

Reeves *et al.* (1961) compared rates of infection and transmission of WEE virus by *C. tarsalis* captured in traps while seeking and taking blood from a susceptible chicken. The infection rates averaged about 4 times higher than the transmission rates. The results suggested that relatively few mosquitoes survive more than one cycle of blood feeding and oviposition. It was demonstrated that the risk of infection was higher for single chickens in a trap than for individuals in a flock. Another report (Reeves *et al.*, 1964) gave evidence that temperature is an important factor in the development of high transmission rates of WEE virus. Hess *et al.* (1963) reported on the relationship between temperature and antibody rates for WEE and SLE viruses in sentinel flocks of domestic chickens exposed during one transmission season.

G. Feeding Habits and Winter Survival of Mosquito Vectors

Reeves *et al.* (1963) reported on the feeding habits of *C. tarsalis* around farm yards in Kern County, California (Lat. 35° N.), based upon improved techniques of producing precipitating antisera in birds (Tempelis and Reeves, 1962; Tempelis and Lofy, 1963). Screening antisera for identifying the blood of birds, mammals, reptiles, and amphibia, and more specific antisera for the common orders and families of vertebrates were used in capillary tube tests on the contents of mosquito stomachs. In summer, 84.4% had fed on birds and most of the remainder on various mammals. In winter, the increased number feeding upon passerine birds was thought to be due to greater availability. Latent infections with WEE virus have been experimentally produced in passerine birds (Reeves *et al.*, 1958a).

Reeves *et al.* (1958b) and Bellamy and Reeves (1963) observed marked reduction in the number of blood-fed *C. tarsalis* in natural resting places in late autumn in Kern County. Fat bodies were enlarged and ovaries undeveloped. WEE virus was isolated from the mosquitoes in October and November but not in December. In January, blood-fed mosquitoes again became numerous and virus isolations could again be made. Burdick and Kardos (1963) and Nelson (1964) studied the seasonal age distribution of *C. tarsalis* populations in the same locality by means of both tracheation and dilatation techniques and found that nulliparous females with enlarged fat bodies predominate from October to January with a transitional period starting in September. Gonotrophic dissociation could not be demonstrated in the laboratory and it was assumed that blood feeding ceased and fat accumulation was the result of a carbohydrate diet (Bellamy and Reeves, 1963). Bennington *et al.* (1958) reported similar findings in Colorado (Lat. 40° N.). Rush *et al.* (1963) reported failure to isolate the virus of WEE from overwintering *C. tarsalis* in Oregon (Lat. 44° N.). Harwood and Halfhill (1964) found that a photoperiod of 8 hours gave larger fat bodies and smaller ovaries than a 16-hour period in a laboratory colony of *C. tarsalis* fed 10% sucrose and held at 22°C. However, the experimentally induced fat body never equaled in size and compactness that seen in naturally hibernating *C. tarsalis*.

References

Aitken, T. H. G., Jonkers, A. H., and Worth, C. B. (1963). *Anais Microbiol. Univ. Brasil* **11A**, 67–77.

Altman, R. M. (1963). *Am. J. Trop. Med. Hyg.* **12**, 425–434.

Arthur, D. R. (1951). *Parasitology* **41**, 66–81.

Arthur, D. R. (1952). *Parasitology* **42**, 187–191.

Balashov, Yu. S. (1957). *Otd. Parazitol. Sborn.* **17**, 137–167.

Barnett, H. C. (1956). *Am. J. Trop. Med. Hyg.* **5**, 86–98.

Bellamy, R. E., and Reeves, W. C. (1963). *Ann. Entomol. Soc. Am.* **56**, 314–323.

Benda, R. (1958). *J. Hyg. Epidemiol. Microbiol. Immunol. (Prague)* **2**, 314–344.

Benda, R., Fuchsová, M., and Danes, L. (1962). *Acta Virol. (Prague)* **6**, 46–52.

Bennington, E. E., Sooter, C. A., and Baer, H. (1958). *Mosquito News* **18**, 299–304.

Bertram, D. S., and Bird, R. G. (1961). *Trans. Roy. Soc. Trop. Med. Hyg.* **55**, 404–423.

Blaskovic, D. (1963). *Anais Microbiol. Univ. Brasil* **11A**, 115–118.

Blaskovic, D., and Rehacek, J. (1962). "Biological Transmission of Disease Agents" (K. Maramorosch, ed.), p. 135. Academic Press, New York.

Boorman, J. P. T. (1960a). *Ann. Trop. Med. Parasitol.* **54**, 8–14.

Boorman, J. P. T. (1960b). *Trans. Roy. Soc. Trop. Med. Hyg.* **54**, 362–365.

Bruno-Lobo, M., and Shope, R., eds. (1963). Seminar on Arboviruses, Seventh International Congresses on Tropical Medicine and Malaria, Rio de Janeiro. *Anais Microbiol. Univ. Brasil* **11A**, 1–293.

Burdick, D. J., and Kardos, E. H. (1963). *Ann. Entomol. Soc. Am.* **56**, 527–535.
Burgdorfer, W. (1959). *J. Infect. Diseases* **104**, 101–104.
Burgdorfer, W., and Eklund, C. M. (1959). *Am. J. Hyg.* **69**, 127–137.
Causey, C. E. (1963). *Anais Microbiol. Univ. Brasil* **11A**, 119–121.
Chamberlain, R. W. (1963). *Anais Microbiol. Univ. Brasil* **11A**, 89–93.
Chamberlain, R. W., and Sudia, W. D. (1961). *Ann. Rev. Entomol.* **6**, 371–390.
Chamberlain, R. W., Sudia, W. D., and Gogel, R. H. (1964). *Am. J. Hyg.* **80**, 254–265.
Christophers, S. R. (1960). "*Aedes aegypti* (L) The Yellow Fever Mosquito: Its Life History, Bionomics and Structure." Cambridge Univ. Press, London and New York.
Clements, A. N. (1963). "The Physiology of Mosquitoes." Macmillan (Pergamon), New York.
Collins, W. E. (1963). *Mosquito News* **23**, 96–99.
Danes, L., Libich, J., and Benda, R. (1962). *Acta Virol. (Prague)* **6**, 37–45.
Darlington, C. D., Mattingley, P. F., and Smith, C. E. G. (1960). *Trans. Roy. Soc. Trop. Med. Hyg.* **54**, 89–134.
Day, M. F. (1955). *Exptl. Parasitol.* **4**, 387–418.
Day, M. F. (1957). *Trans. N. Y. Acad. Sci.* **19**, 244–251.
Downs, W. G., Spense, L., and Aitken, T. H. G. (1962). *Am. J. Trop. Med. Hyg.* **11**, 841–843.
Edney, E. B. (1957). "The Water Relations of Terrestrial Arthropods." Cambridge Univ. Press, London and New York.
Eklund, C. M. Kohles, G. M., and Kennedy, R. C. (1962). "Biology of Viruses of the Tick-Borne Encephalitis Complex" (H. Libíková, ed.), pp. 401–406. Academic Press, New York.
Ernek, E. (1963). *Anais Microbiol. Univ. Brasil* **11A**, 149–151.
Fedorov, Yu. V. (1958). *Tr. Tomskogo Nauchn.-Issled. Inst. Vaktsin Syvorotok. Min. Zdravookh. SSSR* **9**, 27–32.
Gebhardt, L. P., and Hill, D. W. (1960). *Proc. Soc. Exptl. Biol. Med.* **104**, 695–698.
Gebhardt, L. P., Stanton, G. J., Hill, D. W., and Collett, G. C. (1964). *New Engl. J. Med.* **271**, 172–177.
Gould, D. J., Barnett, H. C., and Suyemoto, W. (1962). *Trans. Roy. Soc. Trop. Med. Hyg.* **56**, 429–435.
Grayston, J. T., Wang, S. P., Hu, S. M. K., Chu, I. H., and Yen, C. H. (1962). *Am. J. Trop. Med. Hyg.* **11**, 126–161.
Gregson, J. D. (1960). *Acta Trop.* **17**, 49–79.
Griffiths, R. B., and Gordon, R. M. (1952). *Ann. Trop. Med. Parasitol.* **46**, 311–319.
Haddow, A. J., Davies, C. W., and Walker, A. J. (1960). *Trans. Roy. Soc. Trop. Med. Hyg.* **54**, 517–522.
Han, S. G., and Pogodina, V. V. (1964). *Acta Virol. (Prague)* **8**, 22–29.
Harwood, R. F., and Halfhill, E. (1964). *Ann. Entomol. Soc. Am.* **57**, 596–600.
Hayes, R. O., Daniels, J. B., Maxfield, H. K., and Wheeler, R. E. (1964). *Am. J. Trop. Med. Hyg.* **13**, 595–606.
Hess, A. D., Cherubin, C. E., and LaMotte, L. C. (1963). *Am. J. Trop. Med. Hyg.* **12**, 657–667.
Hoogstraal, H., Kaiser, M. N., Traylor, M. A., Gaber, S., and Guindy, E. (1961). *Bull. World Health Organ.* **24**, 197–212.

Hoogstraal, H., Kaiser, M. N., Traylor, M. A., Guindy, E., and Gaber, S. (1963). *Bull. World Health Organ.* **28**, 235–262.
Hoogstraal, H., Traylor, M. A., Gaber, S., Malabatis, G., Guindy, E., and Helmy, I. (1964). *Bull. World Health Organ.* **30**, 355–367.
Huang, C. H., and Wong, C. (1963). *Acta Virol. (Prague)* **7**, 322–330.
Hughes, T. E. (1954). *Ann. Trop. Med. Parasitol.* **48**, 397–404.
Hurlbut, H. S. (1964). *J. Med. Entomol.* **1**, 301–307.
Hurlbut, H. S., and Thomas, J. I. (1960). Virology **12**, 391–407.
Kissling, R. E. (1960). *Ann. Rev. Microbiol.* **14**, 261–282.
Kozuch, O., Nosek, J., Ernek, E., Lichard, M., and Albrecht, P. (1963). *Acta Virol. (Prague)* **7**, 430–433.
Kundin, W. D., Liu, C., Hysell, P., and Hamachige, S. (1963). *Arch. Ges. Virusforsch.* **12**, 514–536.
LaMotte, L. C., Jr. (1958). *Am. J. Hyg.* **67**, 101–108.
LaMotte, L. C., Jr. (1960). *Am. J. Hyg.* **72**, 73–87.
Lavoipierre, M. M. J., and Hamachi, M. (1961). *Nature* **192**, 998–999.
Lavoipierre, M. M. J., and Riek, R. F. (1955). *Ann. Trop. Med. Parasitol.* **49**, 96–113.
Lavoipierre, M. M. J., Dickerson, G., and Gordon, R. M. (1959). *Ann. Trop. Med. Parasitol.* **53**, 235–250.
Libíková, H., ed. (1962). "Biology of Viruses of the Tick-Borne Encephalitis Complex." Academic Press, New York.
Malkova, D., and Frankova, V. (1959). *Acta Virol. (Prague)* **3**, 210–214.
Malkova, D., and Kolman, J. M. (1964). *Acta Virol. (Prague)* **8**, 10–13.
Maramorosch, K., ed. (1962). "Biological Transmission of Disease Agents." Academic Press, New York.
Maramorosch, K. (1963). *Ann. Rev. Entomol.* **8**, 369–414.
Miles, J. A. R. (1960). *Bull. World Health Organ.* **22**, 339–371.
Miles, J. A. R. (1964). *Bull. World Health Organ.* **30**, 197–210.
Nelson, R. L. (1964). *Am. J. Hyg.* **80**, 242–253.
Nosek, J., Gresikova, M., and Rehacek, J. (1961). *Acta Virol. (Prague)* **5**, 112–116.
Orr, C. W. M., Hudson, A., and West, A. S. (1961). *Can. J. Zool.* **39**, 265–272.
Pavlovskii, E. N., and Solovyev, V. D. (1940). *Arkh. Biol. Nauk. Leningrad* **59**, 111–117.
Pavlovskii, E. N., and Solovyev, V. D. (1941). *Trudy Voyenno-Med. Akad. Krasn. Armii Moskva Leningrad* **25**, 9–18.
Philip, C. B., and Burgdorfer, W. (1961). *Ann. Rev. Entomol.* **6**, 391–412.
Reeves, W. C., Hutson, G. A., Bellamy, R. E., and Scrivani, R. P. (1958a). *Proc. Soc. Exptl. Biol. Med.* **97**, 733–736.
Reeves, W. C., Bellamy, R. E., and Scrivani, R. P. (1958b). *Am. J. Hyg.* **67**, 78–89.
Reeves, W. C., Bellamy, R. E., and Scrivani, R. P. (1961). *Am. J. Hyg.* **73**, 303–315.
Reeves, W. C., Tempelis, C. H., Bellamy, R. E., and Lofy, M. F. (1963). *Am. J. Trop. Med. Hyg.* **12**, 929–935.
Reeves, W. C., Bellamy, R. F., Geib, A. F., and Scrivani, R. P. (1964). *Am. J. Hyg.* **80**, 205–220.
Rush, W. A., Kennedy, R. C., and Eklund, C. M. (1963). *Am J. Hyg.* **77**, 258–264.

Scherer, W. F., Buescher, E. L., Moyer, J. T., Kitaoka, M., McClure, H. E., Rosenberg, J. T., Flemings, M. B., Izumi, I., Okuno, T., Southam, C. M., Noguchi, A., Gresser, I., Ogata, T., Hardy, J. L., Scanlon, J., McCown, J., Bullock, H. R., Yoshi, M., and Okada, Y. (1959). *Am. J. Trop. Med. Hyg.* **8**, 644–722.
Singh, K. R. P., Pavri, K., and Anderson, C. R. (1963). *Nature* **199**, 513.
Smith, C. E. G. (1960). *Trans. Roy. Soc. Trop. Med. Hyg.* **54**, 113–129.
Smith, C. E. G. (1964). *In* "Host-Parasite Relationships in Invertebrate Hosts" (A. E. R. Taylor, ed.), pp. 1–31. Blackwell, Oxford.
Stamm, D. D., and Newman, R. J. (1963). *Anais Microbiol. Univ. Brasil* **11A**, 123–133.
Sulkin, S. E., Allen, R., and Sims, R. (1963). *Am. J. Trop. Med. Hyg.* **12**, 800–814.
Sun, W. K. C. (1964). *J. Med. Entomol.* **1**, 277–284.
Tatchell, R. J. (1964). *Parasitology* **54**, 423–440.
Tempelis, C. H., and Lofy, M. F. (1963). *Am. J. Trop. Med. Hyg.* **12**, 825–831.
Tempelis, C. H., and Reeves, W. C. (1962). *Am. J. Trop. Med. Hyg.* **11**, 294–297.
Thomas, L. A. (1963). *Am. J. Hyg.* **78**, 150–165.
Thomas, L. A., and Eklund, C. M. (1960). *Proc. Soc. Exptl. Biol. Med.* **105**, 52–55.
Thomas, L. A., and Eklund, C. M. (1962). *Proc. Soc. Exptl. Biol. Med.* **109**, 421–424.
Thomas, L. A., Eklund, C. M., and Rush, W. A. (1958). *Proc. Soc. Exptl. Biol. Med.* **99**, 698–700.
Varma, M. G. R., Webb, H. E., and Pavri, K. M. (1960). *Trans. Roy. Soc. Trop. Med. Hyg.* **54**, 509–516.
Whitman, L., and Aitken, T. H. G. (1960). *Am. Trop. Med. Parasitol.* **54**, 192–204.
Woodall, J. P., and Bertram. D. S. (1959). *Trans. Roy. Soc. Trop. Med. Hyg.* **53**, 440–444.
Work, T. H. (1963). *Anais Microbiol. Univ. Brasil* **11A**, 241–255.
Worth, C. B. (1963). *Anais Microbiol. Univ. Brasil* **11A**, 111–114.
Zoltowski, Z., and Wroblewska-Mularczykowa, Z. (1961). *Med. Doswiadczalna Mikrobiol.* **13**, 241–249.

AVIAN TUMOR VIRUSES

Peter K. Vogt*

Department of Pathology, University of Colorado Medical School, Denver, Colorado

I. Introduction . . . 294
II. The Elementary Particles . . . 295
A. Physical Properties . . . 295
B. Ultrastructure . . . 300
C. Purification . . . 301
D. Chemical Composition . . . 302
E. Host-Controlled Properties . . . 305
F. Antigens of Avian Tumor Viruses . . . 308
G. Criteria for Avian Tumor Virus Classification . . . 311
III. Characteristics of Virus Synthesis and Release . . . 312
A. Rous Sarcoma Virus (RSV) . . . 312
B. Avian Myeloblastosis Virus, BAI Strain A (AMV) . . . 324
C. The Infectious Cycle of Other Avian Tumor Viruses . . . 331
D. Synopsis of Avian Tumor Virus Multiplication . . . 335
IV. The Defectiveness of RSV . . . 336
A. Experiments Supporting the Postulate That RSV Is Defective . . . 336
B. The Role of the Helper Virus . . . 340
C. Helper-Controlled Properties of RSV . . . 343
D. Helper-Independent Properties of RSV . . . 348
E. The Significance of Defectiveness . . . 349
V. Principles Governing Avian Tumor Virus Infection *in Vivo* . . . 352
A. Factors of Importance in the Individual Avian Host . . . 352
B. Natural Transmission and Persistence of Avian Tumor Viruses in Chicken Populations . . . 361
VI. RSV in Mammalian Hosts . . . 364
A. *In Vivo* Studies . . . 364
B. *In Vitro* Studies . . . 371
VII. Conclusion . . . 372
References . . . 374

* Supported by Public Health Service Research Grant No. Ca 06655 from the National Cancer Institute and by Grant No. E 302 from the American Cancer Society.

Mancher gibt sich viele Müh'
Mit dem lieben Federvieh;
Einesteils der Eier wegen,
Welche diese Vögel legen,
Zweitens: Weil man dann und wann
Einen Braten essen kann;
Drittens aber nimmt man auch
Ihre Federn zum Gebrauch
In die Kissen und die Pfühle,
Denn man liegt nicht gerne kühle.—
Wilhelm Busch, "Max and Moritz"
Munich, 1865*

I. Introduction

The avian tumor viruses form a well-defined group of related pathogens. The virus particles of the various strains cannot be distinguished from each other by physical techniques. Their morphology and architecture are identical at the present-day level of resolution. Antigenic relationships and differences between the numerous strains can be demonstrated with several serological techniques. The results of such immunological comparisons strengthen the concept that all avian tumor viruses are related. Close similarities between different strains are also found in the cellular biology of virus replication. Finally, all avian tumor viruses are pathogenic for their natural host, the chicken. The degree of pathogenicity covers an extremely wide range from such highly virulent forms as Rous sarcoma virus and avian myeloblastosis virus to the largely avirulent field strains of avian leukosis virus, which may be altogether innocuous in many of the infected animals. The natural occurrence of avian tumor viruses is inversely related to their virulence; virus-induced sarcomas or acute granulocytic leukemias are encountered comparatively seldom, whereas infection with less virulent forms of avian leukosis virus is so frequent that it accounts for the greater number of avian neoplasms.

In the present review the term leukosis virus will be used to

* Many labor 'neath the yoke
for their darling feathered folk,
in the hope that hens in season
eggs produce will 'yond all reason;
or when this has run its course,
mamma hen becomes the roast;
and as well their feathers snug
keep one warm as bug in rug.
For who, when comfort lies in feathers,
shiver would in winter weathers.
(Translated by Dr. Elizabeth Macintyre)

designate any avian tumor virus strain which causes neoplastic disorders originating predominantly in the hematopoietic system. The majority of all avian tumor viruses belongs in this category. Most of the remaining strains cause primarily solid tumors of connective tissue origin and may be classified as avian sarcoma viruses. The experimental work during the past few years has focused on a few prototypes, notably, Rous sarcoma virus (RSV), Rous associated virus (RAV), avian myeloblastosis virus strain BAI A (AMV), strain R erythroblastosis virus, and strain RPL 12 avian leukosis virus.

Research on avian tumor viruses may conveniently be classified into several fields according to the main object of study: properties of the virus particle, cellular biology of viral carcinogenesis and multiplication, response of the organism to infection, and epidemiology of avian tumor viruses. The present survey will follow this subdivision. Most of the older literature has been covered in the excellent and exhaustive reviews of Claude and Murphy (1933), Foulds (1934), Rous (1936), and Engelbreth-Holm (1942). Certain aspects of the avian tumor viruses have also been summarized in more recent publications, such as the problems of virus purification and the properties of avian tumor virus particles (Harris, 1953, Beard *et al.*, 1955, 1963; Beard, 1957b, 1961), the cytochemistry of avian leukemias (Thorell, 1963), the electron microscopy of cells infected with avian tumor viruses (Haguenau and Beard, 1962), and the etiology, pathogenesis, and transmission of avian virus tumors (Beard, 1957a, 1958, 1962, 1963a,b Darcel, 1960). Although the present paper will aim at comprehensiveness, it would be unnecessarily repetitious to consider in detail such data as are already adequately surveyed in the literature. In these areas it should suffice briefly to state and document some of the important principles which have emerged from recent work. The main emphasis of this review on the avian tumor viruses will then rest on the cellular biology of virus multiplication and carcinogenesis.

II. The Elementary Particles

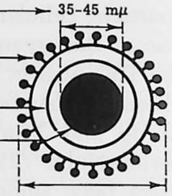
In the following section the basic properties of avian tumor virus particles will be discussed. The important data are compiled in Table I. A complete account of the old literature on RSV may be found in the review by Harris (1953), and more recent work on AMV is discussed by Beard (1961) and Beard *et al.* (1963).

A. Physical Properties

1. Size, Density, and Weight

Experiments using filtration through membranes of graded pore size indicate that infectious RSV has a diameter between 75 and 100 mμ (Elford and Andrewes, 1935). Data obtained by ultracentrifugation suggest a diameter of 90 mμ for RSV and 144 for AMV (Kahler *et al.*, 1954a,b; Sharp and Beard, 1954). These values do not represent a real, consistently found difference in the diameters of RSV and of AMV, but reflect merely differences in the measuring techniques and their interpretation. As will be seen in a later paragraph, the indirect estimates

TABLE I
BASIC PROPERTIES OF AVIAN TUMOR VIRUSES[a]

Dimensions and schematic structure:	
Diameter of the central core	35–45 mμ
Outer membrane with peripheral knobs	
Inner membrane	
Nucleoid	
Diameter of the particle	80–120 mμ
Buoyant density:	
1.06 and 1.16–1.19, depending on experimental conditions	
Particle weight:	
7×10^{-10} μg	
Sedimentation constant:	
In the order of 600 S	
Inactivated by ether, chloroform, deoxycholate, saponin	
pH stability:	
Stable between pH values of 5 to 9, optimum at neutrality	
Half-life time of virus infectivity at 37°C.:	
In the order of 2 to 6 hours, dependent on virus strain, source, and incubation medium (see also Table II)	
Chemical composition:	
RNA: 2.2%	1×10^7 molecular weight units per particle
Protein: About 60%	
Lipid: 35%	
DNA: Not more than 0.1%	Not more than 5×10^5 molecular weight units per particle
Host-controlled properties:	
1. The presence of host cell antigens	
2. The presence of triphosphatase and polynucleotide phosphorylase in AMV grown in cells containing these enzymes at their surface	

[a] References to the data listed may be found in the text.

of avian tumor virus diameters are in approximate agreement with direct electron microscopic measurements which assign diameters from 80 to 120 mμ to the elementary particles. The sedimentation constants of

avian tumor viruses lie between 500 and 655 S [RSV: 550 S and 655 S (Kahler *et al.*, 1954b); AMV: 500 to 600 S (Sharp and Beard, 1954).] The dry weight of a single particle of AMV has been estimated as 7.5×10^{-10} μg. (Bonar and Beard, 1959). AMV has a density of 1.06 in bovine serum albumin solution (Sharp and Beard, 1954). This value is considerably lower than the ones reported for RSV: 1.15 in sucrose density gradient and 1.18 in a rubidium chloride gradient (Kahler *et al.*, 1954a; Crawford, 1960). Again, no controlled comparisons of the densities of RSV and AMV have been made, but one may suspect that the variance of the reported values reflects mainly differences in experimental conditions. RAV, the helper virus of RSV, has been found to have the same buoyant density in a rubidium chloride gradient as does RSV (Hanafusa *et al.*, 1964b). Crawford (1960) observed that RSV from sarcoma tissue had approximately the same density as RSV obtained from tissue cultures, but both preparations contained particles ranging in density from 1.16 to 1.18. This variation appears to reflect the presence of different amounts of lipid in individual virus particles. The viral lipid is presumably derived from the cell membrane and is acquired by the virus during its maturation and release from the cell. Morphological observations on the process of virus release (to be discussed in a later section) make it understandable that the amount of lipid acquired by individual particles should vary. In addition to this inconstancy in the amount of lipid, the composition of the lipid may change with different host cells, leading in turn to dissimilarities in the density of virus particles produced by different cell types. Such host-controlled variations of viral density have been observed in Newcastle disease virus, which has a mode of maturation similar to that of the avian tumor viruses (Stenback and Durand, 1963).

2. *Sensitivity to Lipid Solvents*

Lipid appears to be essential for the integrity of avian tumor virus particles. Treatment of virus suspension with ethyl-ether completely abolishes the infectivity of the preparations. This ether sensitivity has been demonstrated for RSV, for a field strain of avian lymphomatosis virus, and for avian sarcoma virus strain 13 (Andrewes and Horstmann, 1949; Drayton, 1961; Friesen and Rubin, 1961; Wallbank *et al.*, 1962). Electron microscopic observations on ether-treated AMV indicate that the outer membrane of the virus is dissolved by ether (Bonar *et al.*, 1963a). Sensitivity to ether classifies the avian tumor viruses, together with ribonucleic acid (RNA) viruses of similar architecture, the myxo- and the arboviruses. The presence of structurally essential lipid in these three viral groups reflects similar mechanisms of virus maturation and release involving the plasma membrane (Franklin, 1958).

3. *Heat Sensitivity and pH-Dependence of Stability*

Preparations of avian tumor virus lose their infectivity upon storage at all but extremely low temperatures (minus 60°C. or below). Table II lists the published half-life times for several avian tumor viruses at 37°C.; the average lies around 260 minutes. The variation between individual reported values may be due to several factors: (1) The composition of the medium in which the virus is suspended has an effect on the heat sensitivity of avian tumor viruses (Bryan *et al.*, 1950a,b). (2) The tissue of origin may influence the heat sensitivity of avian tumor viruses (Rauscher and Groupé, 1960b). (3) Various strains of the same virus may show different degrees of heat sensitivity (Prince, 1960). The rate of heat inactivation of avian tumor viruses is a function of the temperature. The half-life time for RSV drops from 240 minutes at 39°C. to 8.5 minutes at 50°C. and 0.7 minutes at 60°C. (Dougherty, 1961). A similar relation has been found with AMV. If suspended in chicken plasma, this virus has a half-life time of 300 minutes at 37° which is reduced to 3 to 5 minutes at 56°C. (Eckert *et al.*, 1955b). The half-life time of other avian tumor viruses at 56°C. is probably not significantly different from that reported for RSV or AMV, namely, approximately 5 minutes. The customary inactivation at 56°C. for 30 minutes is therefore not sufficient to free chicken sera to be used in tissue culture from high titers of an avian leukosis virus which may be present. The lability of avian tumor viruses must also be considered when storing frozen virus samples. Even at —15°C. the half-life time of AMV is less than a week and that of strain R erythroblastosis at —22°C. is about 50 days (Eckert *et al.*, 1955b; Bonar *et al.*, 1957a). Only below —60°C. can avian tumor viruses be stored over several years without loss of infectivity (Bryan *et al.*, 1954). The exact chemical basis for the heat sensitivity of avian tumor viruses is not known, but it appears that oxidative processes lead to inactivation and that such inactivation is at least partially reversible by reducing agents (Pirie and Holmes, 1931; Engelbreth-Holm and Fredericksen, 1938). More recent experiments suggest that sulfhydryl groups may be involved in the heat inactivation of avian tumor viruses (Schmidt, 1957). Whatever the nature of the inactivation process, it is substantially slowed down by freeze-drying of the virus (Carr and Harris, 1951; Schmidt, 1963). The heat inactivation of avian tumor viruses follows first-order kinetics. However, careful measurements by Hanafusa and co-workers have revealed a definite shoulder in the survival curves of some avian tumor virus preparations (Hanafusa *et al.*, 1964b). The degree of this deviation from first-order kinetics can be considerably reduced by using sonicated preparations, indicating that aggregation of virus particles is the main cause for the shoulder of the inactivation curve. The stability of avian tumor viruses shows only little variation with pH in the range from pH 5 to pH 9. Outside this range the inactivation rates are markedly increased (Bryan *et al.*, 1950a,b; Eckert *et al.*, 1955c; Bonar *et al.*, 1957a).

TABLE II
HALF-LIFE TIME OF VARIOUS AVIAN TUMOR VIRUSES AT 37°C.

Virus	Half-life time (minutes)	Reference
Bryan standard RSV	About 360	Bryan *et al.* (1950a)
	180	Rubin (1955), (1957)
	200	Prince (1960)
	36 and 360, depending on tissue of origin	Rauscher and Groupé, (1960b)
	240	Dougherty (1961)
	380	Drayton (1960)
Bryan high-titer RSV	540	Prince (1960)
	220	Friesen and Rubin (1961)
	100–120	Hanafusa *et al.* (1964b)
Field strain of avian leukosis virus	200	Friesen and Rubin (1961)
AMV (BAI, strain A)	300	Eckert *et al.* (1955a)
	150	Vogt (1963)
Strain R erythroblastosis virus	540	Bonar *et al.* (1957a)
RAV	100–120	Hanafusa *et al.* (1964b)

4. *Resistance to Ultraviolet Light*

A noteworthy property of avian tumor viruses is their comparatively high resistance to inactivation by ultraviolet (UV) light (Baker and Peacock, 1926; Sturm *et al.*, 1932). RSV is about 10 times as resistant to ultraviolet irradiation as is Newcastle disease virus, although both viruses are similar in size, structure, and RNA content, and have the same sensitivity to X-rays (Rubin and Temin, 1959; Rubin, 1960c). Resistance to ultraviolet light appears to be typical of all avian tumor viruses, since it has also been found with a field strain of avian leukosis virus and with RAV (Friesen and Rubin, 1961; Levinson and Rubin, unpublished results). At present there exists no satisfactory explanation for this unusual property of avian tumor viruses. However, available evidence appears to rule out the repair of UV-inactivated avian tumor virus by interaction with the host cell genome or by photoreactivation (Rubin, 1960c; Friesen and Rubin, 1961).

B. *Ultrastructure*

1. *Shadowed Preparations*

Early electron microscopic observations on the avian tumor viruses were done with shadowed preparations of partially purified virus. These revealed approximately spherical particles with a diameter between 100 and 120 mμ (Sharp and Beard, 1952; Sharp *et al.*, 1952, 1955). More recent electron micrographs of purified AMV have confirmed these early observations (Bonar *et al.*, 1963a).

2. *Thin-Sectioned Preparations*

In thin sections the avian tumor virus particles consist of an outer membrane, 80 to 90 mμ in diameter, and an inner, electron-dense core of 35 to 45 mμ in diameter. A second membrane can be seen between the outer envelope and the inner core (Bernhard *et al.*, 1956, 1958; Haguenau *et al.*, 1958).

3. *Negatively Stained Preparations*

This general architecture of avian tumor virus particles has been confirmed by studies on negatively stained preparations. In addition, the negative-staining technique has revealed the presence of knob-like protrusions, extending from the outer virus envelope (Dourmashkin and Simons, 1961; Bonar *et al.*, 1963a). These structures are probably proteins and bear a distant resemblance to the hemagglutinins of the myxoviruses. However, no hemagglutinating activity is associated with the envelope of avian tumor viruses. The inner core of avian tumor viruses appears to consist of a tubular structure, possibly with a helical arrangement of subunits, similar to the nucleocapsid of the myxoviruses (Eckert *et al.*, 1963). Under certain conditions of preparation avian tumor viruses may exhibit a pronounced pleomorphism with regard to size and shape, often leading to the appearance of tailed forms. There is no evidence which would indicate that such tails are an essential characteristic of the virus or fulfill a specific function in the infectious cycle (Bonar *et al.*, 1963a).

C. *Purification*

1. *Purification from Blood Plasma*

Although many basic biological problems of avian tumor virus infection and carcinogenesis can be solved using crude virus preparations, biochemical investigations require the availability of fast and efficient purification procedures. It is well to remember that recent progress in the biochemistry of animal virus infections has been made with those viruses which can be easily purified from tissue culture material. The purification of avian tumor viruses is complicated by two factors: (1) the instability of the virus and (2) the presence of host material in the viral envelope, making the separation of virus from cell debris difficult. The instability of the virus results in a requirement for shortness of the purification procedure and for a high potency of the starting material. Difficulties of purification originating from the presence of host substances at the viral surface suggest the use of starting materials which are largely free of cellular debris. These demands on the starting material are met to very different degrees with different avian tumor viruses.

Plasma from chickens diseased with myeloblastic leukemia comes close to having the optimal properties of a starting material for virus purification. Highly viremic donor animals can be selected and yield plasmas which contain in the order of 1×10^{12} viral particles per ml. In such plasmas there is an approximately 1000-fold excess of AMV particles over contaminating particulate material of similar size. Highly purified preparations of AMV can therefore be obtained by differential centrifugation. It has been estimated that the maximum of nonviral impurities in such preparation lies between 1 and 5% (Bonar and Beard, 1959). Strain R erythroblastosis virus has been purified in similar manner from the plasma of diseased chickens. However, due to lower virus concentrations in the starting material, a higher proportion of contaminating particulate material remains associated with the virus (Bonar *et al.*, 1963b). In general, the plasma of viremic chickens should be a suitable source for obtaining milligram quantities of purified virus. In contrast to tissue homogenates, there is no bulk of particulate host material difficult to separate from the virus. In addition, specific immune tolerance of donor animals to avian leukosis virus can be induced by injecting the virus into the embryo or newly hatched bird (Rubin, 1962c). Such tolerance results in the failure of infected birds to produce virus-neutralizing antibodies and in the maintenance of a long-lasting, high-level viremia, making possible repeated bleedings of the same donor bird.

2. Purification from Solid Tumors

Purification of avian tumor viruses becomes much more difficult when homogenates of solid tumors such as Rous sarcomas are used as starting material. Under these conditions there is an extreme preponderance of cellular particulate material with physicochemical properties similar to those of the virus. A large variety of purification procedures have been tried with such material, including differential centrifugation, chromatography, and enzymic digestion of host impurities. A review of these procedures leads to the conclusion that "purified" RSV preparations, obtained from solid tumors, contain not more than about 1% viral material (Bryan and Moloney, 1957). Better results can be achieved by the use of fluorocarbon for the removal of host impurities (Epstein, 1958a). As judged by electron micrographs, such RSV preparations are largely free of particulate host material. However, they still contain considerable quantities of gelatinous impurities and cannot be used for the chemical analysis of the virus.

3. Purification from Tissue Culture Material

The purification of virus from animal material has as its main purpose the chemical, structural, and immunological characterization of virus particles. However, experiments using radioactive tracers in studies on the cellular events during avian tumor virus multiplication and carcinogenesis call for purification procedures applicable to tissue culture-grown virus. The purification of RSV from tissue culture fluid by equilibrium density gradient centrifugation in rubidium chloride represents a successful step in this direction (Crawford, 1960). RSV obtained in this way from tissue culture contains very little nonviral particulate material, as judged from electron micrographs of thin-sectioned pellets. The recovery of virus particles lies around 60%, and the loss of infectivity during the purification procedure is only about 33% (Crawford and Crawford, 1961). Progress in the purification of other viruses with a lipid-containing envelope has recently been made using chromatography on columns of calcium phosphate and on cellulose phosphate (Reda and Rott, 1962; Laver, 1962). These promising techniques are awaiting application to avian tumor virus material.

D. Chemical Composition

1. Over-all Composition

Meaningful chemical analyses can only be carried out with virus preparations of high purity. Because of the difficulties in purifying certain avian tumor viruses, the chemical composition has only been determined in a few favorable instances. The most extensive data exist on AMV. This virus contains 35% lipid, about 60% protein, and 2.2% RNA. Trace amounts of deoxyribonucleic acid (DNA) found in purified preparations of AMV are not considered an essential component of the virus (Bonar and Beard, 1959; Allison and Burke, 1962). DNA was also found to be virtually absent from a purified sample of RSV (Crawford and Crawford, 1961). The amount of RNA per particle, expressed in molecular weight units, was found to be 9.8×10^6 for AMV and 9.5×10^6 for RSV (Bonar *et al.*, 1963b; Crawford and Crawford, 1961). It is not known whether this RNA occurs in one or several molecules.

2. Detailed Analysis of Viral RNA and Protein

In some cases the chemical analysis of avian tumor virus has been carried to greater detail. For AMV the amino acid composition of the total protein has been determined (Purcell *et al.*, 1962). Base compositions of the viral RNA are available for RSV, AMV, and strain R erythroblastosis (Crawford and Crawford, 1961; Thorell, 1962; Bonar *et al.*, 1963b; Trávníček *et al.*, 1964; Robinson and Pitkanen, unpublished). The values for RSV given by Crawford and Crawford (1961) are based on the analysis of one purified virus sample only (Table III). They differ markedly from those of Robinson and Pitkanen for purified viral RNA. The RSV samples analyzed contain RAV in approximately 10-fold excess over RSV. The base compositions of such preparations may therefore be primarily a reflection of their RAV content. It is not known whether the base compositions of RAV- and of RSV-RNA differ significantly from each other, and whether a variation in the proportion of RAV to RSV in the test material would influence the results of base analyses. The base composition of AMV, determined with a microtechnique, bears almost no resemblance to the results obtained with conventional methods of nucleic acid analysis (Thorell, 1962). This discrepancy may be due to technical errors. Pending further investigations of this problem, the base compositions of AMV determined with conventional techniques deserve to be regarded with more confidence (Bonar *et al.*, 1963b; Trávníček *et al.*, 1964). Table III shows that there are obvious dissimilarities in the base compositions of the three viruses listed and that there is no resemblance to the base composition of host RNA. The previously reported similarity in the composition of RSV and chick cell RNA was probably due to insufficient purification of the virus (Bather, 1957).

TABLE III
BASE COMPOSITION[a] OF AVIAN TUMOR VIRUS RNA AND HOST CELL

Material		Cytosine	Adenine	Guanine	Uracil	Reference
RSV (Bryan high-titer strain) and RAV		30.4	29.9	20.5	19.2	Crawford and Crawford (1961)
RSV (Bryan high-titer strain) and RAV		24.2	25.1	28.3	22.4	Robinson and Pitkanen, unpublished
AMV (BAI, strain A)		17	27	21	33	Thorell (1962)
		23.6	22.8	31.0	22.5	Bonar, *et al.* (1963b)
		27.2	22.6	29.5	20.8	Trávníček *et al.* (1964)
Strain R erythroblastosis		22.6	20.9	36.4	20.0	Bonar *et al.* (1963b)
Chick embryo RNA		27	20	33	20	Ada and Perry (1955)
Chicken liver cytoplasmic RNA		31	20	35	14	Bather (1957)
Chicken spleen cytoplasmic RNA		29	19	37	15	Bather (1957)
Chick cell RNA	28 S	30.7	17.2	36.0	16.1	Robinson and Pitkanen, unpublished
	18 S	26.4	21.8	31.1	20.7	
	4 S	28.2	18.7	33.1	20.0	
RNA from chicken leukemic myeloblasts		28	20	38	14	Bonar *et al.* (1963b)
Chicken leukemic myeloblasts						Trávníček *et al.* (1964)
Total RNA		30.3	18.0	34.0	17.7	
Microsomal RNA		29.3	18.5	35.3	16.8	
Transfer RNA		30.4	20.0	30.3	19.3	

[a] In moles per 100 moles of recovered base.

3. *Ultrastructural Localization of Chemical Components in the Virus Particle*

Electron microscope techniques give information on the localization of the main chemical components within the virus particle. In virus pellets of RSV fixed with potassium permanganate the central core of

the particles is partially digestible with ribonuclease (Epstein, 1958b; Yamaguchi, 1962). Additional treatment with trypsin still leaves one of the viral membranes intact; whether this is the inner or outer membrane is not clear from the published electron micrographs. Treatment of the virus particles with papain leaves only the central core. Deoxyribonuclease has no effect on the appearance of RSV particles in the electron microscope. Lipid solvents and phospholipase A destroy the structural integrity of the virus membranes as well as that of the central core. A similar effect of lipid solvents is demonstrable with AMV (Bonar *et al.*, 1963a). These experiments indicate that the central core of the avian tumor virus particles contains RNA, and that protein is present in the three major structural components of the virus, the outer and inner membranes and the central core. The results also suggest that lipid occurs in all three structures, although this conclusion needs further confirmation because of possible nonspecific effects of lipid solvents and phospholipase A.

E. *Host-Controlled Properties*

1. *Presence of Host Antigens in Avian Tumor Virus Particles*

The outer coat[*] of avian tumor viruses is derived from the cell membrane. Although this membrane has become altered through the incorporation of viral antigens, some of its original properties persist, conferring upon the virus certain traits of the host cell. The primary function of the host material in the virus envelope appears to be a structural one, necessary for the architectural integrity of the virus. However, the host material retains specific characteristics, such as enzymic or antigenic activities which once were connected with distinct tasks in the host cell. There is no evidence that these specific host properties become integrated functions of the viral infectious cycle. It seems rather that they are merely accidental and entirely phenotypical to the virus, since they can change with different host cells without affecting the viability of avian tumor viruses.

The presence of host antigens in avian tumor viruses is evident from the extensive studies of Eckert and co-workers on the antigenic constitution of AMV (Eckert *et al.*, 1955d). Substances reacting with antiserum to normal chicken tissue and to Forssman antigen were found to be inseparable from the virus by differential centrifugation or electrophoresis. The precipitation of AMV with antiviral serum from the chicken results in coprecipitation of the normal chick antigens and Forssman

[*] In the present review the term "viral coat" will be used synonymously with "viral envelope," and "core" will be equivalent to "nucleocapsid."

antigens of such preparations. It appears reasonable to assume that the host antigen present in avian tumor viruses is derived from the cell membrane during the process of virus maturation and release. In this respect it is of interest that influenza virus, which is completed at the cell surface in a way very similar to that of the avian tumor viruses, also contains host antigens (Knight, 1946). While there is general agreement that host antigens are incorporated in avian tumor virus particles, conflicting data exist as to the role of the antigens in the neutralization of the virus infectivity by antibody. Earlier reports contain evidence for, as well as against, the neutralization of RSV by antiserum to normal chicken tissue (Gye and Purdy, 1933; Amies and Carr, 1938; Kabat and Furth, 1941; Barrett, 1941). A more recent investigation of the problem demonstrates several important differences between the neutralization of RSV by antiviral serum of the chicken and the apparent neutralization caused by rabbit serum to normal chicken tissue (Rubin, 1956). The neutralization with antiviral serum does not require complement and the virus-antibody complex is virtually nondissociable by dilution. The action of normal tissue antibody requires complement and is dependent on the antibody concentration inoculated into the test animal, but independent of the antibody concentration with which the virus is incubated. After penetration into the host cell, virus becomes inaccessible to the neutralizing action of antiviral antiserum but antiserum to normal tissues remains active in suppressing the formation of Rous tumors, even if given 2 days after infection. These observations suggest that the apparent neutralization of RSV by antisera against normal chicken tissues is not due to a direct interaction between antibody and virus but is a consequence of the cytocidal action of antiserum on host cells. This action seems to affect primarily the ability of the cell to divide and to form a tumor. Additional support for these observations on RSV and its interaction with antibody against host tissue comes from the work of Borsos (1958), which demonstrates that antichicken serum has no neutralizing effect on the ability of RSV to induce hemorrhagic lesions in chick embryos. Likewise, no neutralizing activity by antichicken serum of the ability of RSV to cause hemorrhagic disease in rats is demonstrable (Rychlíkova and Svoboda, 1959).

AMV and strain R erythroblastosis virus also show loss of infectivity after exposure to antibodies against normal chicken serum or against chicken tissue. AMV infectivity is also reduced by Forssman antiserum (Eckert *et al.*, 1955d; Beard *et al.*, 1957). However, little information is available on possible differences between the neutralization of these avian leukosis viruses by antiviral antibody of the chicken and their reaction with antiserum to normal tissues. The antigens involved in both

reactions seem to be different; the activity of normal tissue antiserum can be completely removed by absorption with chicken tissues, whereas the neutralizing capacity of antiviral antibodies remains unchanged by such a procedure (Eckert, 1955, quoted by Rubin, 1956). This raises the question whether an action on the host cell rather than on the virus is responsible for the neutralization of AMV and of strain R erythroblastosis virus by antiserum to normal host tissue. This possibility is not rigorously excluded by the published experiments. Another explanation for the effect of normal tissue antiserum on avian leukosis viruses may be found in the frequent inapparent infection of normal chickens with a naturally occurring avian leukosis virus. Such chickens are likely to harbor high titers of virus in their tissues, and antiserum against such material may be expected to contain antibodies against virus-specific antigens. This possibility has been considered by Eckert and co-workers but was dismissed as unlikely (Eckert *et al.*, 1955d). It certainly would not explain the activity of Forssman antibodies against AMV. In considering the available data on the interaction of avian tumor virus and antisera to host tissue, one cannot escape the impression that the problem would greatly profit from a reinvestigation, using tissue culture techniques to determine avian tumor virus infectivity and to control such important parameters as the concentration of antibody and of complement. Such an investigation would be aided by the fact that RSV can be supplied with antigenically different coats (Hanafusa *et al.*, 1964a). A comparison of RSV having the antigenicity of its natural helper virus, RAV, and of RSV coated with the antigens of AMV or strain R erythroblastosis could be carried out in the same tissue culture system, under strictly equivalent and comparable conditions.

2. *Presence of Host Enzymes in Avian Tumor Virus Particles*

AMV obtained from the peripheral blood of chickens diseased with myeloblastosis contains a triphosphatase which is active with adenosine triphosphate or inosine triphosphate as substrates (Mommaerts *et al.*, 1952; Green and Beard, 1955). This enzyme is firmly associated with the virus, it sediments together with the virus particles in the ultracentrifuge, and it cannot be separated from the virus by electrophoresis (Sharp *et al.*, 1954; Eckert *et al.*, 1954b). Besides the triphosphatase a polynucleotide phosphorylase complex can be found in preparations of AMV (Říman and Thorell, 1960). This enzyme complex is most active with guanosine diphosphate as substrate but acts also on the diphosphates of adenosine, uridine, and cytidine. The specific activity, expressed in liberated inorganic phosphate per mg. nitrogen per unit time, is only about one tenth that of the triphosphatase. Ultracytochemical studies show that AMV acquires the triphosphatase activity from the cell membrane during the process of virus release (Novikoff *et al.*, 1962; de Thé *et al.*, 1963a, 1964). Certain host tissues are composed of cells which have a high adenosinetriphosphatase activity at their surfaces, for instance, blast cells occurring in the cortex of the thymus, or myeloblasts in the blood stream of leukemic birds. In cases where such host cells are the main source of the virus, over 90% of the virus particles are carriers of triphosphatase activity. The enzyme occurs only at the external surface of the virus, associated with the virus envelope which is directly derived from the triphosphatase-rich cell membrane. Since the enzyme activity can also be demonstrated on the surface of uninfected cells, it is not virus-induced. On the other hand, cells which do not normally possess adenosinetriphosphatase at their surface do not develop such a property after infection with AMV. An example for this is AMV-induced nephroblastomas. AMV produced by such tumors is largely free of adenosinetriphosphatase (Thorell, 1961). The lack of the enzyme on the virus is matched by the absence of cytochemically demonstrable triphosphatase activity from the surface of most nephroblastoma cells (de Thé *et al.*, 1963b). The erythroblasts circulating in the peripheral blood during the terminal stages of virus-induced erythroblastosis are free from cytochemically detectable adenosinetriphosphatase activity (de Thé *et al.*, 1964). Consequently, purified preparations of virus from the blood of chickens diseased with erythroblastosis do not contain the enzyme (Bonar *et al.*, 1957b).

F. *Antigens of Avian Tumor Viruses*

1. *Antigenicity of the Viral Envelope and Viral Interrelationships Revealed by the Similarity of Coat Proteins*

The envelope of avian tumor viruses contains virus-specific proteins which are antigenic in the avian host and stimulate the production of neutralizing antibodies. Fluorescein-conjugated chicken sera of high neutralizing potency yield a strong staining reaction with avian tumor virus-infected cells. At least part of the fluorescent antibody staining may therefore represent the same immune reaction which leads to neutralization of virus infectivity. However, the correlation between fluorescent-staining ability and neutralizing potency is not always perfect. Occasionally, sera may be found which stain virus antigen on the surface of avian tumor virus-infected cells but fail to exert a neutralizing effect on viral infectivity (Vogt, unpublished). Neutralization and cross-neutralization have long since served to detect relationships between different strains of avian tumor viruses. The early experiments

of Andrewes (1931, 1933) demonstrate an antigenic relationship between several avian sarcoma viruses. Among the avian leukosis viruses, antibody against AMV strongly neutralizes strain R erythroblastosis virus; however, immunological differentiation of the two viruses may be achieved by specific precipitation (Beard *et al.*, 1957). Reciprocal cross-neutralization exists between RSV, AMV, RPL 12, and their respective antisera (Beard, 1957a). A close immunological relationship has also been demonstrated between a field strain of avian leukosis virus and RSV (Friesen and Rubin, 1961). In a few instances unilateral cross-neutralization has been reported. The neutralization of RSV by Fujinami antiserum is not matched by a cross-reaction between RSV antibody and Fujinami virus (Andrewes, 1933). Likewise, the neutralization of RSV by erythroblastosis antiserum is not mirrored by a reaction between RSV antibody and erythroblastosis virus (Beard, 1957a). An explanation for this lack of reciprocality in some immune cross-reactions between avian tumor viruses has not yet been found.

RSV itself occurs in several strains which can be antigenically differentiated. The Harris strain of RSV is only remotely related to the Bryan RSV; most avian sera prepared against either one or the other do not neutralize the heterologous virus (Simons and Dougherty, 1961). Another strain, RSV(29), is closely related to, but not identical with, the Bryan RSV (Simons and Dougherty, 1963). The Schmidt-Ruppin strain of RSV is also antigenically distinct, showing little similarity to the Bryan RSV. However, there is some cross-neutralization between Schmidt-Ruppin RSV and AMV antiserum (Sarma *et al.*, 1964a). The relationship between AMV and Schmidt-Ruppin RSV is also borne out by fluorescent antibody studies, which reveal staining of Schmidt-Ruppin virus-infected cells with fluorescent AMV antibody (Vogt, unpublished). These are only a few of the antigenic similarities between avian tumor viruses reported in the literature. But they suffice to demonstrate a network of antigenic cross-relationships which strengthens the concept that all avian tumor viruses are members of one group of pathogens.

The study of antigenic relationships between avian tumor viruses is complicated by a steady increase in the number of virus strains, and by the finding of antigenic heterogeneity in several strains previously thought to be pure. Immunological heterogeneity has been demonstrated in the Bryan high titer strain of RSV, which consists of at least three antigenic variants (Hanafusa, 1965; Vogt, 1965). Similar antigenic heterogeneity has been found in the Bryan standard strain and the Schmidt-Ruppin strain of RSV as well as in AMV (Ishizaki and Vogt, unpublished). Such results underline the necessity for cloning of the virus strains prior to any immunological comparisons. One might expect that some of the reported immunological relationships will be traced back to the presence of some ubiquitous avian leukosis virus inadvertently included in avian tumor virus stocks during passage in naturally infected chickens. In future work strain differences, rather than similarities, may therefore become more prominent. Ultimately, antigenic relationships between avian tumor viruses will have to be expressed in quantitative terms. This will be greatly simplified by the possibility of wrapping defective RSV in any avian tumor virus coat and thus using the accurate RSV focus assay for neutralization tests of the noncytopathic avian tumor viruses (Hanafusa *et al.*, 1964a).

2. *Avian Tumor Virus Antigenicity in Mammals*

Syrian hamsters or guinea pigs bearing a sarcoma induced by the Schmidt-Ruppin strain of Rous sarcoma virus develop complement-fixing antibodies which are specific for an antigen present in the mammalian Rous sarcoma (Huebner *et al.*, 1963). The same, or a closely related antigen, can also be found in tissue cultures infected with any of several other avian tumor viruses, including Bryan RSV, erythroblastosis virus, RPL 12, and field strains of avian leukosis virus (Huebner *et al.*, 1964; Armstrong *et al.*, 1964). Most of the antigen is not sedimentable at centrifugal speeds sufficient to spin down intact avian tumor virus, and must therefore be smaller than complete virus. However, some antigenic activity is found in virus pellets, indicating that the complement-fixing antigen may be part of the avian tumor virus particle. The location of the antigen in the virus particle is not known with certainty, but circumstantial evidence suggests that it is part of the viral core. The antigen-containing hamster tumor cells are free of complete RSV, and the complement-fixing mammalian antibodies fail to neutralize RSV. The complement-fixing antigen found in avian tumor virus-infected cells appears to be group, rather than strain, specific. No antigenic differences between various strains of avian tumor virus have been seen in complement fixation tests. The existence of such a group-specific avian tumor virus antigen lends further justification for combining all avian tumor viruses in one category. Mammalian complement-fixing antibodies against the avian tumor virus group-specific antigen are of diagnostic value and permit the rapid detection of inapparent avian tumor virus infections (Sarma *et al.*, 1964b). Another technique of producing complement-fixing antibodies against RSV is the injection of a virus-adjuvant mixture into rabbits (Fink, 1964). The viral antigens involved in this reaction have not been identified.

Antigens stimulating the production of complement-fixing antibodies in rabbits have also been found after treatment of AMV with Tween 80 and ether or with sodium laurylsulfate (Eckert *et al.*, 1964a,b). The size of the antigen recovered from AMV preparations by Tween-ether treatment is about 3 S, and its density 1.06. The antigenic material obtained from AMV by degradations with sodium laurylsulfate is of similar size, but can be separated into two components having densities of 1.26 and 1.33, respectively. After absorption with normal chicken tissue, antiserum against the Tween-ether-prepared material fixes complement with the homologous antigens as well as with two antigenic components obtained from AMV by sodium laurylsulfate treatment. Only one of these components (density 1.26) gives complement fixation with antiserum against sodium laurylsulfate-split AMV. The antigens obtained from AMV preparations by Tween-ether or sodium laurylsulfate extraction appear to be components of complete AMV particles, since rabbit antibodies prepared against the split products react also with intact virus. This reaction may be largely blocked by preincubation of the virus preparations with AMV-neutralizing antibody obtained from the chicken. The blocking effect suggests that chicken and rabbit antisera contain antibodies directed against similar or identical viral antigens. Whether or not these antigens are involved in the neutralization reaction and are located at the viral surface remains to be determined in future tests. The physical data on the viral split products suggest a considerable residual complexity of the material, and it is possible that they contain internal as well as external viral antigens.

G. *Criteria for Avian Tumor Virus Classification*

Some of the most obvious differences among avian tumor viruses are revealed by the various neoplastic responses elicited in the avian host by different virus strains. Most avian tumor viruses are capable of inducing several distinct neoplastic diseases, but under a given set of conditions one particular response usually prevails. In this sense it is justifiable to use descriptive terms like myeloblastosis virus, erythroblastosis virus, or lymphomatosis virus. However, the oncogenic spectra of all thoroughly studied strains are broad, and considerable overlaps are encountered. Visceral lymphomatosis, for instance, may result from infection by either one of several distinct strains of avian tumor viruses, for example, AMV, RPL 12, strain R erythroblastosis virus, or even RSV (Beard, 1962; Burmester *et al.*, 1959a,b; Burmester and Walter, 1961). For this reason the descriptive terminology based on symptoms found in the avian host is inaccurate and can be misleading (Beard, 1963b). Whether all this multiplicity of carcinogenic effects exerted by single strains of avian tumor virus is attributable to the pluripotency of genetically homogeneous virus, or whether the heterogeneity found in many strains of avian tumor virus contributes to the broadness of the oncogenic spectrum, can only be decided after critical experiments with cloned virus have been carried out. This cloning must be based on the isolation of viral progeny from single lesions produced by a single virus. Limiting dilution techniques are of doubtful reliability. Even such cloning experiments, however, will not obviate the necessity for a system of avian tumor virus classification which is not entirely dependent on host response. Some criteria which may be useful in such a system of classification are discussed in the following paragraph.

Abundant experience with antibody neutralization and the fluorescent antibody staining of avian tumor viruses indicates that these two reactions based on the antigenicity of the viral coat reveal strain differences most readily. The specificity of these reactions will doubtlessly be enhanced by the use of cloned virus as antigen. Besides antigenicity, there are two other known properties of the viral coat, namely, host range and interference patterns (see Sections IV,C,2 and 3). Judged by present methods of testing, these properties appear to respond much less to small differences between antigenically closely related strains (Vogt, unpublished). It is therefore possible to make a first crude classification of any avian tumor virus by determining its host range on various genetic lines of chicken cells or its ability to interfere with prototype strains of RSV. An exact classification can then be accomplished by differential neutralization or fluorescent antibody staining. In the case of defective RSV, classification is more complicated. Since the envelope of this virus must be supplied by an associated avian tumor virus (see Section IV,B,2), a characterization of the virus envelope merely serves to name the helper. For an unequivocal identification of such defective RSV the use of coat-independent markers is mandatory, in addition to a characterization of the helper virus. Unfortunately, not many such coat-independent properties have been recognized yet. Some will be discussed in Section IV,D.

III. Characteristics of Virus Synthesis and Release

A. *Rous Sarcoma Virus (RSV)*

1. *Measurement of Infectivity*

The infectivity of RSV preparations can be assayed in the avian organism using subcutaneous inoculation in the wing web of young adult chickens. Virus titers are estimated from the latent period of tumor induction or by an end-point technique. There are extensive studies on the characteristics of these assay methods and on the conditions under which they yield reliable results (Bryan, 1946, 1954, 1955). RSV can also be assayed by tumor formation on the chorioallantoic membrane of the developing chick embryo. There is a linear correlation between the concentration of RSV in the inoculum and the number of tumors produced (Keogh, 1938). The direct proportionality between tumor counts and virus concentration implies that one RSV particle is sufficient to cause a tumor (Rubin, 1955, 1957; Edlinger, 1959). The assay of RSV on the chorioallantoic membrane is complicated by a large variability in the tumor counts and by the existence of embryos which are resistant to even the highest doses of virus (Rubin, 1955; Groupé *et al.*, 1957; Prince, 1958a; Vigier, 1959; Dougherty and Simons, 1962; Vrba, 1963). In a survey of several chicken lines the fraction of these "nonreactors" was found to vary from 5 to 75% of the inoculated embryos (Prince, 1958b). The failure of certain chick embryos to respond to RSV inoculation is due to a genetically determined host resistance against the virus inoculum (Prince, 1958b; Bower, 1962). Nonresponsiveness to inoculation with RSV could also arise from congenital infection of the embryo with a field strain of avian leukosis virus. A virus-induced resistance of this kind can be demonstrated in tissue cultures derived from embryos which had been congenitally infected with an avian leukosis virus. The resistance is also found in young chickens inoculated with RAV during embryonic life (Rubin, 1960a, 1962a). There is no reason to doubt that such viral interference would also influence the RSV assays on the chorioallantoic membrane and contribute to the high variability encountered with this method. The *in vivo* techniques for the assay of RSV have gone almost out of use in recent years. The requirements for large numbers of birds to be kept under observation for extended periods of time and the insufficient control over important factors influencing the outcome of the assay make these procedures inadequate for the quantitative investigation of virus-cell interactions.

Most difficulties of the *in vivo* methods are eliminated by the tissue culture assay of RSV. In cultures of chicken fibroblasts RSV induces a distinct morphological change of the infected cells. The normally elongated and flattened fibroblasts become spherical and refractile sarcoma cells (Manaker and Groupé, 1956). This virus-induced tranformation of chick embryo cells is the basis for the quantitative assay of RSV *in vitro* (Temin and Rubin, 1958). Low multiplicities of infection result in the formation of distinct foci of transformed cells. The number of these foci is directly proportional to the virus concentration in the inoculum. A single particle of RSV is therefore sufficient to cause a focus in tissue culture. It should be pointed out that this basic relationship is also found with defective strains of RSV. The defectiveness does not impair the ability of RSV to bring about cellular transformation in infected fibroblasts. A single particle of defective RSV is still capable of initiating a focus. Only the maturation of virus progeny requires the cooperation of a second virus in the case of defective RSV. Since the focus technique is based on the ability of RSV to transform the cell and not on the ability to produce virus progeny, defectiveness does not affect the validity of the assay procedure. RSV foci can be counted on the seventh day after infection with an inverted microscope using a low magnification. Macroscopically identifiable foci are obtained by extending the incubation time (Dougherty and Simons, 1962), by increasing the incubation temperature and repeated feeding of the assay cultures (Adams and Groupé, 1963), or by differential staining with pyronin and azure B (Siminoff and Reed, 1963). The assay of Rous sarcoma cells by the focus technique is somewhat more complicated and a correct interpretation became only possible after the basic parameters of RSV multiplication in chick fibroblasts had been established (Rubin, 1960b). The number of cells registering as infectious centers after exposure to RSV increases linearly with the virus concentration, but only up to a level of 1 to 10% of the cell population. At this point a ceiling is reached and further increases in the virus concentration do not result in a higher proportion of infectious centers. This ceiling is always observed when plating for infective centers is carried out shortly after infection. It is due to the fact that freshly infected cells have a low probability to initiate a focus on the assay culture. This inefficiency of the infective center assay has two causes: (1) Cells plated shortly after infection are still in their latent period and will release virus or become transformed only after a delay which may vary from cell to cell. (2) The assay cultures lose their competence to support focus formation on about the second day after preparation. The delay of virus release and transformation and the concurrent decrease of competence in the assay cultures act synergistically to lower the fraction of infected cells initiating a Rous sarcoma focus. However, the efficiency of the infective center assay can be considerably increased by postponing the plating of the cells for one or more days after infection. Infected cells have then reached a stage where virus release and transformation may be coincident with the time of plating, i.e., with the period of maximum competence of the assay cultures. Under these conditions most cells exposed to a high dose of RSV will show up as infective centers, indicating that the majority of (if not all) cells in such a culture can be infected with a single dose of RSV. The same factors which cause the inefficiency of the infective center assay also affect the assay of free virus by the

focus technique. It can be estimated that about 1 in 10 RSV infections is recorded by the focus assay as it is commonly carried out (Rubin, 1960b). This estimate is also supported by tissue culture assays of RSV in which sensitive end-point techniques are used involving several transfers of the assay cultures in order to detect single infectious units (Vogt, 1964).

These data on the inefficiency of the RSV focus assay are of importance for the interpretation of the particle to infectious unit ratio. In RSV-infected cultures the ratio of avian tumor virus particles to focus-forming units is 750 (Crawford and Crawford, 1961). However, from 60 to 90% of the particles produced by such a culture are Rous-associated virus (RAV), which cannot be distinguished morphologically from RSV. The number of RSV particles per focus-forming unit is therefore much smaller, it lies in the order of 75 to 200. Taking into account the above-mentioned inefficiency of the focus assay and some loss of particles due to incomplete adsorption of the inoculum, one can conclude that about 1 in 5 to 1 in 15 RSV particles obtained from tissue culture fluid is potentially infectious.

2. *Kinetics of Virus Growth and Maturation*

Some of the basic characteristics of RSV growth are evident from studies using *in vivo* systems. RSV placed on the chorioallantoic membrane penetrates into susceptible ectodermal cells and becomes inaccessible to neutralizing antibody within 3 hours after infection (Harris, 1954; Rubin, 1956). Virus entry into the host cell is followed by an eclipse period of approximately 15 hours during which less than 1% of the input infectivity is recoverable by grinding up infected membranes. The end of the eclipse is marked by the reappearance of newly synthesized virus which then increases rapidly until 6 days (Prince, 1958c). Virus release from infected cells is continuous, extending over prolonged periods of time. In a population of Rous sarcoma cells an equilibrium between virus production and heat inactivation of the virus becomes established. This results in a constant ratio of infectious virus to the number of cells (Rubin, 1955, 1957). More detailed information on the multiplication of RSV comes from the use of tissue culture systems (Vigier and Goldé, 1959; Temin and Rubin, 1959; Prince, 1960; Vogt and Rubin, 1961; Goldé and Vigier, 1961). Under conditions of high multiplicities of infection the main features of RSV growth are as follows: Chick fibroblasts eclipse RSV very rapidly; 1 hour after infection 95% of the cell-associated inoculum has disappeared. New virus synthesis can be demonstrated at 12 to 14 hours and is followed by an exponential rise of free and cell-associated virus. This rise is due to an increase in the number of cells entering the state of virus synthesis as well as to an increase in the rate of virus synthesis per individual cell. The increment in the rate of virus production per infected cell is about 100-fold, occurring between 4 hours and 4 days. The fraction of cells actively releasing virus is very small at 24 hours but increases to include most cells by 3 to 4 days. This scatter in the onset of virus release by individual cells indicates an asynchrony in virus synthesis rather than in virus release, since there is no retention of completed virus in Rous sarcoma cells. Because of this asynchrony the latent period differs from cell to cell. The time at which 63% of the infected cells have entered the state of virus synthesis is 25 hours after infection. At about the third day after infection the rise of virus titers levels off, indicating that an equilibrium is reached between the rate of virus production and the rate of thermal inactivation of the released virus. The ratio of focus-forming units to cells at this stage ranges from 1 to 10. An actual decline of virus titers may occur from day 5 on, due to exhaustion of the culture medium. However, the events of virus multiplication do not seriously impair the reproductive capacity of the infected cell; Rous sarcoma cells can release virus and divide.

During the ascending part of the growth curve the amount of free virus exceeds that of cell-associated virus. More than 90% of the cell-associated virus is located at the outer cell surface and can be either removed from intact cells by treatment with trypsin or neutralized with specific immune serum. Even the small proportion of cell-associated RSV which is inaccessible to trypsin and antibody may not be intracellular, but merely sheltered in cytoplasmic vacuoles. From this true ratio of extracellular to intracellular virus obtained during the ascending part of the growth curve, the release time of RSV can be determined according to a formula published by Rubin *et al.* (1955). The release time is defined as the time which elapses from the completion of the virus until its appearance outside the cell. For RSV this interval is 3 minutes or less. This short release time strongly suggests that RSV is completed near the cell surface and gains its infectivity only during the process of leaving the cell. Under these circumstances the process of maturation becomes synonymous with that of virus release.

3. *Light Microscopic Observations*

Completion of RSV at the cell membrane is also strongly suggested by studies with fluorescent antibody. Viral antigen becomes first detectable 24 hours after infection of a chick fibroblast culture. It occurs then in the form of small granules at the external cell surface (Vogt and Luykx, 1963). This predominance of the cell membrane as a site for viral antigen continues through the entire growth cycle. As the infection advances, the small granules of viral antigen enlarge and coalesce to cover a significant portion of the cell surface. Concurrently with the rounding of the infected cells, viral material may become detached from the cell surface (Vogt and Rubin, 1961). A similar course of events has been observed in RSV-infected turkey cells (Bergs and Groupé, 1962a). The involvement of the cell periphery in RSV production is also evident from observations on sarcomas growing in the avian host (Mellors and Munroe, 1960). The viral subunits which are assembled at the cell surface into complete virus appear to be synthesized throughout the cytoplasm. Especially during the advanced stages of infection the cytoplasmic location of viral antigen is conspicuous (Mellors and Munroe, 1960; Malmgren *et al.*, 1960; Noyes, 1960; Vogt and Rubin, 1961). The nuclei of Rous sarcoma cells in tissue culture are free of viral antigen. In specimens from chicken sarcomas specific fluorescence can occasionally be seen at this cellular site (Mellors and Munroe, 1960; Malmgren *et al.*, 1960). The significance of these rare antigen-containing nuclei is not clear and the question of nuclear participation in RSV synthesis should be carefully reinvestigated.

As will be discussed in another context later, the immunochemical staining characteristics of Rous sarcoma cells are not controlled by RSV itself, but by a second avian tumor virus which directs the completion of RSV synthesis. Nevertheless, observations made with the fluorescent antibody technique are truly relevant to the problem of RSV synthesis, as RSV adopts the antigenic coat of the second avian tumor virus participating in the infectious cycle.

RSV induces distinct cytological alterations in the infected cell (Halberstaedter *et al.*, 1941; Lo *et al.*, 1955; Moses and Kohn, 1963). Most prominent at low magnifications is the change in cell morphology from the normal spindle-shaped fibroblast to a rounded, refractile Rous sarcoma cell (Manaker and Groupé, 1956). The morphology of the transformed cell is to a certain extent controlled by the infecting virus (Temin, 1960; Dougherty *et al.*, 1963; Purchase and Okazaki, 1964). Temin has described two mutants of RSV which affect the shape of the infected cell differently. The more common one, termed "morph r," causes the familiar rounding of the fibroblast. Infection with the other one, designated as "morph f," leaves the host cell elongated but induces a marked refractility (Temin, 1960). The transforming activity of RSV in tissue culture is not restricted to fibroblasts. Upon infection with RSV epithelial cells become transformed in a similar way as do fibroblastic cells. A beautiful example of such epithelial transformation may be found in the chick embryo iris culture studies by Ephrussi and Temin (1960).

As fibroblasts in tissue culture become transformed by RSV, they acquire the ability to grow in several layers, whereas the growth of normal fibroblasts is largely restricted to monolayers by the action of contact inhibition (Temin and Rubin, 1958; Abercrombie and Heaysman, 1953, 1954; Rubin, 1961b). Direct inspection as well as cinematographic studies suggest that Rous sarcoma cells are not subject to contact inhibition (Dougherty and Trevan, 1963). It appears that this lack of contact inhibition in the locomotory behavior of Rous sarcoma cells is so obvious to even the casual observer that no quantitative study on this phenomenon has yet been made. However, the extent of cell-clustering in Rous sarcoma foci is not invariable, it depends on the genetic constitution of the virus as well as on environmental conditions. Compared to the Bryan high-titer strain, the Schmidt-Ruppin strain of RSV causes only little piling up of the infected cells. Foci formed after infection with the Bryan high-titer strain have a distinct, sharp border, whereas foci induced by the Schmidt-Ruppin strain are diffuse, with normal-appearing and transformed cells interdispersed. It is clear from such observations that the extent to which contact inhibition is lost and abnormal mobility is gained may vary considerably with Rous sarcoma cells transformed by different virus strains. The importance of environmental conditions for the RSV-induced cellular transformation is most clearly demonstrated by the effect of fetal calf serum on the process of transformation. If such serum is incorporated in the nutrient agar used to overlay RSV-infected cultures focus formation by low multiplicities of RSV will be suppressed. At the same time, the rate of virus synthesis remains comparable to that of the controls or is even increased (Rubin, 1960d). These marked influences of genetic and environmental factors on RSV-mediated transformation and loss of contact inhibition call for quantitative investigations using the techniques developed by Abercrombie and Heaysman (1954) for the analysis of the cellular locomotory behavior of RSV infected cells. The significance of unrestricted mobility of a malignant cell for its invasiveness has been convincingly pointed out in a recent review and requires no additional comment (Abercrombie and Ambrose, 1962). The reason for the lack of contact inhibition in the locomotion of Rous sarcoma cells is not clear. Since contact inhibition is mediated through cell surface properties (Abercrombie and Ambrose, 1958) the coating of the cell with viral antigen offers itself as a possible explanation for the failure of cell contacts to restrict cellular movements (Vogt and Rubin, 1961, 1962; Rubin, 1961b). However, this explanation must be rejected for the following reasons: (1) RSV-infected cells become covered with viral antigen also in the presence of fetal calf serum, when cellular transformation is suppressed (Vogt, unpublished observations). (2) In infections with avian leukosis virus a similar accumulation of viral antigen occurs at the cell surface without being accompanied by a comparable loss of contact inhibition (Rubin and Vogt, 1962; Vogt and Rubin, 1963). (3) Nonproducing Rous sarcoma cells, infected with RSV but not with a helper virus, are transformed and show a pronounced tendency to cluster in the absence of any viral antigen at their surfaces (Vogt, 1964). It appears, therefore, that the coating of Rous sarcoma cells with viral antigen is neither required nor sufficient for the loss of contact inhibition. Another property of Rous sarcoma cells may have profound effects on the cell surface and may thus influence the cellular locomotory behavior. This is the secretion of acid mucopolysaccharides (Erichsen *et al.*; 1961, Grossfeld, 1962). The occurrence of these substances at the cell surface may also account for the drastically lowered adhesion of Rous sarcoma cells to glass or plastic and to each other as well as to normal neighboring cells. Initial information on a possible link between the appearance of acid mucopolysaccharides and the loss of contact inhibition could be gained by testing for mucopolysaccharide secretion under various conditions of virus production and virus-induced transformation. If mucopolysaccharides were of significance for the loss of contact inhibition one should expect an increased synthesis of this material in nonproducing Rous sarcoma cultures despite the absence of complete virus formation. In contrast, RSV-producing cells which fail to transform in the presence of fetal calf serum should show mucopolysaccharide levels which are not significantly elevated over those of the control. Another outstanding cytological feature of RSV-infected fibroblasts is their basophilia, probably representing aberrant RNA synthesis (Goldé, 1959; Rubin, 1960b; Hampton and Eidinoff, 1962). The basophilic material is often found in a well-defined paranuclear zone, a cellular location which also contains high concentration of viral antigen (Vogt and Rubin, 1961).

4. *Electron Microscopy of RSV Infection*

When thinly spread Rous sarcoma cells are examined as whole cell mounts with the electron microscope, viral particles of about 100 $m\mu$ diameter are readily demonstrable at the periphery and on the surface of the cells (Claude *et al.*, 1947; Epstein, 1956). By obtaining sequential fluorescence and electron micrographs from the same cell, it can be shown that the viral particles at the cell surface bind specific RSV antibody. These particles are therefore identified as RSV (Vogt and Luykx, 1963). The technique of using whole mounts of thinly spread fibroblasts for electron microscopy allows the rapid scanning of many cells for the presence of viral particles at their surface, but it does not provide information on the fine structural aspects of the virus and its association with the cell. The morphological details of the cell-virus relationship are revealed by the study of thin-sectioned Rous sarcoma cells with the electron microscope (Gaylord, 1955; Epstein, 1957a,b; Bernhard *et al.*, 1956; Haguenau *et al.*, 1958, 1960; Nishiumi *et al.*, 1961). In material obtained from chicken sarcomas the paucity of virus particles is striking. However, the number of such particles seen in thin sections of tumor samples roughly corresponds to the infectivity of the particular tumor. Cells infected with RSV *in vitro* usually present no difficulty in demonstrating RSV particles. Complete virus particles which have the size and structure discussed in Section II,B occur at two cellular sites: at the cell surface and inside cytoplasmic vacuoles. Frequently, the cytoplasm also shows a marked hypertrophy of the endoplasmic reticulum. It seems possible that these areas of densely packed lamellae correspond to the basophilic zones observed with the light microscope and to the cytoplasmic centers of viral antigen synthesis seen after fluorescent antibody staining. The cytoplasm of Rous sarcoma cells is remarkably free of structures which could be interpreted as incomplete assembly stages of the virus. In an occasional cell granules of the approximate size of the viral nucleoid plus the inner membrane can be seen. The granules are less electron dense than the nucleoids of mature virus particles and appear to be surrounded by ribosomes. At present the relationship of these structures to the process of virus synthesis is not known. In the earlier electron microscopic investigations support for the completion of RSV at the cell surface was only circumstantial. It consisted of the close association between structurally complete virus and the cell membrane and the absence of such virus particles from the interior of the cell. Only recently has the actual process of virus completion and release been seen in electron micrographs (Heine *et al.*, 1962a; Haguenau *et al.*, 1962). Virus particles emerge as buds from the cell membrane and a constriction forming at the base of the bud effects the separation of the virus from the cytoplasm. Completed virus particles have a tendency to remain attached to the cell surface and may accumulate at this site to form clusters of several hundred particles. It is conceivable that this accumulation of released virus at the cell surface is at least in part caused by the secretion of viscous mucopolysaccharides from the Rous sarcoma cell.

A highly conspicuous but very little understood morphological feature of Rous sarcoma cells is the enlargement of the nucleolus (Bernhard, 1958; Haguenau *et al.*, 1961). Infection with other avian tumor viruses does not induce such a drastic increase in the nucleolar size. Hypertrophy of the nucleolus is commonly found in neoplastic cells (Bernhard, 1958) and the nucleolar changes seen in Rous sarcoma cells may therefore be primarily connected to viral carcinogenesis rather than to virus multiplication.

5. *Biochemical Events in RSV Multiplication*

As has been pointed out in a previous section of this review, biochemical studies of virus multiplication are greatly facilitated by the existence of a rapid and efficient purification technique, applicable to tissue culture-grown virus, viral protein, or viral RNA. Several important problems, such as the fate of labeled virus after entry in the cell, the source of building blocks used in the synthesis of viral macromolecules, or any comparison of nucleic acid base composition involving the viral RNA require such purification procedures. In the Rous sarcoma system these methods are still far from perfect and experiments which include virus purification have therefore been comparatively rare (Temin, 1964a,b). A more indirect analysis of biochemical events during RSV synthesis can be carried out with the aid of various substances interfering with nucleic acid metabolism (Fairman, 1959; Rich *et al.*, 1960, 1962; Goldé and Vigier, 1963; Eidinoff *et al.*, 1963; Bather, 1963; Temin, 1963b, 1964b; Bader, 1964 and private communication; Vigier and Goldé, 1964a,b; Force and Stewart, 1964). These experiments indicate that RSV infection can be divided into at least two stages with regard to nucleic acid replication and function. The initiation of RSV infection appears to require both functionally intact DNA as well as actual DNA synthesis. The requirement for functional DNA, acting as a template in RNA synthesis, is suggested by the effect of actinomycin D on RSV infection. Low concentrations of this antibiotic, given early in infection, strongly and irreversibly inhibit the production of RSV. A need for DNA synthesis in the early phases of RSV infection (the first 48 hours) may be deduced from the inhibitory effect exerted on RSV growth by antagonists of DNA synthesis. These antagonists are: amethopterin, fluorodeoxyuridine, 5-fluorouracil, bromo- and iododeoxyuridine, mitomycin C, and cytosine arabinoside.

After RSV infection is established, nucleic acid antagonists cause a different pattern of inhibition. DNA synthesis does not seem to be necessary for the continuous production of RSV by Rous sarcoma cells. The unimportance of DNA synthesis during the advanced stages of infection is indicated by the observation that antagonists of DNA synthesis such as amethopterin, cytosine arabinoside, mitomycin C, 5-fluorouracil, or fluorodeoxyuridine do not interfere with RSV synthesis when given after the onset of virus production. However, RSV synthesis appears to retain a requirement for DNA function even in established Rous sarcoma cells as suggested by the inhibitory effect of actinomycin D on RSV production under these conditions. The effect of actinomycin D on the continuation of RSV synthesis is quantitatively different from that on the initiation of RSV infection. Higher concentrations of actinomycin are required to interrupt RSV synthesis than are necessary to prevent it.

The interpretation of these findings with nucleic acid antagonists leaves much room for speculation. The effect of actinomycin D on the early stages of RSV infection is generally assumed to result from the interaction between inhibitor and host-cell DNA. It is then concluded that the participation of host-cell DNA is required in some early phase of RSV synthesis, possibly to code for an enzyme which uncoats the viral RNA (Vigier and Goldé, 1964b). The requirement of DNA synthesis, or a process intimately linked to DNA synthesis, appears to be supported by the finding of comparable effects with a variety of inhibitors. Experiments using any one of these DNA inhibitors alone would allow a range of different explanations not necessarily involving DNA synthesis. Several of the inhibitors used have been shown to be pleiotropic, causing symptoms which are not explicable by their mechanisms of action as DNA antagonists. However, the fact that several of these antagonists, having different mechanisms of action, influence RSV infection in a similar way suggests that they all act on the same target, namely, DNA synthesis. This conclusion is strengthened by the finding that the suppression of avian tumor virus growth by inhibitors of DNA synthesis can be prevented by adding metabolites which specifically abolish the effect of the antagonist on DNA synthesis. Thus, addition of thymidine to the cultures prevents the inhibition of RSV and of RAV synthesis by bromodeoxyuridine, iododeoxyuridine, fluorodeoxyuridine, and amethopterin. Cytosine arabinoside has no effect on avian tumor virus growth in cultures which have been supplied with deoxycytidine (Bader, personal communication). This apparent need for DNA synthesis during infection with an RNA virus has so far only been found in the avian tumor virus group. The infectious cycle of other RNA viruses may depend on DNA function, but not on DNA synthesis (Barry *et al.*, 1962; Kingsbury, 1962; Rott and Scholtissek, 1964; Buthala, 1964). A noteworthy result is the failure to detect any differences in the sensitivity to DNA antagonists between RSV and RAV (Bader, personal communication). The findings with inhibitors can therefore not be called upon to explain the high efficiency with which RSV induces a malignant transformation or to support a hypothetical genetic interaction between RSV and cell genome.

The importance of DNA synthesis for the initiation of RSV growth has been taken to mean that the genetic message of RSV is transcribed to DNA and that this RNA-generated DNA serves as a template for the synthesis of new viral RNA (Temin, 1964b). Evidence for this proposal is tenuous. The production of the viral DNA template would presumably be suppressed by antagonists of DNA synthesis and actinomycin D would prevent the new viral DNA from priming RNA synthesis. In accordance with this view are tests which have failed to turn up evidence for viral RNA synthesis in actinomycin-treated Rous sarcoma cells. However, the same tests have also failed to provide a basis for the rigorous exclusion of such synthesis (Temin, 1964b). Even in untreated Rous sarcoma cells the amount of viral RNA produced is so small that it does not change the fingerprint pattern of the cellular RNA (Delihas and Evans, 1963). The postulate of a viral DNA in Rous sarcoma cells leads one to expect that RSV-RNA should specifically hybridize with single-stranded DNA obtained from Rous sarcoma cells, but not with DNA from normal chicken fibroblasts. A recent report suggests that such RNase-resistant hybrids of Rous cell DNA and RSV-RNA might be found (Temin, 1964a). The experimental data supporting the formation of these RNA-DNA hybrids are, however, still debatable, and much further work will be required before this question can be considered solved.

The metabolic characteristics of Rous sarcoma cells differ in several respects from those of normal chicken cells, either of the chorioallantoic membrane or of chick embryo cell cultures. Rous sarcoma cells show an increased utilization of glucose, higher activities of glycolytic enzymes, and an increased lactate production (Levine *et al.*, 1960, 1961, 1962; Ashmore *et al.*, 1961; Weber *et al.*, 1961). Amino acids become accumulated in Rous sarcoma cells at faster rates and the activity of amino acid-activating enzymes as well as the rate of incorporation of amino acids into proteins are increased over the values of control cells (Wagle *et al.*, 1961, 1963; Love *et al.*, 1964). Changes in the nucleic acid metabolism are indicated by autoradiographic studies. Nucleolar RNA shows a faster rate of labeling in Rous sarcoma cells than in uninfected chick fibroblasts, and thymidine incorporation into DNA is enhanced markedly (Hampton and Eidinoff, 1962). In agreement with cytological findings, the amount of RNA per cell increases concurrently with the onset of virus-induced transformation. Similar increments have been demonstrated for the amount of protein per cell. The amount of DNA in Rous sarcoma cells shows no significant increase over that of normal cells (Goldé, 1962). These alterations in metabolic activities are not specific properties of Rous sarcoma cells, but are also found in neoplastic cells of a wide variety of different etiologies (Burk, *et al.*, 1941). The magnitudes of the changes are too large to be understandable on the basis of virus synthesis alone. They appear to reflect largely the neoplastic nature of Rous sarcoma cells. In this respect it would be of interest to study the metabolism of Rous sarcoma cells which are not engaged in virus production, such as the nonproducer cells arising from solitary infection with the Bryan high-titer strain of RSV or mammalian Rous sarcoma cells which usually fail to produce virus.

B. *Avian Myeloblastosis Virus BAI Strain A (AMV)*

1. *Titration of AMV*

There exist no less than six different methods for the titration of AMV. One may therefore justly suspect that none of them meets fully all requirements of a convenient quantitative assay technique. The usefulness of the various procedures varies greatly in different situations and it is therefore necessary to discuss advantages and shortcomings.

a. Infectivity Measurements in the Chicken. Several different kinds of dose responses can be used to assay AMV in 3-day-old chickens. Linear relationships have been observed between the logarithm of virus dose and the reciprocal of the latent period and between the logarithm of the virus dose and the incidence of positive responses expressed in probit units (Eckert *et al.*, 1951). A more satisfactory relationship for quantitative purposes is that between the logarithm of the virus dose and the median logarithm of the latent period. This relationship is also linear and allows infectivity titrations which may be expected to lie between ±35% of the true value (Eckert *et al.*, 1954a). It should be pointed out, however, that this precision is only reached by using chickens of high and uniform susceptibility to AMV. Even then the method cannot be employed with low doses of virus, because under these conditions the latent period may be unduly extended, probably by the onset of an immune response in the chicken. The decisive drawbacks of this assay method are practical ones. For example, 80 to 250 chickens are needed to titrate a single virus sample. An average of 10 blood smears per chicken must be made during the course of the experiment to determine the latent period. For these reasons most laboratories will find this technique unsuited for the routine titration of even a modest number of virus samples.

b. Adenosinetriphosphatase as an Estimate of the Number of Virus Particles. AMV contains adenosinetriphosphatase which is firmly associated with the viral particles (Eckert *et al.*, 1954b; Sharp *et al.*, 1954). Since the enzymic activity found in AMV preparations is proportional

to the number of viral particles, it can be used to determine virus concentrations. In the presence of calcium ions, which activate AMV-bound adenosinetriphosphatase, a plasma sample containing 10^{12} viral particles will split approximately 0.8 mg. inorganic phosphorus from adenosinetriphosphate in a reaction time of 1 minute (Mommaerts *et al.*, 1954). Microtests for determining enzyme activity in plasmas from diseased birds are available and can be applied to the routine testing of large numbers of samples (Beaudreau and Becker, 1958; Becker *et al.*, 1962). A prerequisite for this method is the presence of adenosinetriphosphatase in all, or nearly all, virus particles. Since the enzyme is not an integral part of the virus this prerequisite is not always fulfilled. Only AMV liberated from cells which themselves show adenosinetriphosphatase activity at their surface contains the enzyme. For instance, more than 90% of the viral particles obtained from the blood plasma of birds diseased with myeloblastosis carry adenosinetriphosphatase at their surface (de Thé *et al.*, 1964). In contrast, virus produced by nephroblastoma cells is largely free of the enzyme (Thorell, 1961; de Thé *et al.*, 1963b). In accord with these findings are earlier observations on the correlation of the adenosinetriphosphatase activity and virus particle number. Only in plasmas obtained from young birds (19 to 31 days) is the ratio of virus particles to enzyme activity constant. In older chickens (34 to 80 days) considerable variations of this ratio are observed (Mommaerts *et al.*, 1954). In these older animals a larger contribution of nonmyeloblastic infected cells to the population of virus particles in the blood plasma should be expected. From these data one may conclude that the adenosinetriphosphatase assay for the estimate of virus particles can be applied only to those preparations of AMV derived solely from tissues which endow the virus with this enzyme, predominantly myeloblasts. It must also be kept in mind that the enzymic estimate of AMV activity does provide information only on the number of virus particles, not on their infectivity. Since infectivity and adenosinetriphosphatase have different rates of thermal inactivation, their ratios vary in different plasmas (Eckert *et al.*, 1955b). Another limitation of the enzymic technique is its lack of sensitivity—requiring concentrations of at least 10^9 particles per ml. plasma. This requirement for high concentrations of virus and the fact that a property of the viral surface is measured establishes some technical resemblance to the hemagglutination test of certain cytocidal viruses. However, in the latter an intrinsic property of the virus is measured, whereas the enzymic assay of AMV relies on a host-controlled trait of viral particles. The adenosinetriphosphatase assay is at present the fastest *in vitro* test available for any avian tumor virus.

c. Infectivity Measurements in the Chick Embryo. Infectivity titrations of AMV in the chick embryo are of theoretical importance, mainly for two reasons (Baluda and Jamieson, 1961): (1) Using 3-day-old chicks one finds a variable proportion of animals resistant to even the highest doses of virus (Eckert *et al.*, 1951, 1954a; 1955a). This resistance is not observed after intraembryonal inoculation. (2) Inoculation into 3-day-old chickens yields a titration curve with no clear-cut end point, but rather a continuous decrease in the incidence of positive responses over a wide range of dilutions. Titration in the chick embryo reveals a well-defined end point. The incidence of negative responses around the end point conforms with that expected from the zero term of the Poisson distribution. These observations on the susceptibility of chick embryos to AMV and on the shape of the titration curve strongly support the hypothesis that a single particle of AMV is sufficient to induce the disease in the animal. It appears that the failure of demonstrating this fundamental aspect of host-virus relationship after inoculation of 3-day-old chickens is due to immune reactions developing in the hatched bird. The titration of AMV in the chick embryo involves inoculation of the virus in the main vein of the chorioallantoic membrane. This procedure requires dexterity but can be carried out on a routine basis with large numbers of eggs. The assay is based on the presence or absence of the disease in the hatched chicken. Its major drawbacks are the lack of precision inherent in any end-point technique and the requirement for extensive animal facilities.

d. Infectivity Assay Based on AMV-Induced Cellular Transformation in Chick Embryo Yolk Sac Cultures. When cultures of chicken hematopoietic tissue are infected with AMV *in vitro,* small, rounded cells appear which can be identified as myeloblasts by light microscopic inspection (Beaudreau *et al.*, 1960a; Baluda and Goetz, 1961). At the onset of transformation the myeloblasts occur in distinct foci. In yolk sac cultures the number of these foci shows a rough correlation to the amount of AMV in the inoculum. However, this correlation is not sufficiently reproducible to serve as the basis for a quantitative assay of AMV. The reason for this is probably to be found in the fact that assay cultures are kept in fluid medium, as agar overlays suppress the cellular transformation. Under these conditions progeny virus becomes freely distributed over the cell sheet and the resulting secondary infections obscure the relationship between the inoculum and the number of myeloblastic foci (Baluda and Goetz, 1961). At present the *in vitro* assay of AMV by cellular transformation is therefore an end-point technique in which individual cell cultures score as either positive or negative and thus give only qualitative information. Other solidifying components for a nutrient overlay such as plasma, methylcellulose, or hydrolyzed starch should be investigated for possible use in this system. They may facilitate the development of a focus assay for AMV, based on the enumeration of discrete myeloblastic areas in AMV-infected tissue cultures. Such an assay would be more precise and more economical than the present end-point technique.

e. Assay of AMV by Virtue of Its Ability to Induce Cellular Resistance to RSV. Chick embryo fibroblast cultures infected with AMV develop a strong cellular resistance to superinfection with RSV. This resistance can be used to assay AMV in tissue culture (Vogt and Rubin, 1963). Again, it is possible to design an end-point technique in which the AMV titer is determined from the highest virus dilution still capable of inducing resistance to RSV. This method is somewhat laborious because repeated transfers of the assay cultures are necessary until a clear-cut end point of AMV-induced resistance develops. The lack of precision presents similar problems as with other end-point titrations. The advantage of the procedure is a very high sensitivity, since even single infectious units of AMV can multiply sufficiently during the course of the assay to produce interference with RSV. A second interference assay for AMV avoids the shortcomings of the end-point titration but is less sensitive. In this second version the degree of resistance induced by serial dilutions of an unknown virus sample is compared with that of a known standard. Such a comparison requires only a single transfer of the assay cultures and gives virus titers relative to that of the standard. The precision of this method is superior to that of an end point technique.

f. Assay of AMV by Fluorescent Focus Counts. In chick embryo fibroblasts infected with AMV viral antigens appear at the external cell surface. These antigens can be stained with specific fluorescent antisera without prior fixation of the cells. At low multiplicities of infection cells producing viral antigen occur in discrete foci. The number of these foci in a culture dish is directly proportional to the virus concentration in the inoculum and can therefore be used to assay the virus (Vogt and Rubin, 1963; Vogt, 1963). The technique is analogous to the plaque assay of cytocidal viruses or to the focus assay of RSV. At present it is the only assay technique of AMV which is based on counts of discrete virus-induced lesions in tissue culture and combines economy with precision. The sensitivity of the fluorescent focus assay is approximately the same as that of the RSV focus assay, namely, 1 in 10 infectious units is detectable as a focus-forming unit. The main obstacles to a more widespread adoption of this method are technical ones. The fluorescent antibody staining involves much additional handling of the cells, and reliable recognition and counting of AMV foci with the fluorescent microscope may become a tiresome procedure even for the experienced.

2. *Kinetics of AMV Growth and Maturation*

Data for the initial phases of AMV multiplication are so far available only from studies with chicken fibroblasts (Vogt and Rubin, 1963; Vogt, 1963). Active virus production can be detected on the first day after infection. This is followed by an exponential rise of virus titers lasting for 2 to 3 days. Between the third and fourth day after infection the virus titers level off at a plateau of about 5 fluorescent focus forming units per cell. Similar to RSV, an equilibrium between virus production and the thermal inactivation of the released virus appears to become established. Also in resemblance to RSV growth, there is more free virus than cell-associated virus during the initial exponential rise of virus titers. The preponderance of cell-associated virus over free virus during the equilibrium phase of the growth curve results from an accumulation of virus progeny at the external cell surface. Less than 1% of the cell-bound virus is truly intracellular during these late stages of infection. As with RSV, most of the virus associated with intact cells is removed or neutralized by the combined action of trypsin and antibody. Applying the formula of Rubin *et al.* (1955) for the release time of a virus, it can be shown that AMV is released from the cell exceedingly fast, within less than 3 minutes after it is completed. This short release time strongly suggests that the final assembly of AMV takes place near the cell surface. Further proof for this view is furnished by cytological observations to be discussed in a later paragraph. The characteristics of established AMV infections have also been studied in cultures of malignant myeloblasts derived from diseased birds, or from chicken bone marrow infected with AMV *in vitro* (Beaudreau, *et al.*, 1960b). Malignant myeloblasts release virus continuously and divide over periods of many months. The ratio of viral particles to cells is characteristic for a given myeloblast population and remains fairly constant during repeated subculturing. The rate of virus release varies between 5 and 40 particles per cell per hour. It appears likely that in most diseased animals a large proportion of the malignant myeloblasts actively produces virus. Support for this comes from infective center assays with myeloblasts obtained from the blood of moribund chickens (Vogt and Rubin, 1963). Most of these cells are capable of inducing resistance to challenge with RSV when plated on chick embryo fibroblast cultures. However, it should be pointed out that, on occasion, highly leukemic chickens may be found which have no detectable AMV in their blood (Eckert *et al.*, 1952). The conditions leading to such noninfectious myeloblastosis remain to be investigated.

3. *Light Microscopic Studies on AMV-Infected Cells*

Tissue cultures derived from hematopoietic tissues of the chicken respond to AMV infection with a cellular transformation which is analogous to the neoplasia induced by AMV *in vivo* (Beaudreau *et al.*, 1960a, Baluda and Goetz, 1961). In most hematopoietic tissues of the chicken the *in vitro*-induced transformation leads to the appearance of immature granulocytes, predominantly myeloblasts and myelocytes. Occasionally, transformed cells may resemble other types of immature blood cells such as erythroblasts or plasmablasts (Baluda *et al.*, 1963). Not all of the cells infected with AMV also become transformed. The ability to respond to AMV infection with a neoplastic transformation appears to be restricted to certain target cells. The incidence of such targets is correlated with the granulopoietic activity of a given tissue. This suggests that the susceptibility to the carcinogenic action of AMV belongs to primitive precursors of granulocytes. Cell types which do not become transformed by AMV are nevertheless capable of supporting AMV multiplication. Information on the production of viral antigen during the course of AMV infection comes from studies with chick embryo fibroblasts (Vogt and Rubin, 1963). The first newly synthesized viral antigens become demonstrable on the second day after infection. They are confined to the cell surface where they accumulate throughout the later stages of infection. Intracytoplasmic antigen is encountered comparatively rarely. This is in contrast to the development of viral antigen in RSV-infected fibroblasts, which in all other respects show a close resemblance to their AMV-infected counterparts. Preliminary experiments with myeloblasts appearing in chick embryo yolk sac cultures after exposure to AMV, indicate that in these malignant cells viral antigen is also located at the cell surface (Moscovici and Vogt, unpublished results).

4. *Electron Microscopy of AMV Infection*

A considerable number of electron microscopic studies deal with the AMV-infected cell. The most significant contribution of these studies to the understanding of AMV infection concerns the mechanism of virus maturation. Myeloblasts obtained from the blood of leukemic chickens are remarkably free of any morphological evidence of virus production. Infrequently, virus particles may be seen at the cell surface or in cytoplasmic vacuoles (Parsons *et al.*, 1958; Bonar *et al.*, 1959). Cultured in a medium with a high content of chicken serum, these myeloblasts undergo characteristic changes. The number of lysosome-like structures in the cytoplasm increases. Viral particles are not only found at the cell membrane, but also in the lysosomes—at the latter site in apparently incomplete form (Bonar *et al.*, 1959, 1960). Recent cytochemical studies provide unequivocal evidence that the lysosome-associated viral particles represent forms of degradation and that the site of virus maturation in the myeloblast is the cell surface (de Thé *et al.*, 1964). These cytochemical investigations demonstrate the presence of triphosphatase, diphosphatase, and acid phosphatase in the lysosome-like cytoplasmic granules of the myeloblasts. In contrast, AMV particles produced by myeloblasts contain only nucleotide triphosphatase, but are free of cytochemically detectable amounts of diphosphatase and acid phosphatase. Likewise, the cell membrane of myeloblasts is positive for nucleotide triphosphatase, but negative for diphosphatase and acid phosphatase. The dissimilarity between lysosomal and viral enzyme patterns, and the resemblance of viral and cell membrane enzyme content are strong support in favor of AMV maturation at the cell membrane. As already mentioned, the enzyme content of virus and cell membrane is also correlated in other host tissues. The presence or absence of nucleotide triphosphatase on the viral surface depends entirely on the presence or absence of this enzyme on the host cell membrane (Novikoff *et al.*, 1962, de Thé *et al.*, 1963,a,b, 1964). On the other hand, the lysosome-like granules of myeloblasts are involved in phagocytosis, and are therefore ingestive organelles rather than productive ones (Heine *et al.*, 1963a). Recent electron microscopic work with myeloblasts also documents the successive stages of AMV maturation (de Thé *et al.*, 1964). The pronucleoid of the virus can be seen to condense adjacent to the cell surface, apparently inducing the formation of a cytoplasmic bud. Later stages of AMV formation show such cytoplasmic buds of the approximate diameter of the virus particle well-developed and with outer and inner viral membranes and the viral nucleoid discernible. Through constriction at their base the buds become detached from the cell to form mature viral particles. During the process of virus elaboration the viral nucleoid has an electron-transparent center. After detachment from the cell the nucleoid shrinks somewhat to become uniformly electron-dense. The cellular site and course of virus maturation are principally the same in other AMV-infected tissues. Budding activity of the cell membrane is particularly pronounced in cells of AMV-induced nephroblastomas (Dmochowski *et al.*, 1961; Heine *et al.*, 1962b). This mode of virus formation does not seem to be confined to neoplastic cells, but can also be seen in nonneoplastic cells of various organs, such as the thymus, liver, and pancreas (de Thé *et al.*, 1963a,c; Heine

et al., 1963b). Intracytoplasmic structures, similar to those found in Rous sarcoma cells, and interpreted as being ribonucleoprotein particles in association with incomplete virus, may also be seen in AMV-infected cells (de Thé *et al.*, 1963a; Dmochowski *et al.*, 1964). As in RSV infection, the relation of these cytoplasmic structures to virus synthesis is still a matter of speculation.

5. *Biochemical Experiments with Malignant Myeloblasts*

Little is known concerning the biochemical events of AMV multiplication. Studies carried out so far have concentrated on malignant myeloblasts. With these cells only the fully established mechanism of virus synthesis can be studied, but not the early phases of virus-cell interaction. During exposure of cultured myeloblasts to H^3-uridine the RNA of the various cellular fractions becomes labeled, with a time course of labeling that is characteristic for each fraction. The incorporation of H^3-uridine into viral RNA follows a pattern which resembles that of the transfer RNA, and is dissimilar to the time course of labeling in the RNA of other cellular fractions and to the specific radioactivity of the cellular nucleotide pool (Sverak *et al.*, 1962). The conclusion that the transfer RNA serves as a precursor of the viral RNA is supported only very indirectly by the experimental data.

Two remarkable biochemical traits of myeloblasts should still be mentioned, neither of which seems to be directly related to virus synthesis. (1) Myeloblasts have the ability to utilize lactate at a rate that is even higher than the rate of lactate production by the cell (Becker *et al.*, 1959). This property puts the myeloblasts in a unique position compared to other malignant cells, which show rates of lactate production which by far exceed their capacity to utilize it. To these latter ones belong the Rous sarcoma cell and the malignant erythroblasts of avian erythroblastosis (Weber *et al.*, 1961; Becker *et al.*, 1959). (2) Myeloblasts in culture have a requirement for relatively high concentrations of folic acid (Becker *et al.*, 1962). Continuous growth of myeloblasts can be obtained only at folic acid levels that exceed 0.1 μg. per ml. An experimental explanation for this unusual requirement is unavailable at present.

C. *The Infectious Cycle of Other Avian Tumor Viruses*

1. *Assay and Virus Growth*

Several of the techniques discussed in connection with the assay of AMV are also applicable to the assay of other noncytopathic avian tumor viruses. In fact, the first avian tumor virus titrated by its ability to interfere with the growth of Rous sarcoma virus was a field strain of avian leukosis virus, causing predominantly lymphocytic neoplasms (Rubin, 1960a; Rubin *et al.*, 1961). The same principle of interference with RSV has also been applied to the assay of RAV, an avian leukosis virus occurring in stocks of Rous sarcoma virus (Rubin and Vogt, 1962). Virus assays may be based again on end-point dilutions or on comparisons of the degree of interference induced by known and unknown virus samples under identical conditions. Basically, it should be possible to develop analogous interference assays for all noncytopathic avian tumor viruses. The only restriction imposed on the design of such assays is the specificity of the interference observed among avian tumor viruses. As will be shown in Section IV,C,3, virus-induced resistance with avian tumor viruses is only found if interfering virus and challenge virus are antigenically closely related. This requirement for a close antigenic similarity must of course govern the choice of the challenge Rous sarcoma virus in the design of an interference assay. The fluorescent focus assay is likewise applicable to the titration of other noncytopathic avian tumor viruses. It has been used for studies with RAV, with strain R avian erythroblastosis virus, and with the Harris strain of Rous sarcoma virus (Vogt, 1964, and unpublished results). Since the fluorescent antibody staining shows a high degree of strain specificity, it is also possible to assay combinations of antigenically dissimilar avian tumor viruses differentially with antisera which stain only one virus strain in the same mixture. An end-point titration in tissue culture may also be based on the production of complement-fixing antigens detectable with mammalian antisera (Sarma *et al.*, 1964b). For virus titrations in the animal host, the technique of intravenous inoculation in 11-day-old chick embryos appears to be well suited. Studies on four strains of avian leukosis virus show that a given virus dilution induces a higher incidence of neoplasms within a shorter latent period after intraembryonal inoculation, as compared to inoculation in 2-week-old chickens (Piraino *et al.*, 1963). However, even if the virus is inoculated in chick embryos, an observation period of 40 to 50 days is necessary for an estimate of the virus titer by an end-point technique.

As is evident from the initial studies with the interference assay of avian leukosis viruses, field strains of lymphomatosis virus are capable of multiplying in chick fibroblast cultures (Rubin, 1960a). The growth curve is qualitatively similar to that of RSV except for a somewhat slower rate of virus multiplication (Rubin, unpublished). Fibroblasts infected with lymphomatosis virus release virus throughout the life of the culture without undergoing morphological transformation.

Growth curves of RAV have been determined with the interference assay and with the fluorescent focus assay (Hanafusa *et al.*, 1964b; Vogt, 1964). The first newly synthesized virus is detected 12 hours after infection and maximum virus production is reached by 48 hours. The growth of RAV very closely parallels that of RSV. This may be of significance for the interaction between these two viruses during maturation, and will be taken up in Section IV,C,4.

Avian erythroblastosis virus can be maintained in chicken tissue culture for extended periods of time (Ruffili, 1938). Virus production is continuous and proceeds even in purely fibroblastic cultures. No morphological changes in the infected fibroblasts are observed (Doljanski and Pikovski, 1942). Likewise, no microscopically identifiable malignant transformation has been seen in chicken bone marrow cultures infected with avian erythroblastosis virus *in vitro* (Lagerlöf, 1960a). However, circumstantial evidence indicates that chicken bone marrow cells exposed to avian erythroblastosis virus do acquire malignant properties. If such cells are implanted into the avian host, they induce a systemic erythro-leukemia. The early onset of this disease indicates that it is, at least in part, a result of the dividing injected cells, and differentiates it from the virus-induced form of the disease which shows a longer latent period (Lagerlöf, 1960b,c,d).

2. *Light and Electron Microscopic Investigations*

Kinetic measurements on the growth of noncytopathic avian tumor viruses are not yet detailed enough to indicate the cellular site of virus completion. But studies with the fluorescent antibody technique amply demonstrate the involvement of the cell membrane in virus synthesis. In avian erythroblastosis the surface of the circulating malignant cells is virtually covered with viral antigen (Iwakata, 1961a,b). A similar occurrence of viral antigen at the cell membrane can be seen in chick fibroblast cultures inoculated with strain R erythroblastosis virus. Several separate and antigenically different isolates of avian leukosis virus from various commercial flocks of chickens also show preferential accumulation of viral antigen at the surface of the infected cells (Vogt, unpublished observations). RAV induces a pattern of viral antigen formation which is almost identical to that of RSV. Viral antigen can be found in the cytoplasm and at the cell surface (Rubin and Vogt, 1962). The only significant difference to RSV is the lack of cellular transformation in RAV-infected cells. Even when fluorescent antibody staining indicates maximal rates of virus synthesis in a RAV-infected fibroblast, the shape of the cell does not deviate appreciably from the normal. Chicken fibroblasts infected with field strains of avian leukosis virus likewise retain their normal morphology (Rubin, 1960a).

At the electron microscopic level avian erythroblastosis virus has been studied in great detail (Iwakata and Amano, 1958; Iwakata and Ichikawa, 1960; Benedetti and Bernhard, 1958; Dmochowski *et al.*, 1958; Heine *et al.*, 1961). Two main types of association between virus and cell have been found. The first is a reflection of virus synthesis, and is clearly demonstrated in thin sections of erythroblasts. All stages of virus formation can be observed at the surface of these cells. A small area of the cell membrane first shows increased electron density and thickness and a second membrane may be seen underlying the thickened area. Both membranes participate in the formation of a cytoplasmic bud which becomes constricted at its base and is thus liberated from the cell to form a virus particle. The newly released virus particles have a comparatively electron-transparent center; the dense nucleoid found in the majority of the virus particles appears to be formed only after release, presumably as a condensation of material that was associated with the inner membrane. The second type of interaction between erythroblastosis virus and cell, as seen in the electron microscope, has probably no connection with virus synthesis. Especially in macrophages, but also in erythroblasts, large numbers of particles can be found densely packed in cytoplasmic areas surrounded by a membrane. The presence of these complete virus particles in the cytoplasm appears to reflect a high phagocytic activity of the cell. Evidence for this comes from the observation that inert particular material is concentrated in the cytoplasm of the same cell types in a similar way as are complete virus particles (Heine *et al.*, 1961). The ultrastructural morphology of cells infected with Fujinami sarcoma virus closely resembles that of Rous sarcoma cells (Mannweiler and Bernhard, 1958).

Several reports in the literature draw attention to the occurrence of virus particles in normal chickens and chick embryos (Benedetti *et al.*, 1956; Benedetti, 1957; Febvre, 1958; Karrer and Cox, 1960; Zeigel, 1961). These viral particles are morphologically indistinguishable from avian tumor viruses, and it is reasonable to assume that their presence in normal animals represents an infection with one of the ubiquitous strains of avian leukosis virus. The ultrastructural features of such infections are therefore relevant to the study of avian tumor viruses. Particularly noteworthy is, again, the involvement of the cell membrane in virus formation. In fact, some of the clearest electron micrographs depicting sequential stages of avian tumor virus formation at the cell surface come from thin sections of normal chicken pancreas (Zeigel, 1961). No other structural manifestations of virus synthesis are seen besides the budding activity at the cell surface.

D. *Synopsis of Avian Tumor Virus Multiplication*

The infectious cycles of all avian tumor viruses are remarkably similar. The cardinal features, such as long latent period, continuous release, and virus completion at the cell surface, are encountered in every avian tumor virus studied so far, and are compiled in Table IV. These resemblances are important and serve further to define the avian tumor viruses as a group. Yet such a unified and apparently simple picture also implies an important shortcoming of the studies on avian tumor virus multiplication, namely, the failure to detect a basis for the profound biological diversity in the avian tumor virus group. The reported differences in the multiplication cycles of avian tumor viruses are mainly quantitative, with the largest variations being found in the rate of virus production (Rubin, 1964a). Qualitative differences are minor and of questionable significance (Dmochowski *et al.*, 1964). In view of such close similarities in the mode of virus production the striking differences in the carcinogenic spectra of various avian tumor viruses are all the more puzzling. Why does AMV not transform fibroblasts? A simple answer would be a tissue specific susceptibility to AMV, but fibroblasts, as well as other avian cells which show no neoplastic response to AMV infection, are nevertheless capable of supporting AMV growth. It is becoming more and more obvious that present methods of analysis have not yet even touched on such important questions as the tissue specificity of viral carcinogenesis. It is probable that future efforts in this direction will come closer to uncovering the mechanisms of viral carcinogenesis than have past investigations of the kinetics of virus growth.

TABLE IV
COMMON FEATURES OF AVIAN TUMOR VIRUS GROWTH

Latent period:	Comparatively long, 12–18 hr.
Time at which maximal rate of virus synthesis is reached:	2–3 days after infection
Location of cell-associated virus:	External cell surface
Location of viral antigen in infected cells:	Cytoplasm and cell surface
Cellular site of virus assembly and maturation:	Cell surface
Release time:	Short, less than 3 min.
Type of virus release:	Continuous, by budding of the cell membrane without drastic impairment of cell viability

IV. The Defectiveness of RSV

A. *Experiments Supporting the Postulate That RSV Is Defective*

1. *Rous Sarcoma Cells That Fail to Produce Infectious RSV*

Avian virus tumors usually contain the etiological agent in infectious form, at least at some stage of their development. Virus synthesis and viral carcinogenesis are therefore not incompatible at the tissue level. But even at the level of the single cell transformation and virus production may go hand in hand. For Rous sarcoma cells this is clearly demonstrated by an investigation of the infectious center assay (Rubin, 1960b). These experiments establish unequivocally that an individual chick fibroblast may become transformed and release virus. However, this coexistence of the reproductive and carcinogenic viral functions in the same cell does not imply their mutual dependence on each other. It is already evident from the observations on the suppression of focus formation by fetal calf serum that virus synthesis does not necessarily entail neoplastic transformation (Rubin, 1960d). This conclusion is strengthened and amplified by the recent finding that some clones of RSV-infected cells may, at least temporarily, show normal cellular morphology and yet release infectious virus (Trager and Rubin, 1964). The reverse, namely, RSV-induced cellular transformation without concomitant virus production, may also be found under certain conditions with the Bryan standard and the Bryan high-titer strains of RSV. This transformed, virus-free state of RSV-infected cells has given rise to the concept of defectiveness of RSV, which will be discussed in the following section.

Two lines of investigation have led to the unexpected finding of Rous sarcoma cells that fail to produce infectious virus. One is a study on the effect of various feeder cells on virus synthesis by RSV-infected cells, and the other is an attempt to define the relationship of RSV to RAV, an avian leukosis virus present in many stocks of RSV and immunologically indistinguishable from RSV (Temin, 1962, 1963a; Hanafusa *et al.*, 1963). In both studies single foci of Rous sarcoma cells are isolated and tested for active virus production. Only a certain proportion of such foci is found to release virus; the remainder does not synthesize infectious RSV. The most important factor determining the incidence of nonproducing foci is the multiplicity of infection. At high multiplicities of infection all single foci actively produce RSV. At low multiplicities of infection the majority of the isolated foci fails to synthesize RSV. Culture conditions comprise another factor of influence on the proportion of nonproducers found among isolated RSV foci. A given inoculum of RSV plated on a culture of chicken cells may result in the appearance of only virus-producing foci, but may yield a large percentage of nonproducers if plated on sparsely seeded chicken cells growing on a feeder layer of X-irradiated mouse cells. The effect of the heterologous feeder cells on the incidence of nonproducing foci is also found if chick embryo cells are transferred soon after infection on mouse cells. If the chicken cells are transferred several days after infection, the heterologous feeders have no influence on the incidence of nonproducers. Nonproducer cells can be propagated in tissue culture for extended periods of time—at least several months. They divide at a rate which appears to be faster than that of noninfected control cells. In cultures where normal chick fibroblasts are used as feeders these eventually become outgrown by nonproducer cells. A differential decline in the growth rate of normal fibroblasts, especially after several weeks of culture, may also contribute to the final predominance of nonproducer cells. The nonproducer cells remain transformed throughout the period of culture. Regular tests show that most nonproducer cultures remain free of virus indefinitely. Not only do these cells fail to release virus, but they are also devoid of infectivity if broken up by freezing and thawing or by sonic vibration. However, spontaneous RSV production is encountered in some nonproducer cultures. The appearance of this synthetic activity is invariably accompanied by the production of RAV. Complete synthesis of RSV can be predictably activated in nonproducer cultures by superinfection with any avian leukosis virus. In such a case the vast majority, if not all, of nonproducer cells become producers of RSV and of the superinfecting virus. Superinfection with an avian tumor virus is the only way by which RSV maturation can be activated in nonproducer cells. X- and UV-irradiation as well as amethopterin and mitomycin C are ineffective in this respect, and so is superinfection with members of the myxovirus group, which are structurally related to the avian tumor viruses, and also mature at the cell surface. The process of activation must occur in the nonproducer cells. Simple mixing of cell-free supernatant from nonproducer cells with other avian tumor viruses does not produce any active RSV.

In contrast to the initiation of Rous sarcoma virus infection, activation of nonproducers is not inhibited by antagonists of DNA synthesis (Temin, 1964a). This finding suggests a way to test the role of DNA synthesis in avian tumor virus infection. In normal fibroblasts RAV production is inhibited by antagonists of DNA synthesis (Bader, private communication). If this reflected an effect on the manufacture of viral

RNA, it should also be operative in nonproducer cells. In this case activation in the presence of DNA antagonists should result only in the production of RSV, not of RAV. If, on the other hand, a function already established in the nonproducer cell can substitute for the process inhibited by DNA antagonists, then RAV as well as RSV should be synthesized after activation of nonproducers under conditions where DNA synthesis is inhibited. In this case a connection between the required DNA synthesis and the viral genome would be unlikely.

2. The Nonproducing State of Rous Sarcoma Virus: Explanation of Its Origin

The observations discussed in the previous paragraph lead to the following conclusions (Hanafusa *et al.*, 1963): The nonproducing state of Rous sarcoma cells is a consequence of solitary infection by RSV. A significant proportion of nonproducers is therefore found only among foci induced with a high dilution of RSV. There is good evidence that all solitary infections of the Bryan high-titer strain lead to cellular transformation without concomitant virus production. Whenever there is RSV synthesis in a single focus induced by the Bryan high-titer strain there is also production of RAV, indicating that a double infection has occurred. This is true, not only of RSV foci which actively release virus at the time of their isolation, but also of those nonproducer cultures which begin to synthesize RSV "spontaneously" after several transfers. The absence of RSV synthesis after solitary infection by RSV, and its presence after double infection with RSV and RAV, indicate that RAV infection is necessary for the completion of RSV synthesis. This is strongly supported by the finding that experimental superinfection of nonproducer cultures with RAV invariably activates RSV production. The Bryan high-titer strain of RSV must therefore be considered defective, unable to direct complete virus synthesis without the assistance of another avian tumor virus acting as helper virus in the same cell. The requirement for a helper virus in RSV production explains the presence of high titers of RAV in stocks of Rous sarcoma virus. RAV is the natural helper virus of RSV. The preponderance of RAV over RSV in RSV stocks guarantees double infection with the defective virus and its helper with all except the most diluted inocula. The need for the cooperation of a second avian tumor virus in Rous sarcoma virus synthesis also explains the effect of heterologous feeder cells on the incidence of nonproducer foci. These feeders are not susceptible to RAV infection, and RAV is therefore less abundant in such cultures, reducing the chances of double infection for chicken cells. Another means by which the probability of secondary RAV infection is diminished is the incorporation of RSV antibody in the agar overlay of the culture from which single foci are to be picked. Since RSV antiserum also neutralizes RAV, this procedure suppresses the spread of newly produced RAV during the incubation of the cultures, and minimizes the danger of secondary RAV infections during the process of focus isolation. "Spontaneous" RSV synthesis in nonproducer cultures probably arises from accidental contamination of the focus with RAV at the time of isolation or during one of the successive transfer manipulations. The fact that even after prolonged culture RSV production can be activated in virtually all nonproducer cells by superinfection with RAV indicates that the RSV genome is present in these cells. Despite its defectiveness RSV must therefore be capable of initiating the multiplication of its own genome in the transformed cell. This suggests that a helper virus is needed only for the final stages of RSV synthesis and maturation, and that the early events of virus replication take place in the absence of a helper.

To summarize, all observations made on the origin and properties of nonproducing Rous sarcoma cells are in accord with the hypothesis that RSV is defective. A helper virus appears to be needed for some late stage of RSV synthesis, whereas early events of RSV infection, such as the virus-induced transformation of the cell and the replication of the RSV genome, can proceed without the assistance of a second virus. Since most of the decisive experiments have been carried out with the Bryan high-titer strain of RSV, the statement of defectiveness must at present be restricted to this virus (Hanafusa *et al.*, 1963). But the results obtained with the Bryan standard strain (Temin, 1962, 1963a) are fully explicable by the hypothesis of defectiveness. The finding of RAV in this and other strains of RSV makes it likely that the defectiveness of the Bryan high-titer strain will not remain an isolated discovery. It should be pointed out that not all workers in the field consider the RSV nonproducer cells as resulting from a defectiveness of the virus. As an alternative it has been suggested that the nonproducing state reflects a close interaction of the viral and the cell genomes (Temin, 1964a). This suggestion is not contradicted by observed facts, but it fails to provide an explanation for the well-documented role of the helper virus and for the observation that RSV strains which induce a nonproducer state upon solitary infection appear to have no unique envelope antigen of their own.

3. Cytological Evidence for Defectiveness

There are two main cytological criteria for RSV infection: the cellular transformation visible in the light microscope, and the appearance of viral antigen demonstrable with fluorescent antibody in the UV microscope. The latter criterion is also indicative of virus production. When chick embryo fibroblast cultures infected with a low multiplicity of RSV are checked for these two criteria, it is found that transformation and viral antigen production are not linked to each other. In these cultures three types of infected cells are seen: (1) fibroblasts which elaborate viral antigen but are not transformed, (2) transformed cells which are free of viral antigen, and (3) cells which are transformed and also contain viral antigen. The defectiveness of RSV and the presence of a helper virus in the inoculum suggest that the first type of cells represents solitary infection with RAV, the second type solitary infection with RSV, and the third type combined infection with RAV and RSV. The second type of cells, marked by transformation in the absence of viral antigen, appears of particular significance in relation to defectiveness. If these nonfluorescent RSV foci were indeed the result of a solitary infection by RSV, their incidence should be dependent on the multiplicity of infection in a similar way as is the incidence of nonproducer foci. Experiments with the Bryan high-titer strain of RSV show that this is indeed the case, and the nonfluorescent foci are therefore the cytological equivalent of the nonproducers (Vogt, 1964). The cytological observations on chick fibroblast cultures infected with high dilutions of RSV are also in accord with the postulate that RSV foci engaged in virus synthesis are the product of a double infection with RAV and RSV.

B. The Role of the Helper Virus

1. Absence of Viral Coat Proteins from Nonproducer Cells

As pointed out earlier, an approximate demarcation of helper virus function may be derived from the capabilities of RSV alone, which include the early stages of virus replication. The helper virus appears to be needed for the late steps in RSV synthesis. More definite information on the role of the helper comes from immunological experiments with nonproducer cells (Hanafusa *et al.*, 1964a; Vogt, 1964). Extracts of disrupted nonproducer cells fail to bind RSV neutralizing antibody, whereas extracts from producing cells strongly absorb such antibody. The maximum level of viral coat antigen which could be present in the nonproducer cells without being detected by absorption tests is about 17% of that found in an equal number of RSV-producing cells. A more sensitive indicator for the presence of a viral coat antigen is the induction of an immune response in the chicken. Nonproducer

cells readily induce sarcomas when inoculated into chickens, but the tumor growth is not followed by the production of virus-neutralizing antibodies, nor do the animals develop a cell-bound immunity which would result in resistance to challenge infection with RSV. This is in contrast to the effect of RSV-producing sarcomas, which stimulate the synthesis of neutralizing antibodies as well as the development of a cell-mediated immune response operating against the tumor cells. Further proof for the lack of viral coat antigen in nonproducer cells comes from studies with fluorescent antibodies. Nonproducer cells are entirely free of the antigens which are readily demonstrable with fluorescent chicken antibodies in the cytoplasm and at the surface of RSV-producing and RAV-infected cells. This finding also demonstrates that most, if not all, of the antigens participating in the fluorescent antibody staining are located in the viral coat. It should be emphasized that only the antigen of the RSV coat has been shown to be absent from nonproducer cells. RSV is a relatively complex virus and may therefore be expected to have internal antigens as well. These antigens are not detectable by neutralization tests and also appear to escape detection with the fluorescent antibody technique. In addition to such structural proteins of the viral core, functional viral proteins may be present in nonproducer cells and perform various tasks connected with the replication of the viral RNA. It is therefore to be expected that future work will uncover some RSV antigens in nonproducer cells.

2. Specification of RSV Antigenicity by the Helper Virus

The absence of coat protein from nonproducer cells suggests that the synthesis of this protein is a function of the helper virus. Since nonproducer cells are activable by any avian leukosis virus this suggestion is amenable to experimental test. The prediction implicit in the helper-control of coat protein synthesis is that RSV should have a coat immunologically identical with that of the helper, and that different helper viruses should furnish RSV with immunologically different coat proteins. This prediction has indeed been verified by comparative neutralization experiments with RSV obtained after activation of nonproducers with RAV and RSV prepared by activating other nonproducers with different strains of avian leukosis virus (Hanafusa *et al.*, 1964a).

Preparations of RSV obtained by activating nonproducers with different helper viruses are antigenically distinct from each other and are indistinguishable by neutralization tests from their respective helpers. The influence of the helper virus on RSV antigenicity is further documented by experiments using strain-specific fluorescent antibodies against avian leukosis viruses (Vogt, 1964). Nonproducer cells activated with RAV only bind fluorescent RAV-antibody. Nonproducer cells activated with AMV stain exclusively with AMV antiserum. No matter what antigenicity the parental RSV used to initiate a nonproducer line may have, the progeny virus released after activation always has a coat which is immunologically identical to that of the helper virus. These experiments firmly establish that one missing link in the maturation of RSV is the production of coat protein. This viral component is made under the exclusive direction of the helper virus. It is still undecided whether the influence of the helper virus on RSV maturation goes beyond the mere production of coat protein. It is possible that the helper is also needed to direct the integration of this coat protein with the cell membrane and the induction of the budding which leads to virus release. Nucleocapsids of the helper as well as of RSV appear to become enveloped by these buds at random.

Extracellular RSV is thus wrapped in a borrowed coat. RSV does not contribute genetic information to the manufacture of this coat, nor does it effect the transmission of the coat to progeny virus. The genetic information for the RSV coat must be supplied anew for each viral generation by the helper virus. This allows one to change the viral coat of RSV experimentally by providing a nonproducer line with a new helper virus. The interaction between RSV and the helper occurs at a nongenetic level. It can be considered as a limiting case of phenotypic mixing with one partner, namely, RSV, contributing zero (Hanafusa *et al.*, 1964a).

3. *Bipartition of RSV Infectious Cycle*

The defectiveness of RSV introduces a natural dichotomy in the infectious cycle of this virus (Fig. 1). There are first some early functions of the infectious process: the replication of the RSV genome and the neoplastic transformation of the infected cell. Both are directed by RSV itself. Then there are the late functions of the infection: virus maturation and release, for which the assistance of the helper virus is required. Properties of RSV which remain the same regardless of the helper can therefore be considered as anchored in the RSV genome. They are likely to be of importance for the early stages of infection occurring after the penetration of the virus. They may also be properties of the viral nucleocapsid. Properties of RSV which change with different helpers belong to RSV only phenotypically. They are most likely properties of the borrowed viral envelope, and will therefore affect primarily those phases of the infection in which the envelope plays an important part, namely, adsorption and penetration. These two types of RSV prop-

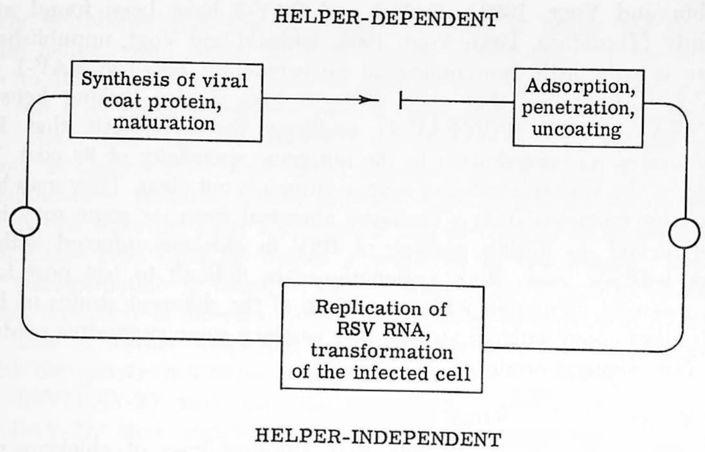


FIG. 1. Dichotomy of RSV infectious cycle.

erties, helper-controlled and helper-independent ones, will be discussed in the following sections.

C. *Helper-Controlled Properties of RSV*

1. *Antigenic Variation*

As has already been discussed, the antigenic specificity of the RSV coat is determined by the helper virus. This is not only the case with major antigenic determinants as are provided by such different helpers as RAV and AMV, but it is also true of the more subtle antigenic variation found within stocks of the Bryan high-titer strain of RSV. Recent observations show that this strain of RSV is highly heterogeneous and consists of at least three different antigenic variants, as determined by neutralization and fluorescent antibody tests. These variants have been designated as RSV(RAV-1), RSV(RAV-2), and RSV(RAV-3) (Rubin, 1964a; Hanafusa, 1965; Vogt, 1965; Ishizaki and Vogt, unpublished). This immunological heterogeneity is due to the presence of at least three different helper viruses in the stocks of the Bryan high-titer strain: RAV-1, RAV-2, and RAV-3. With the same techniques which have been used to demonstrate the specification of coat protein by the helper virus it can be shown that the substrain variation of Bryan high-titer RSV is controlled entirely by the helper virus, i.e., RSV(RAV-1) is antigenically identical to RAV-1, RSV(RAV-2) to RAV-2, and RSV-(RAV-3) to RAV-3. RAV-1 is the helper virus isolated previously

(Rubin and Vogt, 1962). RAV-2 and RAV-3 have been found more recently (Hanafusa, 1965; Vogt, 1965; Ishizaki and Vogt, unpublished). There is only little immunological cross-reaction between RAV-1 and RAV-2. The finding that such cross-reaction is also lacking between RSV(RAV-1) and RSV(RAV-2) reaffirms the hypothesis that RSV itself makes no contribution to the antigenic specificity of its coat. The origin of the various strains of helper viruses is not clear. They may have arisen by mutation from a common ancestral form, or some may have been picked up during passage of RSV in chickens infected with an avian leukosis virus. Both explanations are difficult to test post facto, but a careful immunological comparison of the different strains of RAV with other avian leukosis viruses may produce some suggestive evidence for their common origin.

2. *Intraspecies Host Range*

It is a common observation that different lines of chickens show different degrees of susceptibility to avian tumor viruses. For instance, studies carried out in the animal host indicate that the resistance to RSV encountered with certain chicken lines is of a heritable nature (Greenwood *et al.*, 1948; Waters and Fontes, 1960). This resistance is operative in the embryo as well, where it affects the number of RSV tumors found after inoculation of the chorioallantoic membrane (Prince, 1958b). The genetic resistance to RSV found in the whole animal or embryo is also apparent in tissue cultures derived from resistant birds (Crittenden *et al.*, 1963). This finding demonstrates that genetic resistance is a true cellular property and is not based on a defense mechanism effective only at the organismic level. Resistance of tissue cultures does not only prevent focus formation of RSV but also suppresses virus growth (Payne and Biggs, 1964). Recent experiments demonstrate that the genetic resistance to RSV and other avian tumor viruses is highly selective for certain virus strains (Hanafusa, 1965; Vogt, 1965). In several commercial lines of chickens a resistance is demonstrable which is effective against RSV(RAV-2) but not against RSV(RAV-1). In tissue culture this resistance prevents focus formation as well as virus production of RSV(RAV-2). The same tissue cultures that show this resistance to RSV(RAV-2) are also resistant to RAV-2. After infection with RAV-2 they do not produce viral antigen and do not liberate a virus which would interfere with RSV(RAV-2). When nonproducer lines initiated by RSV(RAV-1) or RSV(RAV-2) are activated by either RAV-1 or RAV-2, the resulting RSV does not only carry the antigenic specificity of the activating helper virus, but it also shares its host range on selectively resistant chick embryo cells. This clearly establishes that the host range of RSV is under the control of the helper virus. Since the only known contribution of the helper virus to RSV is the coat protein it appears reasonable to assume that the viral coat plays a decisive role in determining the host range of RSV. The genetic cellular resistance operates against the helper virus as well as against RSV. One might therefore imagine that it is a lack of helper virus function which prevents RSV growth in the resistant cells. If this were the case, RSV should still be capable of transforming the resistant cells since no helper is required for this process. But no such transformation is found. The resistance must therefore establish a block very early in infection, presumably during adsorption or penetration. This is further supported by the failure of RAV-1 infection to abolish the genetic resistance to RSV(RAV-2). RAV-1 does not interfere with RSV(RAV-2) and can infect the cells selectively resistant to RSV(RAV-2), thus supplying these cells with active helper function. Despite the presence of a helper the cells remain resistant to RSV(RAV-2), which therefore does not seem to accomplish penetration (Hanafusa, 1965). The hypothesis that the genetic resistance affects early processes of the infectious cycle is in keeping with the important role assigned to the helper-controlled viral envelope in determining the specificity of this reaction. It should be noted briefly that the embryos which show a selective genetic resistance to RSV(RAV-2) are also resistant to the Schmidt-Ruppin strain, to the Harris strain of RSV, and to a substrain of AMV designated as AMV-2. They are fully susceptible to RSV(RAV-3) of the Bryan high-titer strain, to the Bryan standard strain of RSV, and to Fujinami virus (Vogt, 1965 and unpublished experiments). The *in vitro* experiments so far have only dealt with the intraspecies host range of RSV. It is quite likely that future work will demonstrate an importance of the viral envelope for the extension of the RSV host range beyond the species of *Gallus domesticus* and even beyond the avian class (Rubin, 1964a).

3. *Interference Patterns between RSV and Other Avian Tumor Viruses*

Various avian leukosis viruses are capable of inducing a strong cellular resistance to challenge infection with RSV in chick fibroblast cultures (Rubin, 1960a; Rubin and Vogt, 1962; Vogt and Rubin, 1963). Experiments on the nature of this resistance show that the plating efficiency of RSV on cultures infected with an avian leukosis virus may be reduced as much as 10^3 to 10^6-fold. Even with this virus-induced resistance, however, RSV focus formation does not require the cooperation of several RSV particles but is initiated by single transforming units of RSV. The resistant cultures are essentially homogeneous with respect to their susceptibility to RSV, the probability for successful infection being decreased to approximately the same extent in each cell (Rubin, 1961a; Hanafusa *et al.*, 1964b). The full extent of the virus-induced resistance to RSV is observed only if the interfering virus is given a headstart in infection (Rubin, 1960a; Ting, 1964). But even simultaneous infection with interfering virus and RSV may result in some resistance to the latter provided the concentration of the interfering virus is very high. In the case of RAV-1 such rapid interference has been demonstrated with multiplicities of infection of around 10 RAV infectious units per cell (Hanafusa *et al.*, 1964b). The quantitative relationship between the amount of interfering virus and the degree of resistance to RSV in these experiments suggests that a single particle of RAV is capable of inducing an immediate partial resistance to RSV in a cell, presumably by lowering the number of freely available penetration sites. Interfering virus given after RSV has entered the cell does not induce resistance, even at high multiplicities of infection. In the focus assay of RSV the inoculum invariably contains RAV also. However, in this case the multiplicities of the interfering virus are by far too low to cause simultaneous interference.

The existence of many different strains of avian leukosis virus raises the question of whether all these agents are able to induce a cellular resistance effective against all strains of RSV. An indication that this is not the case may be found in a recent communication by Hanafusa *et al.* (1964c). Further experimentation has suggested that interference between avian tumor viruses is effective only if interfering and challenge virus are closely related antigenically (Hanafusa, 1964, 1965; Vogt, 1965). For instance, RAV-1 interferes with RSV(RAV-1) and with RSV(RAV-3) but not with RSV(RAV-2). As shown previously, RAV-1 is antigenically identical with RSV(RAV-1), and neutralization as well as fluorescent antibody studies suggest a close antigenic relationship between RSV(RAV-1) and RSV(RAV-3). On the other hand, experiments comparing RSV(RAV-1) with RSV(RAV-2) have failed to reveal such a close immunological relationship. Consequently, RAV-2 interferes only with RSV(RAV-2) but not with RSV(RAV-3) or RSV(RAV-1). The correlation between antigenic similarity and interference implies that a helper virus always interferes most strongly with its corresponding RSV. This has been verified by experiments. In fact, no exception to the rule that interference requires antigenic relationship has been found in extensive tests with 10 different strains of avian leukosis virus and 12 strains of RSV (Vogt, unpublished).

It appears paradoxical that the same virus which guarantees completion of RSV synthesis, if given after RSV has entered the cell, should be most effective in causing resistance if granted first entry into the cell. In order to solve this dilemma, the suggestion has been made that the processes by which a helper virus intervenes in RSV maturation are at the same time specifically suppressive for early events in infection and thus exclude the establishment of a related challenge virus in the same cell (Hanafusa *et al.*, 1964b). This would also explain why the interference is not reciprocal, and cells infected with RSV alone, i.e., nonproducer cells, do not lose their susceptibility to superinfection with avian leukosis viruses. In these cells the same late functions of the infectious cycle, which presumably are suppressive for early events, are lacking. If this hypothesis is correct one would expect reciprocal interference between nondefective RSV strains and antigenically related avian leukosis viruses. The role of the helper virus in determining the interference pattern of RSV suggests that the virus coat is involved in the mechanism of this interference. This would locate the crucial barrier in some early steps of infection, possibly in adsorption, penetration, or uncoating. In this respect there is a close similarity between the virus-induced resistance and the genetically determined resistance discussed in the previous section. At this point brief consideration should be given to the possible role of interferon in the virus-induced resistance to RSV. The participation of interferon in this reaction has been suggested (Allison, 1963), and interferon action has been demonstrated in a RAV stock (Bader, 1962). However, recent attempts to isolate interferon from preparations of avian leukosis viruses have failed (Hanafusa *et al.*, 1964b). The resistance-inducing activity of RAV sediments with the virus and is neutralized by antiviral antibodies. A mere intracellular production and action of interferon is ruled out by the strain specificity of the interference patterns between avian tumor viruses. Interferon is species-specific but not virus-specific, and certainly does not discriminate between substrains of a given virus, as does the interference between avian tumor viruses.

4. Growth Rate

A comparison of the growth curves of RAV and RSV reveals close similarities in all essential points (Hanafusa *et al.*, 1964b). Since helper virus participation is essential for RSV maturation, it is likely that the rate of this maturation is also controlled by the helper. This would mean that the early functions directed by RSV itself proceed at a rate exceeding that of the late functions which are governed by the helper. These final processes in the assembly of progeny virus appear to be the rate-limiting step for the production of RSV as well as for that of the helper virus. This is supported by the finding that helper viruses with different growth rates also impose their rate on the growth of RSV to which they render assistance (Rubin, 1964a).

D. Helper-Independent Properties of RSV

1. Morphology of Transformed Cells and Focus Structure

The helper-controlled characteristics of RSV dramatically affect the fate of the virus during early stages of infection. Because of their conspicuousness in existing test procedures these properties are well investigated. Comparatively little is known, however, about the helper-independent properties of RSV. The cellular transformation induced by RSV does not require a helper virus. It appears reasonable, therefore, to speculate that the specific features of this transformation are controlled by RSV itself. One of these features is the morphology of the transformed cell. Two main types of Rous sarcoma cells have been recognized in tissue culture: fusiform and round ones (Temin, 1960, 1961). If RSV itself controlled the shape of the sarcoma cell, the virus appearing after activation of a fusiform nonproducer line should always induce fusiform transformation itself, regardless of the helper virus used. The same morphogenetic stability should be found with RSV from round nonproducers. Critical experiments designed to test this hypothesis have not yet been carried out. But cursory observations indicate that the proposition is correct. A change in the morphology of the transformed cell has never been reported in connection with a change in the helper virus. Additional virus-controlled morphological features of transformed cells have recently been reported (Purchase and Okazaki, 1964). It will be of interest to see whether these, too, are helper-independent. Another aspect of RSV mediated transformation is represented by the intercellular relationships in a focus. Foci induced by the Bryan high-titer strain consist of a compact area of transformed cells, often piled up in several layers, with no, or few, interdispersed fibroblastic cells. Foci initiated by the Schmidt-Ruppin strain of Rous sarcoma virus are usually larger, less distinctly delineated, show little or no clumping of the transformed cells, and contain fibroblastic cells interdispersed with rounded cells. The Fujinami virus forms foci which have a structure similar to those of the Schmidt-Ruppin strain. Activation of nonproducer lines initiated by the Bryan high-titer strain with helper virus isolated from Fujinami virus leads to the production of RSV which continues to cause only foci of the Bryan high-titer type. Conversely, activation of Fujinami nonproducers by RAV from the Bryan high-titer strain induces the synthesis of a virus which produces only Fujinami-type foci (Vogt, unpublished experiments). Analogous findings

have been made with the Schmidt-Ruppin strain of RSV which may be wrapped in a RAV coat without change of the characteristic focus type (Rubin, 1964a). These observations suggest that the morphology of the transformed cell and the focus type are determined exclusively by RSV itself.

2. The Group-Specific Complement-Fixing Antigen

This antigen is listed here among the helper-independent properties only tentatively. There are at present no decisive experimental data which would justify this classification. However, suggestive support for a helper independence of the complement-fixing antigen derives from results which indicate that this antigen is a component of the viral

TABLE V
INFLUENCE OF HELPER VIRUS ON RSV PROPERTIES

A. Helper-independent functions of RSV:
1. Replication of viral RNA
2. Transformation of the infected cell
   a. Morphology of the transformed cell
   b. Morphology of the RSV focus
3. Group-specific, complement-fixing antigen (?)

B. Helper-controlled functions of RSV:
1. Rate of virus maturation
2. Antigenic specificity of the viral coat
3. Host range
4. Interference patterns

nucleocapsid (Huebner *et al.*, 1964). So far, there is no indication that the helper virus is involved in the formation of this structure. The putative helper independence of the internal complement-fixing antigen is amenable to simple experimental tests. Complement fixation with nonproducer cell lines as antigens should produce a clear answer to this question.

A survey of helper-controlled and helper-independent events in RSV synthesis is presented in Table V.

E. The Significance of Defectiveness

The discovery of defectiveness in the Bryan high-titer strain of RSV has important theoretical implications and opens up new possibilities of experimentation as well. Through the exchange of helper viruses some fundamental properties of RSV and of its helper viruses have been defined as functions of the viral envelope: antigenic variation, host range, and interference patterns. In view of these data on RSV and various helper viruses there can be little doubt that the assignment of these properties to the virus coat is justified for the entire group of avian tumor viruses. As already mentioned, the study of avian tumor virus coat antigens is greatly simplified by the existence of defective RSV. RSV may be used as a carrier for any avian tumor virus coat, and neutralization studies of all avian tumor viruses may be performed by using the accurate RSV focus assay (Hanafusa *et al.*, 1964a). The occurrence of defectiveness outside the Bryan high-titer strain is an important question still only incompletely solved. The following experimental criteria must be fulfilled in order to establish the presence of defective virus in an RSV stock: (1) An avian leukosis virus, acting as helper virus must be demonstrable in the virus preparations. (2) This virus should be immunologically indistinguishable from the RSV which it is suspected to activate. (3) Solitary infection with RSV should lead to the appearance of nonproducer cells. (4) Superinfection of such cells must activate RSV synthesis. The findings made by Temin (1962, 1963a) with nonproducer cells initiated by the Bryan standard strain of RSV are compatible with the hypothesis that this strain also contains defective virus. This is further supported by the isolation of RAV-activable nonproducer cells after solitary infection with Bryan standard RSV, and by the finding of an avian leukosis virus in stocks of the Bryan standard strain of RSV (Shimizu and Rubin, unpublished observations). The fact that this avian leukosis virus is antigenically indistinguishable from Bryan standard RSV strengthens the assumption that it may function as a helper virus (Vogt, unpublished). A similar situation is found with Fujinami virus. Virus stocks contain an avian leukosis virus of an antigenic constitution closely related to, and possibly identical with, that of Fujinami virus. Nonproducing foci of transformed cells may be isolated from cultures infected with a high dilution of virus (Vogt, unpublished results). Observations made in several laboratories indicate that the Schmidt-Ruppin strain of RSV is not defective. No avian leukosis virus which could qualify as a helper has been found in stocks of this strain, nor have foci of nonproducer cells been isolated after solitary infection (Dougherty, personal communication; Rubin, 1964a). Foci initiated by Schmidt-Ruppin RSV always show viral antigen even after conditions of single infection (Vogt, unpublished). Recently, a second virus, possibly belonging to the avian tumor virus group, has been discovered in stocks of Schmidt-Ruppin RSV. However, Schmidt-Ruppin RSV may be cloned, thus eliminating the contaminating virus without loss of the ability to produce complete viral progeny (Dougherty, personal communication). These findings suggest that Schmidt-Ruppin RSV does not require or use a helper virus for the

maturation of its progeny. However, Schmidt-Ruppin RSV may enter a relationship with avian leukosis viruses similar to that of defective RSV and its helper. Double infection of chick fibroblast cultures with Schmidt-Ruppin RSV and RAV boosts RSV production, and results in the adoption of RAV coats by Schmidt-Ruppin RSV (Rubin, 1964a). The low rate of virus production characteristic for cells infected with Schmidt-Ruppin virus alone is apparently due to a limited supply of Schmidt-Ruppin-type coat protein.

The finding that not all RSV strains are defective raises the question whether some nondefective RSV particles occur also in those strains of RSV which have been shown to contain defective virus. Data which permit an estimate of the level of defectiveness are available only for the Bryan high-titer strain. Never during the extensive work with this virus has a producing focus been isolated which did not contain RAV (Hanafusa *et al.*, 1963, 1964a). Fluorescence microscopic inspection reveals that at low multiplicities of infection more than 99% of the RSV foci are free of viral antigen, indicating a nonproducer state (Vogt, 1964). If any nondefective RSV occurs in the Bryan high-titer strain at all, it must be present in exceedingly low quantities. The level of defectiveness in other strains of RSV and the possible occurrence of defectiveness in strains of avian leukosis virus remain to be determined. A problem related to the level of defectiveness concerns the formation of genetic recombinants between RSV and the helper virus, resulting in nondefective RSV. So far there is no indication of such a genetic interaction, but the efforts to detect it have only been cursory. It is plausible that the nature of the defect in RSV is genetic, but present data provide no rigorous proof for this.

The main theoretical implication emerging from the experiments on defectiveness is that virus maturation is not an essential part of RSV carcinogenesis. But even the early processes of RSV infection do not seem to be necessarily and immediately carcinogenic. This is suggested by the finding that RSV can be synthesized by cells which do not show neoplastic transformation (Rubin, 1960d; Trager and Rubin, 1964). RSV-induced malignant transformation may therefore be a process which is not directly linked to any step in virus synthesis. A different view on RSV carcinogenesis envisages defectiveness as the basis for the exceptionally high efficiency with which RSV induces malignant transformation (Rubin, 1964a,b). It is postulated that the replication of the RSV genome is regulated by the late events in virus synthesis. When the late events fail to take place, as in the case of defective RSV, the early replication of viral RNA proceeds unchecked and thus mediates transformation. At present these hypotheses are highly speculative; their merit will depend on their ability to generate guidelines for decisive experimental work.

V. Principles Governing Avian Tumor Virus Infection *in Vivo*

A. *Factors of Importance in the Individual Avian Host*

1. *Pathogenesis in the Chicken*

The pathogenesis of virus-induced avian neoplasms has been adequately covered by several reviews (Claude and Murphy, 1933; Foulds, 1934; Rous, 1936; Beard *et al.*, 1955; Beard, 1957a, 1963a,b; Spencer and Groupé, 1962a). Despite the recent progress in techniques dealing with tumor virus infection at the cellular and subcellular levels, questions of animal pathology remain of continued importance in research on avian tumor viruses. Many problems can be defined and solved only at the animal level, and insights gained by modern *in vitro* techniques must be implemented in the animal. Consequently, the opportunities for studies with conventional pathological methods are by no means exhausted, as is evident from recent papers in the field (Sevoian and Chamberlain, 1962, 1963, 1964; Sevoian *et al.*, 1962, 1964; Fredrickson *et al.*, 1964; Burmester and Fredrickson, 1964; Yamagiwa *et al.*, 1963). However, a detailed analysis of the published data would be beyond the scope of this survey. It should be sufficient here to outline briefly some of the important rules governing the course of avian tumor virus infection in the animal and to point out some of the more recent literature. All thoroughly investigated strains of avian tumor virus have proved to be capable of inducing several forms of neoplastic disease in the chicken. Under a given set of conditions the types and incidences of various growths are characteristic for a distinct strain of avian tumor virus, forming its oncogenic spectrum. The factors which influence this spectrum are (1) the amount of virus inoculated, (2) the route of inoculation, (3) the breed and (more rarely) the sex of the inoculated birds, and (4) previous history of exposure to avian tumor viruses. Virus multiplication includes many more tissues than does viral carcinogenesis. In fact, there appears to be no solid evidence for the existence of tissues which are incapable of supporting avian tumor virus multiplication in an otherwise susceptible avian host. This is well documented by experiments on the tissue distribution of RSV after subcutaneous infection (Munroe and Southam, 1959; Spencer and Groupé 1962a,b). Before a sarcoma becomes detectable at the site of inoculation, virus is present and probably multiplies in all organs tested. High titers of virus continue to be recovered after the primary sarcoma is well developed. Even tissues which are free of tumor foci appear to be actively producing virus. The participation of non-neoplastic tissues in virus production is even more pronounced with avian leukosis viruses, which generally show a low efficiency of malignant transformation. Liver and pancreas take part in AMV synthesis, although there is no evidence of a neoplastic AMV-induced transformation in these tissues (de Thé *et al.*, 1963b; Heine *et al.*, 1963b). A high proportion of non-neoplastic, virus-producing cells is also found in chick embryos congenitally infected with avian lymphomatosis virus and in the viremic birds developing from such embryos (Rubin *et al.*, 1961). In contrast to the remarkable lack of tissue tropism in the multiplication cycle of avian tumor viruses, there is a very strict and well-known tissue specificity of the avian tumor virus-induced carcinogenesis. This suggests that viral carcinogenesis, but not viral multiplication, is dependent on a distinct state of cellular differentiation. The requirement for specific target cells has been demonstrated for AMV which transforms primarily primitive cells of hematopoietic tissues (Baluda, 1962). Another example for tissue specificity in avian tumor virus carcinogenesis is the role of the *bursa Fabricii* in the genesis of lymphomatosis (Peterson *et al.*, 1964). Surgical removal of this lymphoid organ prevents the development of visceral lymphomatosis after injection of strain RPL 12 virus. Removal of the thymus has no similar effect, but thymectomy performed in addition to bursectomy enhances the suppression of lymphoid neoplasms observed after bursectomy alone. Other neoplasms induced by RPL 12 (erythroblastosis and osteopetrosis) are not affected by the removal of the bursa or of the thymus. The findings indicate that the bursa of Fabricius is of crucial importance for the development of virus-induced lymphoid neoplasms in the chicken. Whether this role is based on the supply of a special cell type susceptible to the carcinogenic action of the virus, or whether the bursa releases a humoral factor which renders cells in other lymphoid organs susceptible, cannot be determined from the present data. The clarification of the mechanism involved in the tissue specificity of avian tumor virus carcinogenesis is one of the most challenging tasks for future work.

2. *Genetic Determination of Host Susceptibility*

It has long been recognized that the susceptibility to avian tumor viruses is strongly influenced by the genetic constitution of the avian host. The first animal passages of RSV were successful only in chickens related to the bird from which the tumor was originally isolated (Rous, 1911). This exceedingly narrow host range is no longer a property of RSV after more than fifty years of experimental transmission with constant selection for virulence. Some of the currently used laboratory strains are even transmissible by contact between chickens housed in the same cage (Burmester, 1960). But throughout the laboratory history of RSV genetic differences of animal susceptibility have been observed (Rous and Murphy, 1914b; Carr, 1943a; Greenwood *et al.*, 1948; Prince, 1958b; Waters and Fontes, 1960; Smidova and Rada, 1964). The patterns by which resistance and susceptibility to RSV are inherited have been studied with reciprocal crosses of susceptible and resistant birds from several lines. The results are diverse and do not permit any generalized conclusions. In some lines of chickens susceptibility to RSV appears to be determined by a single, dominant autosomal gene; in others resistance is found to be the dominant trait, and in still other systems crosses produce progeny with intermediate resistance (Waters and Burmester, 1961; Dhaliwal, 1963; Bang and Foard, 1963; Bower, 1962; Bower *et al.*, 1964; Hanafusa *et al.*, 1964c). Genetically determined host resistance is also encountered with a variety of avian leukosis viruses (Eckert *et al.*, 1954c; Gowen, 1958; Waters *et al.*, 1958; Burmester *et al.*, 1960b; Payne and Biggs, 1964). Resistance to avian leukosis virus is often, but not always, correlated with resistance to RSV. As pointed out in Section IV,B,2, genetic resistance to RSV and to other avian tumor viruses is a cellular property persisting in tissue culture (Crittenden *et al.*, 1963; Payne and Biggs, 1964). The decisive interactions appear to occur during the initial stages of infection and involve the viral envelope and the cell membrane (Hanafusa, 1965; Vogt, 1965). Much progress may be expected in the near future from an *in vitro* analysis of genetic resistance to avian tumor viruses. Genetic resistance appears to be directed against infection per se rather than against virus-induced malignant transformation. Resistant strains of chickens also show lower incidence of "naturally occurring" RSV neutralizing antibodies which are common in sensitive strains, indicating that the resistant strain has a lower incidence of infection with field strains of avian lymphomatosis virus (Kenzy *et al.*, 1961).

3. *Homotransplantation*

Karyological techniques permit a differentiation of chicken cells derived from female birds and cells derived from male birds. In the chicken, as in other birds, the female is the heterogametic sex. The diploid chromosome set of the male contains two homologous sex chromosomes among the six largest pairs, the diploid female set contains only one of these chromosomes. These sex chromosomes can therefore serve as simple and reliable markers in following the fate of homotransplanted chicken tumor cells injected into recipients of the opposite

sex (Biggs and Payne, 1959; Pontén, 1962a). Another cytological technique for determining the sex of chicken cells is based on the presence of sex chromatin, which occurs frequently in female and rarely in male nuclei (Morgan and Andrese, 1961). With these techniques homotransplantability of four representative virus-induced tumors in the chicken has been studied.

a. RSV. Transplantation of Rous sarcoma cells into chickens induces a tumor within a shorter latent period than does the inoculation of RSV alone. This suggests that the donor cells represent a substantial part of the cell population during the initial growth of the transplanted sarcoma (Rous, 1913; Dougherty and Morgan, 1962). Experiments with donors and recipients of a different sex show indeed that during the early tumor growth the sex chromatin pattern of the sarcoma is that of the donor animal (Morgan and Andrese, 1962). However, a serial transplantation of such sarcomas reveals that donor-type metaphases decrease rapidly in the tumor; by the second transplant generation the portion of the donor cells has fallen below the 0.5% level (Pontén, 1962d). This finding suggests that Rous sarcoma cells are not serially homotransplantable and that the perpetuation of Rous sarcomas over several transplant generations is mainly based on the continued recruitment of normal cells by RSV infection. At least part of the reason for this may be a difference in histocompatibility between the random bred donors and recipients. The resulting homograft reaction also provides a logical explanation for the regression of Rous sarcomas induced by nonproducer cells and free of virus (Hanafusa *et al.*, 1964a). It is also possible that the growth potential of Rous sarcoma cells *in vivo* is not unlimited even in the absence of a homograft response. Experiments with isotransplanted Rous sarcomas of turkeys suggest this possibility (Bergs and Groupé, 1963). Related to the question of transplantability is the problem of metastases occurring after RSV infection. It appears that in the antibody-free host the more prevalent origin of these secondary growths is viral infection rather than dissipation of tumor cells (Munroe and Southam, 1961). Such a finding is to be expected, since RSV is demonstrable in practically all host tissues before and after the appearance of a tumor at the site of inoculation (Munroe and Southam, 1959; Spencer and Groupé, 1962a,b).

b. AMV. Transplantation of AMV-induced myeloblastosis has led to results similar to those obtained with Rous sarcoma cells (Baluda, 1962). If random-bred animals are used, a strong homograft reaction against the donor cells is observed. No immune reaction is detectable after injecting the leukemic cells into embryos or recipients which belong to the same inbred line as the donor. However, even then the donor cells fail to persist and the leukemias which develop show the karyotype of the recipient. At present this finding is difficult to reconcile with the apparently inexhaustible growth potential which AMV-induced myeloblasts display *in vitro* (Beaudreau *et al.*, 1960b).

c. Erythroblastosis Virus. Compared to Rous sarcoma cells and malignant myeloblasts the erythroblasts obtained from chickens infected with strain R erythroblastosis virus show a higher degree of transplantability (Pontén, 1962b). In highly inbred chickens dividing donor cells persist up to the fifth transplant generation.

d. Strain RPL 12 Leukosis Virus. This is so far the only virus-induced avian neoplasm for which an apparently unlimited transplantability has been demonstrated (Pontén, 1962c). RPL 12 tumor cells grow in homologous as well as in isologous hosts. In this connection it is of interest that the RPL 12 tumor is exceptional also in having a hyperdiploid chromosome set. The increase in chromosome number does not follow a distinct, consistent pattern but appears to involve various chromosomes at random (Pontén, 1963). Rous sarcoma cells grown *in vitro* and *in vivo*, malignant myeloblasts and erythroblasts have a normal or near-normal complement of macrochromosomes and are free of gross detectable aberrations of the microchromosomes (Temin and Rubin, 1958; Bayreuther and Thorell, 1959; Baluda, 1962; Pontén, 1963).

4. Chicken Sarcoma Viruses in Other Avian Species

The natural and most susceptible host for avian tumor viruses is the chicken (Murphy and Rous, 1912). However, some strains of avian sarcoma virus have been found to have an extended host range including several unrelated species of birds. Others may be adapted to grow and cause tumors in a heterologous species by serial transfers in young animals. Finally, heterologous infection may be facilitated by acquired immunological tolerance to chicken antigens.

Among these chicken sarcoma viruses with a naturally broad host range belongs Fujinami virus, which is capable of producing sarcomas in ducks as well as in chickens, and thus is pathogenic in two different avian orders (Fujinami and Hatano, 1929; Gye, 1932). Similar in this respect is strain B77, a chicken sarcoma virus isolated in 1954 from a spontaneous tumor (Thurzo *et al.*, 1963; Thurzo and Smida, 1963). This strain also induces pocks on the chorioallantoic membrane of duck embryos (Smidova and Thurzo, 1964). Within the order of the *Galliformes*, RSV and Fujinami virus cause tumors in pheasants (Andrewes, 1932). The Bryan strain of RSV is pathogenic for turkeys (Groupé and Rauscher, 1957; Spencer and Groupé, 1962a; Harris and Simons, 1962; Harris, 1963). Recently the Japanese quail has been introduced as a laboratory animal. Quail cells in tissue culture are susceptible to RSV-induced transformation (Freeman, 1963).

The gradual adaption of chicken sarcoma viruses to other species is exemplified by the extensive studies of Duran-Reynals with RSV in ducks (Duran-Reynals, 1942, 1947a). Most of the "duck-adapted" strains of RSV have lost their pathogenicity for chickens, but one strain, designated as 14(d)7, shows comparable pathogenicity in chickens and in ducks even after repeated passage in one of the two species. The characteristics of the 14(d)7 strain have recently been confirmed by Kuwata (1964a,b), who also succeeded in selecting additional RSV strains which are pathogenic for ducks and chickens. No immunological comparisons of RSV before and after duck adaption have been reported, and the nature of the adaptive process is not known. The 14(d)7 strain of Duran-Reynals is also pathogenic for pigeons (Duran-Reynals, 1947b; Burges and Duran-Reynals, 1952). The pigeon tumors are free of infectious virus, but virus synthesis is activated upon back-transfer of the tumor in the chicken. In this respect the pigeon tumors induced by strain 14(d)7 resemble the mammalian Rous sarcomas to be discussed in a later section.

An expansion of RSV host range can also be accomplished by inducing immune tolerance in the heterologous hosts. The two systems studied in this respect are infection of turkeys with the Harris strain of RSV and infection of ducks with the Prague strain of RSV (Harris and Simons, 1962; Svoboda, 1962c,d). Turkeys are resistant to the induction of sarcomas by cell-free material of the Harris strain of RSV, and a similar resistance is found in ducks 2 weeks or older injected with cell-free material of the Prague strain of RSV. This resistance is greatly reduced by the injection of chicken blood into duck or turkey embryos or newly hatched birds (Harris, 1956; Svoboda, 1958, 1960a). Injection of chicken blood into older birds is not effective. This indicates that the exposure to chicken blood early in the development of turkeys or ducks makes these animals tolerant to the development of sarcomas induced by certain strains of RSV. This conclusion also implies that control animals are resistant to RSV by virtue of an immune response. The initial development and subsequent regression of tumors in control birds suggest that this immune response consists of a homograft reaction against the Rous sarcoma. This is further supported by the experimental termination of acquired tolerance in turkeys through transfer of spleen cells from RSV-immune donors to tolerant recipients (Harris and Simons, 1962; Harris, 1963). The immune reaction against the tumor must be cell-mediated, since transfer of serum from RSV-immune animals does not abolish tolerance (Svoboda, 1962a,c). The antigens involved in conferring tolerance have been studied in detail (Harris, 1956; Harris and Simons, 1958; Svoboda, 1960b). From this work it appears that a polysaccharide related to human blood group A substance is active in inducing tolerance to RSV in ducks or turkeys. Surprisingly, no material conferring such tolerance has so far been isolated from Rous sarcomas or from RSV preparations, possibly because the available quantities have not been sufficient (Harris, 1963; Harris and Simons, 1962). Of interest is the finding that the development of natural heteroagglutinins to chicken red blood cells is not suppressed in RSV-tolerant ducks, and that tolerance to RSV may be retained in turkeys which have rejected a chicken skin graft (Svoboda, 1960a; Harris, 1956). The available information on the induction and nature of the tolerance to RSV in ducks and turkeys is compatible with the hypothesis that cells infected with certain strains of RSV acquire a new antigen, probably related to blood group A substance. The mechanism by which RSV endows the infected cell with this new antigen is not known.

5. *Virus-Free Tumors*

It is an old observation that the amount of infectious virus recoverable from Rous sarcomas is highly variable and that occasionally no RSV whatever can be found even in highly malignant growths (Rous and Murphy, 1914a). Similar findings have been made repeatedly, and an examination of past and recent publications indicates that there are at least two basically different situations from which Rous sarcomas with low or lacking virus content may arise. Lack of virus in sarcomas initiated with a high dose of RSV is correlated with the age of the tumor and the age of the tumor-bearing animal (Carr, 1943b; Duran-Reynals, 1946; Duran-Reynals and Freire, 1953; Bryan *et al.*, 1955). These data suggest that an immune response of the host against virus or tumor cells might be responsible for the absence of infectivity. Rous sarcomas caused by a low dose inoculum are devoid of virus even in their early stages of development regardless of host age (Bryan *et al.*, 1955; Prince, 1959). There is no indication that the lack of virus in these cases is due to an immune reaction of the chicken. Recent experiments have confirmed the dual origin of noninfective Rous sarcomas and have provided a clarification of the mechanisms involved (Rubin, 1962a,b; Rubin and Hanafusa, 1963; Shimizu and Rubin, 1964). Sarcomas arising from a large dose of virus contain high titers of RSV throughout the second week of growth; this is followed by a rapid decline in virus content, resulting in the virtual absence of virus from sarcomas grown for 30 days or more. Regressions of such old tumors are not uncommon, and animals which recover are immune to challenge infec-

tion with RSV or sarcoma cells. The immune reaction of the host not only affects the complete virus present in the tumor but appears to act also on tumor cells themselves. Sarcoma cells obtained from old tumors show a greatly decreased ability to produce RSV when placed in tissue culture. The nature of the immune reaction responsible for the termination of virus synthesis and cell growth in old Rous sarcomas is indicated by two observations: (1) Although many tumor-bearing chickens develop neutralizing antibody against RSV, there is no correlation between antibody titers in the blood and the failure to recover virus from the tumor. (2) Old and regressing Rous sarcomas are massively infiltrated with lymphocytes. This suggests that the principle cause for noninfectiousness and regression is a cell-mediated immunity directed primarily against the Rous sarcoma cell rather than against RSV. Additional support for this hypothesis is provided by the finding that birds recovering from a Rous sarcoma may be fully immune to challenge infection even in the absence of circulating viral antibody (Prince, 1959), and that the development of RSV neutralizing antibodies in a tumor-bearing chicken does not signal tumor regression (Vigier, 1958; Mendle and Pollard, 1959; Dougherty *et al.*, 1960). The connection between lymphocytic infiltration, lack of infectious RSV, and regression of Rous sarcomas has also been observed by Stenkvist and Pontén (1963). It is highly probably that cessation of virus synthesis and tumor rejection are but two different manifestations of the same immunological phenomenon. In turkeys, the production of RSV antibodies shows a better correlation with the lack of virus in the tumors than it does in chickens; but here, too, sarcomas may be found which are virtually free of RSV, despite the absence of significant antibody titers in the tumor-bearing host (Rauscher and Groupé, 1960a).

Several lines of evidence indicate that the antigens eliciting the immune reaction against Rous sarcomas induced by high doses of virus belong to the virus particles themselves and may be the coat proteins of RSV. Chickens injected with RSV nonproducer cells not only fail to develop RSV neutralizing antibodies, but also do not acquire the cell-bound immunity directed against RSV tumor cells. Rous sarcoma cells which are free of complete virus are apparently devoid of the critical antigen involved in the cell-mediated immune reaction. Induction of immune tolerance to RAV by intraembryonal injection results in a complete inability of the adult birds to mount an immune reaction against Rous sarcomas. Since the only presently known common antigen of RAV and of RSV is that of the viral coat, this same antigen seems to be crucial for the process of tumor regression. It is conceivable that viral coat antigens which become incorporated in the cell membrane mark such a cell for an immune attack. However, the possibility remains that RAV- and RSV-producing cells share more antigens than those of the viral coat. But even if such antigens were involved in the process of Rous sarcoma regression, they would still be antigens which are characteristic of virus production rather than of malignant cells.

An explanation for the virus-free Rous sarcomas arising from a low dose inoculum has been based on the defectiveness of RSV (Rubin and Hanafusa, 1963; Shimizu and Rubin, 1964). In a small inoculum of RSV the probability for solitary infection without RAV is increased, and the resulting tumors may therefore consist of nonproducer cells. Noninfectious Rous sarcomas initiated with a small virus inoculum indeed show all essential characteristics which define nonproducer cells *in vitro*. Cells from such sarcomas explanted in tissue culture fail to release virus, but virus production can be activated predictably by superinfection with RAV. The proportion of nonproducing sarcomas arising from a small RSV inoculum can also be sharply reduced by inoculating an excess of RAV in the same site, thus providing ample opportunity for superinfection of prospective nonproducer cells with a helper virus. Even without an initial excess of RAV, sarcomas of nonproducer cells eventually become superinfected by helper virus and turn virus producers. It is only then, however, that the antigens appear which activate the immunological defense of the host, resulting in eventual regression of such tumors. The experiments substantiating the dual origin of virus-free Rous sarcomas do not rule out the possibility that still other mechanisms exist which lead to noninfectiousness. In fact, the present theories do not fully account for all observations made on virus-free Rous sarcomas, such as the finding of resistance to RSV infection in sarcoma cells cultivated from some noninfective tumors (Prince, 1959; Bergs and Groupé, 1962a,b).

The discovery of a cell-bound immune response to Rous sarcoma raises the question whether similar mechanisms of host defense are also operative against other avian tumor virus neoplasms. The strong decline of susceptibility to tumor formation with increasing age of the animal observed with several avian tumor viruses suggests that immune responses might play an important role in determining the outcome of avian tumor virus infection (Eckert *et al.*, 1955a; Burmester *et al.*, 1960a; Sevoian and Chamberlain, 1963). This supposition is further strengthened by the finding that bone marrow cells obtained from adult chickens are fully susceptible *in vitro* to AMV-induced transformation (Beaudreau *et al.*, 1960a). The reason for the failure of AMV to cause myeloblastosis in adult chickens must therefore be sought at the level of the organism rather than at the level of the single cell. The increased incidence of apparently virus-free plasmas in chickens developing myeloblastosis only after a prolonged latent period constitutes another similarity to the Rous sarcoma system, although the mechanisms involved in producing virus-free tumors need not be the same as those found with RSV (Eckert *et al.*, 1952).

B. *Natural Transmission and Persistence of Avian Tumor Viruses in Chicken Populations*

Avian leukosis virus infection is extremely common in chicken flocks, and there is reason to believe that the overwhelming majority of all chickens become infected with field strains of avian leukosis virus sometime during their lives. This widespread infection is responsible for often devastating outbreaks of visceral lymphomatosis and constitutes, therefore, a considerable economic problem. For this reason epidemiological research on avian lymphomatosis has been of importance for some time. The basic characteristics of horizontal and vertical virus transmission in chicken flocks have been worked out in extensive and patient experimentation by Burmester (1957). These outlines have been completed and extended through the use of *in vitro* techniques for the assay of avian lymphomatosis virus (Rubin, 1960a). Such techniques have facilitated the processing of adequate numbers of serum and tissue samples collected in the field in tests for the presence of virus and virus-neutralizing antibodies. Avian lymphomatosis virus is detected by its ability to interfere with RSV in tissue culture. Antibody to lymphomatosis virus is measured by the direct neutralization of RSV, since it has been shown that field strains of lymphomatosis virus and RSV are immunologically related, although not identical (Rubin *et al.*, 1961; Friesen and Rubin, 1961). Adult chickens of commercial flocks may be divided into two major classes (Rubin *et al.*, 1961, 1962). The first consists of highly viremic birds which have no detectable amounts of virus-neutralizing antibody. The second is made up of birds which do not carry the virus in their blood, but have neutralizing antibodies, indicating past infection. The immune or viremic states of the individual adult chickens are stable. The origin of this bipartite pattern of leukosis virus infection can be best understood by observing the vertical transmission of the virus from one generation to the next, as well as the horizontal transmission within one generation (Burmester and Gentry, 1954, Burmester, 1955, 1962; Burmester and Waters, 1955, Rubin *et al.*, 1961 1962).

Viremic hens regularly transmit the infection to their offspring through the egg. Most of the immune hens produce offspring which are virus-free upon hatching, but some of these immune hens lay infected eggs. It appears that such immune shedders carry an ovarian infection which persists even in the presence of circulating viral antibodies. The viremic or immune state of roosters has no influence on the vertical transmission of lymphomatosis virus. Even highly viremic roosters, with active virus production in testicular cells, fail to produce infected progeny if mated to nonviremic hens. This indicates that the cellular localization of avian lymphomatosis virus is cytoplasmic, a conclusion which is in keeping with the available information on the cellular biology of avian tumor virus infection in general. Embryos produced by "shedder" hens usually contain large quantities of virus. Infectious center measurements suggest that the majority of their cells are infected and engaged in active virus synthesis. Yet the development of such congenitally infected embryos proceeds without detectable deficiencies; the birds hatch and grow up normally. A follow-up of chicks from "shedders" and "nonshedders" shows that the immune or viremic state of the hen has a tendency to be preserved in the offspring. Most congenitally infected chicks are immunologically tolerant to the virus and remain viremic throughout their life. They are a constant source for the horizontal spread of the virus through contact infection. Chicks produced by "nonshedders" and free of virus are partially protected through the first few weeks of life by passively transmitted maternal antibody. By the fourth week after hatching these antibodies have disappeared. At that point proximity of viremic and nonviremic chicks leads to a high incidence of contact infections in the latter. These infections manifest themselves in a transient viremia which is succeeded by the appearance of virus-neutralizing antibodies. Thus, contact infection results in immunity, whereas congenital infection leads to immune tolerance. The progeny generation consists then, again, of two classes of birds, one viremic and derived from "shedder" hens and the other immune, originating from immune hens. However, this conservation of viremia or immunity over several generations is not perfect. As already mentioned, some of the immune hens transmit virus to a high proportion of their progeny. Conversely, an occasional embryo produced by a hen classified as "shedder" may contain only little or no virus at all and become an antibody producer later in life. In addition, contact infection early after hatching can lead to a permanent viremia and immune tolerance in chicks produced by hens which do not infect their progeny (Rubin, 1962c). The incidence of visceral lymphomatosis in virus-tolerant birds is significantly higher than that in immune members of the same population. However, immune animals are not free from lymphomatosis. As

can be concluded from the existence of immune "shedder" hens, antibody production is not completely successful in eliminating the tumor virus from the organism. The noncytocidal virus can persist in the host cell indefinitely and is sheltered there from antibody action. The production of virus-neutralizing antibody is therefore of little protective value for the individual host, although it might be considered useful for the population since it reduces the efficiency of horizontal virus spread. The higher incidence of lymphomatosis in virus-tolerant birds constitutes a selective disadvantage for this group and should eventually result in its elimination. However, there appears to be a constant new recruitment of permanent viremic animals, through immune "shedder" birds and through contact infection during the first few days after hatching, leading to immune tolerance.

The epidemiology of avian leukosis virus infection is also in accord with some observations of long standing. There can be no doubt that the "natural" antibodies to RSV found so frequently in adult chickens are the result of an infection with field strains of avian leukosis virus (Duran-Reynals, 1940; Duran-Reynals *et al.*, 1953; Kenzy, 1953; Kenzy and Neuzil, 1953; Bang, 1958; Bang and Foard, 1963). The transmission of such antibodies via the egg to progeny birds has also been observed many years ago (Andrewes, 1939). The new knowledge in the transmission of avian leukosis virus has been used for the establishment of leukosis-free chicken flocks (Hughes *et al.*, 1963; Levine and Nelsen, 1964). In the experiments of Levine and Nelsen about one fourth of the chickens appear to have escaped infection as judged by the absence of virus and of RSV antibody. Besides viremic and immune birds these apparently unaffected chickens constitute a third, neutral class, which does not seem to be involved in the horizontal or vertical transmission of avian leukosis virus. Since the horizontal spread of avian leukosis virus is highly efficient among susceptible birds, and can even occur by airborne transmission (Sevoian *et al.*, 1963), the existence of uninfected animals in a population where leukosis is endemic is surprising. Two explanations, which are not mutually exclusive may be offered for this finding: (1) A proportion of the birds in the population may be genetically resistant to avian leukosis virus. Such animals do not develop "natural" RSV antibodies (Kenzy *et al.*, 1961). (2) Some birds may have avian leukosis virus antibodies which do not cross-react with RSV. Such a sign of past infection would escape detection by routine tests using RSV as indicator virus for neutralization. The natural routes by which avian lymphomatosis virus is transmitted vertically and horizontally are represented in Fig. 2.

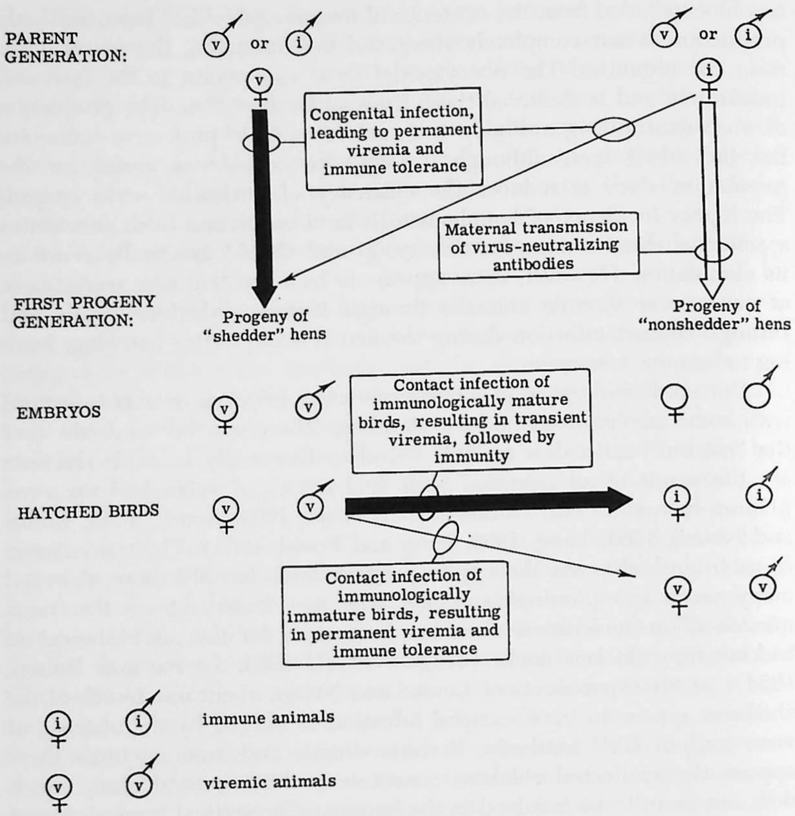


FIG. 2. Vertical and horizontal transmission of avian leukosis virus in chicken flocks (see text for details).

VI. RSV IN MAMMALIAN HOSTS

A. *In Vivo Studies*

1. *RSV Strains Pathogenic for Mammals*

A considerable expansion of the avian tumor virus field has resulted from the recent findings that certain strains of RSV are carcinogenic in mammals. This observation has attracted much attention and at the moment the study of mammalian Rous sarcomas, although still in its infancy, is one of the fastest moving areas in tumor virology. It is

therefore to be expected that a review of the present data will be rapidly outdated by new developments. There appear to be three variants of RSV which are regularly pathogenic in mammals: (1) The "Carr-Zilber" strain is mainly used by laboratories in the Soviet Union and was supplied by Dr. Carr, Edinburgh (Zilber, 1961). (2) The Prague strain, studied by Czechoslovakian groups, was acquired from Dr. Engelbreth-Holm, Copenhagen, in 1952 (Klement and Svoboda, 1963). (3) The Schmidt-Ruppin strain, used predominantly in Sweden and the United States, derives from material provided by Dr. Schmidt-Ruppin, Frankfurt am Main, who in turn received it from Dr. Oberling, Paris, in 1953 (Schmidt-Ruppin, 1964). The origins of these strains, their relationships to each other and to the widely used Bryan strains are not clear. The Prague and Schmidt-Ruppin strains differ from each other with respect to their pathogenicity. Tumors can be induced in adult Syrian hamsters by the Schmidt-Ruppin strain, but not by the Prague strain (Klement and Svoboda, 1963). Antigenic distinctness of the Bryan and Schmidt-Ruppin strains and a relationship of the latter to AMV have already been mentioned (Section II,F,2). Antigenic differences exist also between the Carr-Zilber and the Bryan strains (Munroe and Southam, 1964). Other strains of Rous sarcoma virus, such as the Mill Hill strain and the Harris strain, have not been found to be carcinogenic to a significant extent in the mammal (Ahlström and Jonsson, 1962a; Ahlström *et al.*, 1962; Harris and Chesterman, 1963). The case of the Bryan strain is equivocal, but its carcinogenicity in mammals is certainly of a low order (Munroe and Southam, 1964). Some of the data on the induction of Rous sarcomas in mammals and corresponding references are summarized in Table VI. It can be seen that most of the work has been carried out in common laboratory rodents, but the successful induction of sarcomas in newborn rhesus monkeys indicates that the host range of some RSV strains may encompass a wide variety of mammals. An inoculation of RSV in the adult human performed in 1939 has not resulted in any pathological effects for more than 20 years, although the virus strain used may have been identical or closely related to the one which was later found to be pathogenic in newborn monkeys (Carr, 1960). The pathogenesis of RSV-induced growths is fairly uniform in different species, with sarcomas as the prevailing host response to the virus. An exception is the rabbit in which only benign fibromas are formed. In the case of rats the sarcomas are usually preceded by the formation of chronic hemorrhagic cysts. The histogenesis of these cysts as well as that of rat Rous sarcomas has been studied very extensively (Zilber and Kryukova, 1957; Svet-Moldavsky, 1957; Saldeen, 1962, 1963; Klement and Sácha, 1963; Shapiro and Morgunova,

TABLE VI
SURVEY OF ROUS SARCOMAS IN MAMMALS

Mammalian species	RSV strain[a]	Reference	Growth	Conditions of successful inoculation[b]
Mouse, *Mus musculus*	SR	Schmidt-Ruppin (1959) Ahlström *et al.* (1963b) Schmidt-Ruppin (1964)	Sarcomas	a, b, c
Syrian hamster, *Cricetulus aureus*	PR	Klement and Svoboda (1963)	Sarcomas	a, c
	SR	Ahlström and Forsby (1962) Klement and Svoboda (1963) Schmidt-Ruppin (1964) Klement (1964)	Sarcomas	a, b, c, d
	CA	Munroe and Windle (1963)	Sarcomas	
Chinese hamster, *Cricetulus griseus*	SR	Ahlström *et al.* (1964)	Sarcomas	a, b, c
Rat, *Rattus norvegicus*	CZ	Zilber and Kryukova (1957) Svet-Moldavsky (1957, 1958a,b, 1961) Zilber (1961) Munroe and Southam (1964)	Cysts, sarcomas	a, b, c
	PR	Svoboda (1960c)	Cysts, sarcomas	a, c
	SR	Schmidt-Ruppin (1959) Ising-Iversen (1960) Ahlström and Jonsson (1962b) Klement *et al.* (1963) Schmidt-Ruppin (1964)	Cysts, sarcomas	a, b, c
	[c]	Sokoloff *et al.* (1963)	Cysts	a, b

TABLE VI (*Continued*)

Mammalian species	RSV strain[a]	Reference	Growth	Conditions of successful inoculation[b]
Cotton rat, *Sigmodon hispidus*	CZ	Munroe and Windle (1963) Svet-Moldavsky (1963) Svet-Moldavsky and Svet-Moldavskaya (1964)	Sarcomas	a, c
Guinea pig, *Cavia porcellus*	SR	Ahlström *et al.* (1963a)	Sarcomas	a, c
	CZ	Munroe and Windle (1963)	Sarcomas	
Rabbit, *Oryctolagus cuniculus*	CZ	Zilber and Kryukova (1958) Zilber (1961)	Benign fibromas	a, c
	SR	Ahlström *et al.* (1963b)	Benign fibromas, cysts	a, b, c
Monkey, *Macaca mulatta*	CZ	Munroe and Windle (1963)	Sarcomas	a, c

[a] CZ, Carr-Zilber strain; PR, Prague strain; SR, Schmidt-Ruppin strain.
[b] Tumors were induced by a, inoculation of cellular material from chicken sarcomas; b, inoculation of cell free material from chicken sarcomas; c, inoculation of embryos, newborn, or young animals; d, inoculation of adult animals.
[c] Strain not identified.

1963). Primary tumor induction in the mammal often requires the implantation of cellular material from chicken sarcomas and may be unsuccessful if cell-free preparations are used. However, sufficiently numerous mammalian sarcomas induced by cell-free RSV material are on record to indicate that the apparent requirement for cellular material in some cases is not a basic one (Ahlström *et al.*, 1963b; Munroe and Southam, 1964; Klement, 1964). One might presume that the effect of viable Rous sarcoma cells in the inoculum is to provide large quantities of virus over an extended period of time. So far, only newborn or young animals have been found susceptible to the carcinogenic action of RSV, with the exception of Syrian hamsters which remain susceptible as adults (Ahlström and Forsby, 1962; Klement and Svoboda, 1963).

2. *RSV-Induced Carcinogenesis in the Mammal versus Heterotransplantation of Chicken Sarcoma*

In many cases primary mammalian Rous sarcomas are induced with an inoculum of chicken sarcoma cells. It is therefore important to establish that the tumor in the mammal is composed of host cells and not of chicken cells. There are two main lines of evidence which affirm the host origin of primary mammalian Rous sarcomas and exclude the participation of chicken cells in these growths: (1) Primary mammalian Rous sarcomas do not contain detectable amounts of chicken antigens (Svet-Moldavsky and Skorikova, 1960; Gusev and Svoboda, 1962 Ahlström and Forsby, 1962; Ahlström and Jonsson, 1962b; Svet-Moldavsky and Svet-Moldavskaya, 1964). Serial passage of Rous sarcomas in mammals is even successful in animals preimmunized with chicken antigens (Svoboda, 1962b). (2) No chicken karyotypes are seen among the dividing cells of primary mammalian Rous sarcomas (Ising-Iversen, 1960; Landa *et al.*, 1962a,b). Identifiable metaphases show the chromosomes of the mammalian host (Ahlström and Forsby, 1962; Ahlström and Jonsson, 1962b; Svoboda and Šimkovič, 1963; Ahlström *et al.*, 1964). The primary tumors contain mostly a diploid or near diploid chromosome complement of the host. Only in the Chinese hamster has a tetraploid primary tumor been reported (Ahlström *et al.*, 1964). Upon serial transplantation in mammals ploidy changes as well as structural changes of the chromosomes are observed (Nichols, 1963; Nichols *et al.*, 1963). Further unequivocal support for the direct malignization of mammalian cells is provided by the successful induction of tumors with cell-free virus preparations. An important objection which could be raised against the conclusion that RSV is carcinogenic in mammals is the possibility that the Schmidt-Ruppin strain and other RSV strains are contaminated with such ubiquitous oncogenic agents as polyoma virus. However, neither polyoma virus hemagglutinin nor polyoma virus hemagglutination-inhibiting antibodies are detectable in Rous sarcoma-bearing mammals (Ahlström and Jonsson, 1962b; Ahlström *et al.*, 1963a). The presently available evidence is therefore overwhelmingly in favor of a direct interaction between certain strains of RSV and mammalian cells, leading to malignant transformation.

3. *Transplantability of Mammalian Rous Sarcomas*

Successful homotransplantation has been reported with some of the first mammalian Rous sarcomas described in the literature (Svet-Moldavsky, 1958a,b). Almost any reference listed in Table VI attests to the

generality of this finding. A survey of serial transfers in various species is given by Ahlström *et al.*, 1963b. Perhaps the oldest established line of mammalian Rous sarcoma is the tumor "XC" which has been kept in serial passage since 1960 (Svoboda, 1961; Chýle *et al.*, 1963). In all instances, serial homotransplantation in the mammal is dependent on the injection of viable cells. Mice which have rejected a homograft of Rous sarcoma show a clear-cut resistance to challenge injections with isologous Rous sarcoma cells which cause progressively growing tumors in untreated control animals (Sjögren and Jonsson, 1963). The nature of the antigens responsible for this immune response has not been clearly defined. The antigens are present in mammalian Rous sarcoma cells which are presumably free of virus, whereas cell-free preparations of RSV prove almost ineffective in inducing a specific immunity to Rous sarcoma isotransplants. This would seem to indicate that a cellular, rather than a viral, antigen is of importance for the acquired transplantation resistance to Rous sarcomas in mice. Many of the mammalian Rous sarcomas can also be transplanted back into the chicken, resulting in the growth of Rous sarcomas which in some cases have been shown to contain infectious RSV (Svoboda, 1960c; Svet-Moldavsky, 1961; Ahlström and Forsby, 1962; Ahlström 1963b). Only with Rous sarcomas from guinea pigs have attempts of heterotransplantation into the chicken met with consistent failure (Ahlström, 1963a).

4. *Absence of Free Virus from Mammalian Rous Sarcomas and Evidence for the Persistence of Viral Genetic Material*

Serial passages of Rous sarcomas in mammals can only be accomplished with viable cells, which indicates that the mammalian Rous sarcomas might not contain appreciable amounts of RSV. This is further supported by the failure to demonstrate free RSV in filtrates and subcellular fractions of mammalian tumors, or in tissue cultures of such tumors, by inoculation into chickens (Svoboda *et al.*, 1963; Klement *et al.*, 1963; Šimkovič *et al.*, 1963; Huebner *et al.*, 1964). Furthermore, Rous sarcoma-bearing mammals do not develop RSV-neutralizing antibodies (Svoboda *et al.*, 1963). [A notable exception to this general rule is a single hamster sarcoma studied by Svoboda and Klement (1963). This tumor contained infectious RSV in the cell-free filtrate of the seventh hamster passage.] Despite the widely observed absence of free RSV from mammalian Rous sarcomas, a potential for complete RSV production appears to be preserved and transmitted in the mammalian sarcoma cells. This is indicated by the finding of complete RSV in chicken sarcomas which arise from back-transplants of mammalian Rous sarcoma cells into the avian host (Svoboda, 1960c, 1961, 1962b; Šimkovič *et al.*, 1962a, 1963; Huebner *et al.*, 1964). Such back transplants are successful only with whole cells. Frozen and thawed material from rat tumors does not induce infectious Rous sarcomas in the chicken (Svoboda and Šimkovič, 1963). The injection of X-irradiated cells from the rat Rous sarcoma "XC" into chickens has led to conflicting results, and it is not clear from the published data whether the mammalian tumor cells must be capable of dividing in order to cause an infectious Rous sarcoma in chickens (Vrba and Chaytor, 1964; Chýle, 1964). X- or UV-irradiation of "XC" cells *in vitro* does not induce virus production, but mixed cultivation with chicken fibroblasts does (Šimkovič *et al.*, 1962b; Svoboda and Šimkovič, 1963). A close association of the mammalian sarcoma cells with chicken cells appears, therefore, to be the prerequisite for the activation of RSV synthesis. The nature of this cellular interaction remains to be worked out. It should also be pointed out that activation of RSV production is not always observed when mammalian tumor cells are brought in contact with chicken cells. It has already been mentioned that Rous sarcoma cells of the guinea pig do not induce a tumor when heterotransplanted into chickens (Ahlström *et al.*, 1963a). But even when Rous sarcomas of other mammalian species are used, the outcome of attempts to reactivate virus synthesis by heterotransplantation appears to be largely unpredictable, indicating that the conditions of reactivation are still insufficiently understood (Ahlström *et al.*, 1963b; Klement and Svoboda, 1963; Klement, 1964; Chýle, 1964). Despite such unpredictability of the system, it is probably safe to say that the complete genome of RSV is carried and replicated in some mammalian Rous sarcomas. This perpetuation of the viral genome in the absence of virus synthesis is reminiscent of the avian nonproducer cells resulting from infection with defective RSV. However, the state of the RSV genome in mammalian cells is not likely to be comparable to that in avian nonproducers. The Schmidt-Ruppin strain of RSV does not appear to be defective (Section IV,E), and there is no indication that superinfection with an avian leukosis virus is involved in the activation of RSV synthesis in mammalian tumors. Additional evidence for the presence of viral genetic material comes from the discovery of an avian tumor virus group-specific, complement-fixing antigen in mammalian Rous sarcomas (Huebner *et al.*, 1963, 1964; see also Section II,F,2). The specific complement-fixing antigen is present in Rous sarcomas of all host species which have been tested. This argues against specification of the antigen by the cell genome and favors determination by the genetic material of RSV.

B. *In Vitro Studies*

In tissue cultures of several mammalian species RSV induces a cellular transformation similar to the one observed in chick fibroblast cultures and characterized by the appearance of rounded, refractile cells, which may grow in clumps and occasionally show excessive vacuolization (Table VII). In general, only those strains which are pathogenic for

TABLE VII
TRANSFORMATION OF MAMMALIAN CELLS BY RSV IN TISSUE CULTURE

Mammalian species	RSV strain[a]	Reference
Mouse	SR	Bergman and Jonsson (1962)
Rat	SR	Bergman and Jonsson (1962)
	PR	Svoboda and Chýle (1963)
	BR	Rothschild and Febvre (1963)
African green monkey	SR	Jensen *et al.* (1964)
Man	SR	Jensen *et al.* (1964)
	CZ	Zilber and Shevljaghyn (1964)
Guinea pig	SR	Bergman and Jonsson (1962)
		Ahlström *et al.* (1963a)

[a] SR, Schmidt-Ruppin strain; PR, Prague strain; BR, Bryan strain; CZ, Carr-Zilber strain.

mammals *in vivo* are also effective in tissue culture (Bergman and Jonsson, 1962; Jensen *et al.*, 1964; Zilber and Shevljaghyn, 1964). [A possible exception is the finding of transformation in rat fibroblast cultures infected with the Bryan strain (Rothschild and Febvre, 1963).] The correlation between the ability of RSV to infect mammalian cells *in vivo* and *in vitro* suggests that the host range is controlled at the cellular level and is probably not influenced by organismic responses. It would be of interest to determine whether the viral coat proteins play the same decisive role here as they do in defining RSV host range patterns in chickens. The mammalian cells transformed by RSV *in vitro* share important properties with cells obtained from mammalian Rous sarcomas induced *in vivo*. They produce progressively growing tumors when implanted into animals of the same species (Svoboda and Chýle, 1963). No infectious RSV is detectable in cultures of mammalian cells transformed by RSV *in vitro* (Svoboda and Chýle, 1963;

Jensen *et al.*, 1964). However, virus production can be activated by mixed cultivation of transformed mammalian cells and normal chicken cells (Šimkovič *et al.*, 1962b; Svoboda and Šimkovič, 1963; Svoboda and Chýle, 1963; Jensen *et al.*, 1964). The avian tumor virus group-specific antigen is present in transformed mammalian cultures even after prolonged periods of cultivation (Jensen *et al.*, 1964). The infection of human cells with the Schmidt-Ruppin strain of RSV also causes marked chromosome abnormalities (Nichols *et al.*, 1964), a surprising effect of an RNA virus with a multiplication cycle that appears to be exclusively cytoplasmic. The transformation of mammalian cells by RSV *in vitro* is doubtlessly the strongest evidence for a direct interaction of avian tumor virus and mammalian host. The tissue culture system also offers ideal opportunities to investigate the numerous problems which are created by the inability of mammalian Rous sarcomas to produce complete virus, and the activation of virus synthesis through association with chicken cells.

VII. Conclusion

A review of a scientific field should not only present a concise survey of past and current developments, but should also be helpful in formulating new problems as well as in restating old, unsolved ones which recent work may have put in a different light. Several such problems have been pointed out during the course of this paper. Others will be defined in this last chapter.

The basic anatomy of avian tumor viruses is well established, and to a certain degree morphology has become correlated with function, mainly by the differentiation of coat and core properties in defective RSV. The geometrical as well as chemical architecture of the nucleocapsid needs further study. An immediate question here concerns the size and uniformity of viral RNA molecules, and whether the nucleocapsid contains one or several of such molecules. The antigens of the two major viral structures, nucleocapsid and envelope, await characterization. Advances in this respect will in turn necessitate cytological investigations with fluorescent antibodies which differentiate between the various viral building blocks. A logical step toward higher resolution would then be the use of ferritin-conjugated antibodies for the electron microscopic identification of viral precursors. The structure of the viral envelope is still only very incompletely understood. How does viral antigen become integrated with the double lipid layer that forms the cell membrane? Is it inserted in a lipid matrix, or is it superimposed on the otherwise unaltered cell membrane? Does the addition of viral antigen to the cell surface induce the budding? Which function do the knobs seen

at the viral periphery perform? A considerable gap in the knowledge of avian tumor virus anatomy concerns the inner membrane visible in thin sections of viral particles. There is no solid information as to the origin, chemical and antigenic nature, or function of this structure. The chemical dissection of the virus particle is still in a primitive state, and the long-term goal of determining the primary structure of the main virus-controlled molecules in the particle seems far away.

In the realm of infection and virus multiplication future work will probably turn increasingly toward a molecular analysis of virus growth. The unsatisfactory state of avian tumor virus purification is at present an obstacle to such experiments, but solutions for this difficulty appear to be within reach. On the other hand, the cellular biology of avian tumor virus infection is by no means an exhausted subject. This is particularly true of the avian leukosis viruses, for most of which tissue culture systems are still in a developmental stage. An enormous field for biological exploration has also opened with the successful infection of mammalian cells by RSV. In addition, such specific aspects as the mechanisms of genetic cellular resistance and interference between avian tumor viruses will require much further study before they are ripe for a molecular analysis. A problem which has been touched upon several times during this review is the involvement of the cell nucleus in avian tumor virus infection. The possibility of such nuclear participation is supported only by very indirect evidence, such as the enlargement of the nucleolus, or the rare finding of viral antigen in the nuclear region. Such signs are commonly interpreted as secondary effects of cytoplasmic virus multiplication on the nucleus, rather than a direct and essential contribution of the nucleus to the infectious cycle. However, if the suggested requirement for DNA synthesis in avian tumor virus growth is confirmed, a neutrality of the nucleus would appear unlikely.

Many questions also remain open at the level of the animal organism. One of the more conspicuous ones is the age-dependent decline in susceptibility to viral carcinogenesis observed with several avian leukosis viruses. This decline has probably an immunological basis, but at present such a contention lacks satisfactory experimental backing. The availability of tissue culture tests for virus infectivity and for neutralizing antibodies makes a thorough study of this subject feasible. A related topic concerns the finding of virus-free leukemias, which appear to be more frequent in older birds. The conditions leading to these growths require analysis, bearing in mind the dual origin of noninfectious Rous sarcomas.

A task which should not be overlooked in future work is that of bringing some order into the wide and increasingly confusing array of avian tumor virus strains, by establishing subgroups and relationships to reference strains, and by setting up guidelines for the rapid classification of newly isolated viruses. A logical extension of this project would be the development of a generally accepted and adhered-to nomenclature for avian tumor virus strains. Because of the multitude of such strains this new nomenclature would have to be numerical.

The central and most fundamental problem of the avian tumor virus field is doubtlessly the mechanism of virus-induced malignant transformation. Although the past few years have seen impressive progress in the anatomy and multiplication of avian tumor viruses, advances in the understanding of viral carcinogenesis have been slow. Perhaps the main finding in this respect is the lack of an absolute link between virus synthesis and cellular transformation. Another significant discovery is the involvement of the cell membrane in the malignant behavior of virus-transformed cells. This observation has given weight to nongenetic theories of viral carcinogenesis, which regard cytoplasmic organelles including the cell membrane, as likely primary targets of the cancer-producing virus. The difficulties encountered in the analysis of virus-induced malignant transformation are caused, at least in part, by a lack of information on the normal control mechanisms operating in vertebrate cells. Malignant cellular behavior and unrestricted growth cannot be separated from differentiation and normal control. This clearly defines the area where avian tumor virology has its strongest and most significant ties with general biology.

Acknowledgment

The author wishes to express his gratitude to Dr. Harry Rubin and to Dr. Joseph W. Beard for valuable criticism and to Dr. W. Robinson and Dr. A. Pitkanen for permission to include their unpublished results in Table III.

References

Abercrombie, M., and Ambrose, E. J. (1958). *Exptl. Cell Res.* **15**, 332–345.
Abercrombie, M., and Ambrose, E. J. (1962). *Cancer Res.* **22**, 525–548.
Abercrombie, M., and Heaysman, J. E. M. (1953). *Exptl. Cell Res.* **5**, 111–131.
Abercrombie, M., and Heaysman, J. E. M. (1954). *Exptl. Cell. Res.* **6**, 293–306.
Ada, G. L., and Perry, B. T. (1955). *Nature* **175**, 854–855.
Adams, E. V., and Groupé, V. (1963). *Virology* **21**, 271–273.
Ahlström, C. G., and Forsby, N. (1962). *J. Exptl. Med.* **115**, 839–852.
Ahlström, C. G., and Jonsson, N. (1962a). *Acta Pathol. Microbiol. Scand.* **54**, 136–144.
Ahlström, C. G., and Jonsson, N. (1962b). *Acta Pathol. Microbiol. Scand.* **54**, 145–171.
Ahlström, C. G., Jonsson, N., and Forsby, N. (1962). *Acta Pathol. Microbiol. Scand.* Suppl. 154, 127–129.
Ahlström, C. G., Bergman, S., and Ehrenberg, B. (1963a). *Acta Pathol. Microbiol. Scand.* **58**, 177–190.

Ahlström, C. G., Bergman, S., Forsby, N., and Jonsson, N. (1963b). *Acta. Unio Intern. Contra Cancrum* **19**, 294–298.
Ahlström, C. G., Kato, R., and Levan, A. (1964). *Science* **144**, 1232–1233.
Allison, A. C. (1963). "Viruses, Nucleic Acids and Cancer," pp. 462–484. Williams & Wilkins, Baltimore, Maryland.
Allison, A. C., and Burke, D. C. (1962). *J. Gen. Microbiol.* **27**, 181–194.
Amies, C. R., and Carr, J. G. (1938). *J. Pathol. Bacteriol.* **49**, 497–513.
Andrewes, C. H. (1931). *J. Pathol. Bacteriol.* **34**, 91–107.
Andrewes, C. H. (1932). *J. Pathol. Bacteriol.* **35**, 407–413.
Andrewes, C. H. (1933). *J. Pathol. Bacteriol.* **37**, 27–44.
Andrewes, C. H. (1939). *J. Pathol. Bacteriol.* **48**, 225–227.
Andrewes, C. H., and Horstmann, D. M. (1949). *J. Gen. Microbiol.* **3**, 290–297.
Armstrong, D., Okuyan, M., and Huebner, R. J. (1964). *Science* **144**, 1584.
Ashmore, J., Weber, G., Banerjee, G., and Love, W. C. (1961). *J. Natl. Cancer Inst.* **27**, 863–867.
Bader, J. P. (1962). *Virology* **16**, 436–443.
Bader, J. P. (1964). *Virology* **22**, 462–468.
Baker, S. L., and Peacock, P. R. (1926). *Brit. J. Exptl. Pathol.* **7**, 310–316.
Baluda, M. A. (1962). *Cold Spring Harbor Symp. Quant. Biol.* **27**, 415–425.
Baluda, M. A., and Goetz, I. E. (1961). *Virology* **15**, 185–199.
Baluda, M. A., and Jamieson, P. P. (1961). *Virology* **14**, 33–45.
Baluda, M. A., Goetz, I. E., and Ohno, S. (1963). "Viruses, Nucleic Acids and Cancer," pp. 387–396. Williams & Wilkins, Baltimore, Maryland.
Bang, F. B. (1958). *J. Natl. Cancer Inst.* **20**, 329–338.
Bang, F. B., and Foard, M. (1963). *J. Natl. Cancer Inst.* **30**, 457–466.
Barrett, M. K. (1941). *Cancer Res.* **1**, 543–544.
Barry, R. D., Ives, D. R., and Cruickshank, J. G. (1962). *Nature* **194**, 1139–1140.
Bather, R. (1957). *Brit. J. Cancer* **11**, 611–619.
Bather, R. (1963). *Proc. Am. Assoc. Cancer Res.* **4**, 4.
Bayreuther, K., and Thorell, B. (1959). *Exptl. Cell Res.* **18**, 370–373.
Beard, D., Beaudreau, G. S., Bonar, R. A., Sharp, D. G., and Beard, J. W. (1957). *J. Natl. Cancer Inst.* **18**, 231–259.
Beard, J. W. (1957a). *Ann. N.Y. Acad. Sci.* **68**, 473–486.
Beard, J. W. (1957b). *Texas Rept. Biol. Med.* **15**, 627–658.
Beard, J. W. (1958). *Am. Scientist* **46**, 226–254.
Beard, J. W. (1961). *Nouvelle Rev. Franc. Hematol.* **1**, 81–132.
Beard, J. W. (1962). *Progr. Hematol.* **3**, 105–135.
Beard, J. W. (1963a). *Advan. Cancer Res.* **7**, 1–127.
Beard, J. W. (1963b). *Ann. N.Y. Acad. Sci.* **108**, 1057–1085.
Beard, J. W., Sharp, D. G., and Eckert, E. A. (1955). *Advan. Virus Res.* **3**, 149–197.
Beard, J. W., Bonar, R. A., Heine, U., de Thé, G., and Beard, D. (1963). "Viruses, Nucleic Acids and Cancer," pp. 344–373. Williams & Wilkins, Baltimore, Maryland.
Beaudreau, G. S., and Becker, C. (1958). *J. Natl. Cancer Inst.* **20**, 339–349.
Beaudreau, G. S., Becker, C., Bonar, R. A., Wallbank, A. M., Beard, D., and Beard, J. W. (1960a). *J. Natl. Cancer Inst.* **24**, 395–415.
Beaudreau, G. S., Becker, C. Stim, T., Wallbank, A. M., and Beard, J. W. (1960b). *Natl. Cancer Inst. Monograph* **4**, 167–187.
Becker, C., Beaudreau, G. S., and Beard, J. W. (1959). *J. Natl. Cancer Inst.* **23**, 261–275.

Becker, C., Beaudreau, G. S., Castle, W., Gibson, B. W., Beard, D., and Beard, J. W. (1962). *J. Natl. Cancer Inst.* **29**, 455–481.
Benedetti, E. L. (1957). *Bull. Cancer* **44**, 473–482.
Benedetti, E. L., and Bernhard, W. (1958). *J. Ultrastruct. Res.* **1**, 309–336.
Benedetti, E. L., Bernhard, W., and Oberling, C. (1956). *Compt. Rend. Acad. Sci.* **242**, 2891–2894.
Bergman, S., and Jonsson, N. (1962). *Acta. Pathol. Microbiol. Scand.* Suppl. **154**, 130–133.
Bergs, V., and Groupé, V. (1962a). *J. Natl. Cancer Inst.* **29**, 723–739.
Bergs, V., and Groupé, V. (1962b). *J. Natl. Cancer Inst.* **29**, 739–748.
Bergs, V., and Groupé, V. (1963). *Science* **139**, 922–923.
Bernhard, W. (1958). *Cancer Res.* **18**, 491–509.
Bernhard, W., Oberling, C., and Vigier, P. (1956). *Bull. Cancer* **43**, 407–422.
Bernhard, W., Bonar, R. A., Beard, D., and Beard, J. W. (1958). *Proc. Soc. Exptl. Biol. Med.* **97**, 48–52.
Biggs, P. M., and Payne, L. N. (1959). *Nature* **184**, 1594.
Bonar, R. A., and Beard, J. W. (1959). *J. Natl. Cancer Inst.* **23**, 183–197.
Bonar, R. A., Beard, D., Beaudreau, G. S., Sharp, D. G., and Beard, J. W. (1957a). *J. Natl. Cancer Inst.* **18**, 831–842.
Bonar, R. A., Beaudreau, G. S., Sharp, D. G., Beard, D., and Beard, J. W. (1957b). *J. Natl. Cancer Inst.* **19**, 909–922.
Bonar, R. A., Parsons, D. F., Beaudreau, G. S., Becker, C., and Beard, J. W. (1959). *J. Natl. Cancer Inst.* **23**, 199–225.
Bonar, R. A., Weinstein, D., Sommer, J. R., Beard, D., and Beard, J. W. (1960). *Natl. Cancer Inst. Monograph* **4**, 251–285.
Bonar, R. A., Heine, U., Beard, D., and Beard, J. W. (1963a). *J. Natl. Cancer Inst.* **30**, 949–997.
Bonar, R. A., Purcell, R. H., Beard, D., and Beard, J. W. (1963b). *J. Natl. Cancer Inst.* **31**, 705–714.
Borsos, T. (1958). *J. Natl. Cancer Inst.* **20**, 1215–1221.
Bower, R. K. (1962). *Virology* **18**, 372–377.
Bower, R. K., Gyles, N. R., and Brown, C. J. (1964). *Virology* **24**, 47–50.
Bryan, W. R. (1946). *J. Natl. Cancer Inst.* **6**, 225–237.
Bryan, W. R. (1954). *J. Natl. Cancer Inst.* **15**, 305–314.
Bryan, W. R. (1955). *J. Natl. Cancer Inst.* **16**, 843–863.
Bryan, W. R., and Moloney, J. B. (1957). *Ann. N.Y. Acad. Sci.* **68**, 441–453.
Bryan, W. R., Maver, M. E., Moloney, J. B., Wood, M. T., and White, C. L. (1950a). *J. Natl. Cancer Inst.* **11**, 269–277.
Bryan, W. R., Maver, M. E., Moloney, J. B., Calnan, D., White, C. L., and Wood, M. (1950b). *J. Natl. Cancer Inst.* **11**, 929–937.
Bryan, W. R., Moloney, J. B., and Calnan, D. (1954). *J. Natl. Cancer Inst.* **15**, 315–329.
Bryan, W. R., Calnan, D., and Moloney, J. B. (1955). *J. Natl. Cancer Inst.* **16**, 316–335.
Burges, P. R. F., and Duran-Reynals, F. (1952). *Cancer Res.* **12**, 55–58.
Burk, D., Prince, H., Spangler, J. M., Kabat, E. A., Furth, J., and Claude, A. (1941). *J. Natl. Cancer Inst.* **2**, 201–240.
Burmester, B. R. (1955). *Proc. Soc. Exptl. Biol. Med.* **88**, 153–155.
Burmester, B. R. (1957). *Ann. N.Y. Acad. Sci.* **68**, 487–495.
Burmester, B. R. (1960). *J. Natl. Cancer Inst.* **25**, 307–313.

Burmester, B. R. (1962). *Cold Spring Harbor Symp. Quant. Biol.* **27**, 471–477.
Burmester, B. R., and Fredrickson, T. N. (1964). *J. Natl. Cancer Inst.* **32**, 37–63.
Burmester, B. R., and Gentry, R. F. (1954). *Cancer Res.* **14**, 34–42.
Burmester, B. R., and Walter, W. G. (1961). *J. Natl. Cancer Inst.* **26**, 511–518.
Burmester, B. R., and Waters, N. F. (1955). *Poultry Sci.* **34**, 1415–1429.
Burmester, B. R., Gross, M. A., Walter, W. G., and Fontes, A. K. (1959a). *J. Natl. Cancer Inst.* **22**, 103–127.
Burmester, B. R., Walter, W. G., Gross, M. A., and Fontes, A. K. (1959b). *J. Natl. Cancer Inst.* **23**, 277–291.
Burmester, B. R., Fontes, A. K., and Walter, W. G. (1960a). *N. Natl. Cancer Inst.* **24**, 1423–1442.
Burmester, B. R., Fontes, A. K., Waters, N. F., Bryan, W. R., and Groupé, V. (1960b). *Poultry Sci.* **39**, 199–215.
Buthala, D. A. (1964). *Proc. Soc. Exptl. Biol. Med.* **115**, 69–71.
Carr, J. G. (1943a). *Brit. J. Exptl. Pathol.* **24**, 127–132.
Carr, J. G. (1943b). *Brit. J. Exptl. Pathol.* **24**, 133–137.
Carr, J. G. (1960). *Vet. Record* **72**, 87.
Carr, J. G., and Harris, R. J. (1951). *Brit. J. Cancer* **5**, 95–105.
Chýle, P. (1964). *Folia Biol. (Prague)* **10**, 359–365.
Chýle, P., Klement, V., and Svoboda, J. (1963). *Folia Biol. (Prague)* **9**, 92–98.
Claude, A., and Murphy, J. B. (1933). *Physiol. Rev.* **13**, 246–275.
Claude, A., Porter, K. R., and Pickels, E. G. (1947). *Cancer Res.* **7**, 421–430.
Crawford, L. V. (1960). *Virology* **12**, 143–153.
Crawford, L. V., and Crawford, E. M. (1961). *Virology* **13**, 227–232.
Crittenden, L. B., Okazaki, W., and Raemer, R. (1963). *Virology* **20**, 541–544.
Darcel, C. (1960). *Cancer Res.* **20**, 2–17.
Delihas, N., and Evans, E. (1963). *Nature* **199**, 488–489.
de Thé, G., Heine, U., Sommer, J. R., Arvy, L., Beard, D., and Beard, J. W. (1963a). *J. Natl. Cancer Inst.* **30**, 415–455.
de Thé, G., Ishiguro, H., Heine, U., Beard, D., and Beard, J. W. (1963b). *J. Natl. Cancer Inst.* **30**, 1267–1301.
de Thé, G., Ishiguro, H., Beard, D., and Beard, J. W. (1963c). *J. Natl. Cancer Inst.* **31**, 717–739.
de Thé, G., Becker, C., and Beard, J. W. (1964). *J. Natl. Cancer Inst.* **32**, 201–235.
Dhaliwal, S. S. (1963). *J. Natl. Cancer Inst.* **30**, 323–336.
Dmochowski, L., Grey, C. E., Burmester, B. R., and Walter, W. G. (1958). *Proc. Soc. Exptl. Biol. Med.* **98**, 666–669.
Dmochowski, L., Grey, C. E., Burmester, B. R., and Walter, W. G. (1961). *Texas Rept. Biol. Med.* **19**, 545–579.
Dmochowski, L., Grey, C. E., Padgett, F., Langford, P. L., and Burmester, B. R. (1964). *Texas Rept. Biol. Med.* **22**, 20–60.
Doljanski, L., and Pikovski, M. (1942). *Cancer Res.* **2**, 626–631.
Dougherty, R. M. (1961). *Virology* **14**, 371–372.
Dougherty, R. M., and Morgan, H. R. (1962). *J. Exptl. Med.* **115**, 245–251.
Dougherty, R. M., and Simons, P. J. (1962). *Virology* **18**, 559–566.
Dougherty, R. M., and Trevan, D. J. (1963). *Proc. Am. Assoc. Cancer Res.* **4**, 17.
Doughtery, R. M., Stewart, J. A., and Morgan, H. R. (1960). *Virology* **11**, 349–370.

Dougherty, R. M., Simons, P. J., and Chesterman, F. C. (1963). *J. Natl. Cancer Inst.* **31**, 1285–1311.
Dourmashkin, R. R., and Simons, P. J. (1961). *J. Ultrastruct. Res.* **5**, 505–522.
Drayton, H. A. (1960). *Brit. J. Cancer* **14**, 306–312.
Drayton, H. A. (1961). *Brit. J. Cancer* **15**, 348–352.
Duran-Reynals, F. (1940). *Yale J. Biol. Med.* **13**, 61–76.
Duran-Reynals, F. (1942). *Cancer Res.* **2**, 343–369.
Duran-Reynals, F. (1946). *Cancer Res.* **6**, 529–534.
Duran-Reynals, F. (1947a). *Cancer Res.* **7**, 99–102.
Duran-Reynals, F. (1947b). *Cancer Res.* **7**, 103–106.
Duran-Reynals, F., and Freire, P. (1953). *Cancer Res.* **13**, 376–382.
Duran-Reynals, F., Burmester, B. R., Cottral, G. E., and Bryan, E. (1953). *Cancer Res.* **13**, 408–414.
Eckert, E. A., Beard, D., and Beard, J. W. (1951). *J. Natl. Cancer Inst.* **12**, 447–463.
Eckert, E. A., Sharp, D. G., Beard, D., and Beard, J. W. (1952). *J. Natl. Cancer Inst.* **13**, 533–541.
Eckert, E. A., Beard, D., and Beard, J. W. (1954a). *J. Natl. Cancer Inst.* **14**, 1055–1067.
Eckert, E. A., Sharp, D. G., Mommaerts, E. B., Reeve, R. H., Beard, D., and Beard, J. W. (1954b). *J. Natl. Cancer Inst.* **14**, 1039–1053.
Eckert, E. A., Waters, N. F., Burmester, B. R., Beard, D., and Beard, J. W. (1954c). *J. Natl. Cancer Inst.* **14**, 1067–1080.
Eckert, E. A., Beard, D., and Beard, J. W. (1955a). *J. Natl. Cancer Inst.* **15**, 1195–1207.
Eckert, E. A., Green, I., Sharp, D. G., Beard, D., and Beard, J. W. (1955b). *J. Natl. Cancer Inst.* **16**, 153–161.
Eckert, E. A., Green, I., Sharp, D. G., Beard, D., and Beard, J. W. (1955c). *J. Natl. Cancer Inst.* **15**, 1209–1215.
Eckert, E. A., Sharp, D. G., Beard, D., Green, I., and Beard, J. W. (1955d). *J. Natl. Cancer Inst.* **16**, 593–644.
Eckert, E. A., Rott, R., and Schäfer, W. (1963). *Z. Naturforsch.* **18b**, 339–340.
Eckert, E. A., Rott, R., and Schäfer, W. (1964a). *Virology* **24**, 426–433.
Eckert, E. A., Rott, R., and Schäfer, W. (1964b). *Virology* **24**, 434–448.
Edlinger, E. (1959). *Neoplasma* **6**, 138–143.
Eidinoff, M. L., Bates, B., Perez, A., and de la Sierra, A. (1963). *Proc. Am. Assoc. Cancer Res.* **4**, 18.
Elford, W. J., and Andrewes, C. H. (1935). *Brit. J. Exptl. Pathol.* **16**, 61–66.
Engelbreth-Holm, J. (1942). "Spontaneous and Experimental Leukemia in Animals." Oliver & Boyd, Edinburgh and London.
Engelbreth-Holm, J., and Frederiksen, O. (1938). *Acta. Pathol. Microbiol. Scand.* **37**, 138–144.
Ephrussi, B., and Temin, H. (1960). *Virology* **11**, 547–552.
Epstein, M. A. (1956). *Brit. J. Cancer* **10**, 33–48.
Epstein, M. A. (1957a). *Brit. J. Cancer* **11**, 268–273.
Epstein, M. A. (1957b). *J. Biophs. Biochem. Cytol.* **3**, 851–857.
Epstein, M. A. (1958a). *Brit. J. Cancer* **12**, 248–255.
Epstein, M. A. (1958b). *Nature* **181**, 1808.
Erichsen, S., Eng, J., and Morgan, H. R. (1961). *J. Exptl. Med.* **114**, 435–440.

Fairman, M. (1959). *J. Gen. Microbiol.* **20**, ii–iii.
Febvre, H. (1958). *Bull. Cancer* **45**, 434–444.
Fink, M. A. (1964). *Proc. Soc. Exptl. Biol. Med.* **115**, 580–584.
Force, E. E., and Stewart, R. C. (1964). *Proc. Soc. Exptl. Biol. Med.* **116**, 803–806.
Foulds, L. (1934). *Sci. Rept. Imp. Cancer Res. Fund Suppl.* **11**, 1–41.
Franklin, R. M. (1958). *Experientia* **14**, 346–348.
Fredrickson, R. N., Piraino, F., Okazaki, W., and Burmester, B. R. (1964). *Avian Diseases* **8**, 123–134.
Freeman, G. (1963). *J. Natl. Cancer Inst.* **31**, 761–767.
Friesen, B., and Rubin, H. (1961). *Virology* **15**, 387–396.
Fujinami, A., and Hatano, S. (1929). *Gann* **23**, 67–75.
Gaylord, W. H. I. (1955). *Cancer Res.* **15**, 80–83.
Goldé, A. (1959). *Exptl. Cell Res.* **18**, 528–541.
Goldé, A. (1962). *Virology* **16**, 9–20.
Goldé, A., and Vigier, P. (1961). *Virology* **15**, 36–46.
Goldé, A., and Vigier, P. (1963). *Virology* **20**, 420–432.
Gowen, J. W. (1958). *Ann. N.Y. Acad. Sci.* **71**, 1179–1188.
Green, I., and Beard, J. W. (1955). *J. Natl. Cancer Inst.* **16**, 163–172.
Greenwood, A. W., Blyth, J. S. S., and Carr, J. G. (1948). *Brit. J. Cancer* **2**, 135–143.
Grossfeld, H. (1962). *Nature* **196**, 783.
Groupé, V., and Rauscher, F. J. (1957). *J. Natl. Cancer Inst.* **18**, 507–514.
Groupé, V., Dunkel, V. C., and Manaker, R. A. (1957). *J. Bacteriol.* **74**, 409–410.
Gusev, A. J., and Svoboda, J. (1962). *Folia Biol. (Prague)* **8**, 140–142.
Gye, W. E. (1932). *Brit. J. Exptl. Pathol.* **13**, 458–460.
Gye, W. E., and Purdy, W. J. (1933). *Brit. J. Exptl. Pathol.* **14**, 250–259.
Haguenau, F., and Beard, J. W. (1962). *In* "Ultrastructure of Tumors Induced by Viruses" (A. J. Dalton and F. Haguenau, eds.), pp. 1–50. Academic Press, New York.
Haguenau, F., Dalton, A. J., and Moloney, J. B. (1958). *J Natl. Cancer Inst.* **20**, 633–649.
Haguenau, F., Febvre, H., and Arnoult, J. (1960). *Compt. Rend. Acad. Sci.* **250**, 1477–1479.
Haguenau, F., Febvre, H., and Arnoult, J. (1961). *Perspectives Virol. Symp.* **2**, 160–173, New York.
Haguenau, F., Febvre, H., and Arnoult, J. (1962). *J. Microscopie* **1**, 445–454.
Halberstaedter, L., Doljanski, L., and Tenenbaum, E. (1941). *Brit. J. Exptl. Pathol.* **22**, 179–187.
Hampton, E. G., and Eidinoff, M. L. (1962). *Cancer Res.* **22**, 1061–1066.
Hanafusa, H. (1964). *Natl. Cancer Inst. Monograph* **17**, 543–556.
Hanafusa, H. (1965). *Virology* **25**, 248–255.
Hanafusa, H., Hanafusa, T., and Rubin, H. (1963). *Proc. Natl. Acad. Sci. U.S.* **49**, 572–580.
Hanafusa, H., Hanafusa, T., and Rubin, H. (1964a). *Proc. Natl. Acad. Sci. U.S.* **51**, 41–48.
Hanafusa, H., Hanafusa, T., and Rubin, H. (1964b). *Virology* **22**, 591–601.
Hanafusa, T., Hanafusa, H., and Rubin, H. (1964c). *Virology* **22**, 643–645.
Harris, R. J. C. (1953). *Advan. Cancer Res.* **1**, 233–271.
Harris, R. J. C. (1954). *Brit. J. Cancer* **8**, 731–736.

Harris, R. J. C. (1956). *Proc. Roy. Soc.* **B146**, 59–66.
Harris, R. J. C. (1963). "Viruses, Nucleic Acids and Cancer," pp. 331-343. Williams & Wilkins, Baltimore, Maryland.
Harris, R. J. C., and Chesterman, F. C. (1963). *Proc. Roy. Soc. Med.* **56**, 307–308.
Harris, R. J. C., and Simons, P. J. (1958). *Nature* **181**, 1485–1486.
Harris, R. J. C., and Simons, P. J. (1962). *In* "Mechanisms of Immunological Tolerance" (M. Hašek, A. Lengerová, and M. Vojtíšková eds.), pp. 187–198. Academic Press, New York.
Heine, U., Beaudreau, G. S., Becker, C., Beard, D., and Beard, J. W. (1961). *J. Natl. Cancer Inst.* **26**, 359–388.
Heine, U., de Thé, G., Ishiguro, H., and Beard, J. W. (1962a). *J. Natl. Cancer Inst.* **29**, 111–123.
Heine, U., de Thé, G., Ishiguro, H., Sommer, J. R., Beard, D., and Beard, J. W. (1962b). *J. Natl. Cancer Inst.* **29**, 41–105.
Heine, U., Becker, C., Bonar, R. A., and Beard, J. W. (1963a). *J. Natl. Cancer Inst.* **31**, 731–739.
Heine, U., de Thé, G., Beard, D., and Beard, J. W. (1963b). *J. Natl. Cancer Inst.* **30**, 817–836.
Huebner, R. J., Armstrong, D., Okuyan, M., Sarma, P. S., and Turner, H. C. (1963). *Natl. Acad. Sci. U.S.* **50**, 379–389.
Huebner, R. J., Armstrong, D., Okuyan, M., Sarma, P. S., and Turner, H. C. (1964). *Proc. Natl. Acad. Sci. U.S.* **51**, 742–749.
Hughes, W. F., Watanabe, D. H., and Rubin, H. (1963). *Avian Diseases* **7**, 154–165.
Ising-Iversen, M. (1960). *Acta Pathol. Microbiol. Scand.* **50**, 145–155.
Iwakata, S. (1961a). *Acta. Haematol. Japon.* **24**, 584–590.
Iwakata, S. (1961b). *Ann. Rept. Inst. Virus Res. Kyoto Univ.* **4**, 24–36.
Iwakata, S., and Amano, S. (1958). *Acta Haematol. Japon.* **21**, 154–163.
Iwakata, S., and Ichikawa, Y. (1960). *Acta Haematol. Japon.* **23**, 767–775.
Jensen, C., Girardi, A. J., Gilden, R. V., and Koprowski, H. K. (1964). *Proc. Natl. Acad. Sci. U.S.* **52**, 53–59.
Kabat, E. A., and Furth, J. (1941). *J. Exptl. Med.* **74**, 257–261.
Kahler, H., Bryan, W. R., Lloyd, B. J., and Moloney, J. B. (1954a). *J. Natl. Cancer Inst.* **15**, 331–336.
Kahler, H., Bryan, W. R., Lloyd, B. J., and Moloney, J. B. (1954b). *J. Natl. Cancer Inst.* **15**, 337–339.
Karrer, H. E., and Cox, J. (1960). *J. Ultrastruct. Res.* **4**, 360–371.
Kenzy, S. G. (1953). *Am. J. Vet. Res.* **14**, 455–457.
Kenzy, S. G., and Neuzil, P. V. (1953). *Am. J. Vet. Res.* **14**, 123–128.
Kenzy, S. G., McClary, C. F., and Zander, D. V. (1961). *Avian Diseases* **5**, 337–346.
Keogh, E. V. (1938). *Brit. J. Exptl. Pathol.* **19**, 1–8.
Kingsbury, D. W. (1962). *Biochem. Biophs. Res. Commun.* **9**, 156–161.
Klement, V. (1964). *Folia. Biol. (Prague)* **10**, 321–324.
Klement, V., and Sácha, I. (1963). *Folia Biol. (Prague)* **9**, 343–353.
Klement, V., and Svoboda, J. (1963). *Folia Biol. (Prague)* **9**, 181–188.
Klement, V. Chýle, P., and Svoboda, J. (1963). *Folia Biol. (Prague)* **9**, 412–419.
Knight, C. A. (1946). *J. Exptl. Med.* **83**, 281–294.
Kuwata, T. (1964a). *Arch. Ges. Virusforsch.* **14**, 628–636.
Kuwata, T. (1964b). *Cancer Res.* **24**, 947–956.

Lagerlöf, B. (1960a). *Acta Pathol. Microbiol. Scand.* **49**, 361–372.
Lagerlöf, B. (1960b). *Acta Pathol. Microbiol. Scand.* **49**, 438–454.
Lagerlöf, B. (1960c). *Acta Pathol. Microbiol. Scand.* **49**, 455–466.
Lagerlöf, B. (1960d). *Acta Pathol. Microbiol. Scand.* **49**, 467–479.
Landa, Z., Svoboda, J., and Chýle, P. (1962a). *Folia Biol. (Prague)* **8**, 84–89.
Landa, Z., Svoboda, J., and Jirásek, J. (1962b). *Folia Biol. (Prague)* **8**, 12–15.
Laver, W. G. (1962). *Virology* **18**, 19–32.
Levine, A. S., Stricker, F., Uhl, R., and Ashmore, J. (1960). *Nature* **188**, 229–230.
Levine, A. S., Uhl, R., and Ashmore, J. (1961). *J. Natl. Cancer Inst.* **27**, 597–609.
Levine, A. S., Petraitis, R., and Green, I. (1962). *Nature* **192**, 1271–1272.
Levine, S., and Nelsen, D. (1964). *Avian Diseases* **8**, 358–368.
Lo, W. H. Y., Gey, G. O., and Sharpes, P. (1955). *Bull. Johns Hopkins Hosp.* **97**, 248–256.
Love, W. C., Levine, A. S., and Ashmore, J. (1964). *J. Natl. Cancer Inst.* **32**, 731–738.
Malmgren, R. A., Fink, M. A., and Mills, W. (1960). *J. Natl. Cancer Inst.* **24**, 995–1001.
Manaker, R. A., and Groupé, V. (1956). *Virology* **2**, 838–840.
Mannweiler, K., and Bernhard, W. (1958). *Bull. Cancer* **45**, 223–236.
Mellors, R. C., and Munroe, J. S. (1960). *J. Exptl. Med.* **112**, 963–974.
Mendle, L., and Pollard, M. (1959). *Texas Rept. Biol. Med.* **17**, 218–223.
Mommaerts, E. B., Eckert, E. A., Beard, D., Sharp, D. G., and Beard, J. W. (1952). *Proc. Soc. Exptl. Biol. Med.* **79**, 450–455.
Mommaerts, E. B., Sharp, D. G., Eckert, E. A., Beard, D., and Beard, J. W. (1954). *J. Natl. Cancer Inst.* **14**, 1011–1025.
Morgan, H. R., and Andrese, A. P. (1961). *Experientia* **17**, 309–310.
Morgan, H. R., and Andrese, A. P. (1962). *J. Exptl. Med.* **116**, 329–335.
Moses, E., and Kohn, A. (1963). *Exptl. Cell Res.* **32**, 182–186.
Munroe, J. S., and Southam, C. M. (1959). *Cancer Res.* **19**, 303–306.
Munroe, J. S., and Southam, C. M. (1961). *J. Natl. Cancer Inst.* **26**, 775–783.
Munroe, J. S., and Southam, C. M. (1964). *J. Natl. Cancer Inst.* **32**, 591–623.
Munroe, J. S., and Windle, W. R. (1963). *Science* **140**, 1415–1416.
Murphy, J. B., and Rous, P. (1912). *J. Exptl. Med.* **15**, 119–132.
Nichols, W. W. (1963). *Heriditas* **50**, 53–80.
Nichols, W. W., Levan, A., and Ahlström, C. G. (1963). *Proc. Am. Assoc. Cancer Res.* **4**, 48.
Nichols, W. W., Levan, A., Coriell, L. L., Goldner, H., and Ahlström, C. G. (1964). *Science* **146**, 248–250.
Nishiumi, Y., Okano, H., and Imai, T. (1961). *Proc. Soc. Exptl. Biol. Med.* **107**, 88–90.
Novikoff, A. B., de Thé, G., Beard, D., and Beard, J. W. (1962). *J. Cell Biol.* **15**, 451–462.
Noyes, W. F. (1960). *Virology* **12**, 488–492.
Parsons, D. F., Painter, J. C., Beaudreau, G. S., Becker, C., and Beard, J. W. (1958). *Proc. Soc. Exptl. Biol. Med.* **97**, 839–844.
Payne, L. N., and Biggs, P. M. (1964). *Nature* **203**, 1306–1307.
Peterson, R. D. A., Burmester, B. R., Fredrickson, T. N., Purchase, H. G., and Good, R. A. (1964). *J. Natl. Cancer Inst.* **32**, 1343–1353.
Piraino, F., Okazaki, W., Burmester, B. R., and Fredrickson, T. N. (1963). *Virology* **21**, 396–401.

Pirie, A., and Holmes, B. E. (1931). *Brit. J. Exptl. Pathol.* **12**, 127–133.
Pontén, J. (1962a). *Nature* **194**, 97.
Pontén, J. (1962b). *J. Cellular Comp. Physiol.* **60**, 209–215.
Pontén, J. (1962c). *J. Natl. Cancer Inst.* **29**, 1013–1021.
Pontén, J. (1962d). *J. Natl. Cancer Inst.* **29**, 1147–1159.
Pontén, J. (1963). *J. Natl. Cancer Inst.* **30**, 897–921.
Prince, A. M. (1958a). *J. Natl. Cancer Inst.* **20**, 147–159.
Prince, A. M. (1958b). *J. Natl. Cancer Inst.* **20**, 843–850.
Prince, A. M. (1958c). *Virology* **5**, 435–457.
Prince, A. M. (1959). *J. Natl. Cancer Inst.* **23**, 1361–1381.
Prince, A. M. (1960). *Virology* **11**, 371–399.
Purcell, R. H., Bonar, R. A., Beard, D., and Beard, J. W. (1962). *J. Natl. Cancer Inst.* **28**, 1003–1011.
Purchase, H. G., and Okazaki, W. (1964). *J. Natl. Cancer Inst.* **32**, 579–589.
Rauscher, F. J., and Groupé, V. (1960a). *J. Natl. Cancer Inst.* **25**, 141–159.
Rauscher, F. J., and Groupé, V. (1960b). *J. Natl. Cancer Inst.* **25**, 1391–1404.
Reda, I. M., and Rott, R. (1962). *Zentr. Veterinaermed.* **9**, 158–164.
Rich, M. A., Stonehill, E. H., and Eidinoff, M. L. (1960). *J. Natl. Cancer Inst.* **25**, 1355–1363.
Rich, M. A., Perez, A. G., and Eidinoff, M. L. (1962). *Virology* **16**, 98–99.
Řiman, J., and Thorell, B. (1960). *Biochim. Biophs. Acta* **40**, 565–567.
Rothschild, L., and Febvre, H. (1963). *Compt. Rend. Acad. Sci.* **257**, 3530–3532.
Rott, R., and Scholtissek, C. (1964). *Z. Naturforsch.* **19b**, 316–323.
Rous, P. (1911). *J. Exptl. Med.* **13**, 397–411.
Rous, P. (1913). *J. Exptl. Med.* **18**, 416–427.
Rous, P. (1936). *Am. J. Cancer* **28**, 233–272.
Rous, P., and Murphy, J. B. (1941a). *J. Exptl. Med.* **19**, 52–69.
Rous, P., and Murphy, J. B. (1941b). *J. Exptl. Med.* **20**, 419–432.
Rubin, H. (1955). *Virology* **1**, 445–473.
Rubin, H. (1956). *Virology* **2**, 545–558.
Rubin, H. (1957). *Ann. N.Y. Acad. Sci.* **68**, 459–472.
Rubin, H. (1960a). *Proc. Natl. Acad. Sci. U.S.* **46**, 1105–1119.
Rubin, H. (1960b). *Virology* **10**, 29–49.
Rubin, H. (1960c). *Virology* **11**, 28–47.
Rubin, H. (1960d). *Virology* **12**, 14–31.
Rubin, H. (1961a). *Virology* **13**, 200–206.
Rubin, H. (1961b). *Cancer Res.* **21**, 1244–1253.
Rubin, H. (1962a). *Cold Spring Harbor Symp. Quant. Biol.* **27**, 441–452.
Rubin, H. (1962b). *Ann. N.Y. Acad. Sci.* **101**, 181–184.
Rubin, H. (1962c). *Nature* **195**, 342–345.
Rubin, H. (1964a). *J. Cellular Comp. Physiol.* **64**, 173–179.
Rubin, H. (1964b). *Sci. Am.* **210**, 46–52.
Rubin, H., and Hanafusa, H. (1963). "Viruses, Nucleic Acids and Cancer," pp. 508–525. Williams & Wilkins, Baltimore, Maryland.
Rubin, H., and Temin, H. M. (1959). *Virology* **7**, 75–91.
Rubin, H., and Vogt, P. K. (1962). *Virology* **17**, 184–194.
Rubin, H., Baluda, M., and Hotchin, J. E. (1955). *J. Exptl. Med.* **101**, 205–212.
Rubin, H., Cornelius, A., and Fanshier, L. (1961). *Proc. Natl. Acad. Sci. U.S.* **47**, 1058–1060.

Rubin, H., Fanshier, L., Cornelius, A., and Hughes, W. F. (1962). *Virology* **17**, 143–156.
Ruffili, D. (1938). *Bull. Lega Ital. Lotta Contra Tumori* **12**, 3–16.
Rychlíkova, M., and Svoboda, J. (1959). *Virology* **10**, 545–547.
Saldeen, T. (1962). *Acta Pathol. Microbiol. Scand.* **55**, 151–165.
Saldeen, T. (1963). *Acta Pathol. Microbiol. Scand.* Suppl. **162**, 1–87.
Sarma, P. S., Huebner, R. J., and Armstrong, D. (1964a). *Proc. Soc. Exptl. Biol. Med.* **115**, 481–486.
Sarma, P. S., Turner, H. C., and Huebner, R. J. (1964b). *Virology* **23**, 313–321.
Schmidt, F. (1957). *Z. Krebsforsch.* **61**, 527–533.
Schmidt, F. (1963). *Acta Biol. Med. Ger.* **10**, 615–617.
Schmidt-Ruppin, K. H. (1959). *Strahlentherapie, Sonderbaende* **41**, 26–27.
Schmidt-Ruppin, K. H. (1964). *Oncologia* **17**, 247–277.
Sevoian, M., and Chamberlain, D. M. (1962). *Vet. Med.* **57**, 608–609.
Sevoian, M., and Chamberlain, D. M. (1963). *Avian Diseases* **7**, 97–102.
Sevoian, M., and Chamberlain, D. M. (1964). *Avian Diseases* **8**, 281–310.
Sevoian, M., Chamberlain, D. M., and Counter, F. T. (1962). *Vet. Med.* **57**, 500–501.
Sevoian, M., Chamberlain, D. M., and Larose, R. N. (1963). *Avian Diseases* **7**, 102–105.
Sevoian, M., Larose, R. N., and Chamberlain, D. M. (1964). *Avian Diseases* **8**, 336–346.
Shapiro, V. S., and Morgunova, T. D. (1963). *Vopr. Virusol.* **8**, 346–348.
Sharp, D. G., and Beard, J. W. (1952). *Proc. Soc. Exptl. Biol. Med.* **81**, 75–79.
Sharp, D. G., and Beard, J. W. (1954). *Biochim, Biophys. Acta* **14**, 12–17.
Sharp, D. G., Eckert, E. A., Beard, D., and Beard, J. W. (1952). *J. Bacteriol.* **63**, 151–161.
Sharp, D. G., Mommaerts, E. B., Eckert, E. A., Beard, D., and Beard, J. W. (1954). *J. Natl. Cancer Inst.* **14**, 1027–1037.
Sharp, D. G., Beard, D., and Beard, J. W. (1955). *Proc. Soc. Exptl. Biol. Med.* **90**, 168–173.
Shimizu, T., and Rubin, H. (1964). *J. Natl. Cancer Inst.* **33**, 79–91.
Siminoff, P., and Reed, F. C. III (1963). *Virology* **21**, 284–286.
Šimkovič, D., Valentová, N., and Thurzo, V. (1962a). *Folia Biol. (Prague)* **8**, 221–229.
Šimkovič, D., Valentová, N., and Thurzo, V. (1962b). *Neoplasma* **9**, 104–106.
Šimkovič, D., Svoboda, J., and Valentová, N. (1963). *Folia Biol. (Prague)* **9**, 82–91.
Simons, P. J., and Dougherty, R. M. (1961). *Virology* **15**, 200–201.
Simons, P. J., and Dougherty, R. M. (1963). *J. Natl. Cancer Inst.* **31**, 1275–1283.
Sjögren, H. O., and Jonsson, N. (1963). *Exptl. Cell Res.* **32**, 618–620.
Smidova, V., and Rada B. (1964). *Neoplasma* **11**, 307–309.
Smidova, V., and Thurzo, V. (1964). *Neoplasma* **11**, 310–313.
Sokoloff, B., Enomoto, K., Saelhof, C., Fujisawa, M., and Bird, L. (1963). *Growth* **27**, 29–38.
Spencer, J. H., and Groupé, V. (1962a). *J. Natl. Cancer Inst.* **29**, 397–419.
Spencer, J. H., and Groupé, V. (1962b). *J. Natl. Cancer Inst.* **29**, 421–441.
Stenback, W. A., and Durand, D. P. (1963). *Virology* **20**, 545–551.
Stenkvist, B., and Pontén, J. (1963). *Acta Pathol. Microbiol. Scand.* **58**, 273–282.

Sturm, E., Gates, F. L., and Murphy, J. B. (1932). *J. Exptl. Med.* **55**, 551–444.
Sverak, L., Beaudreau, G. S., and Beard, J. W. (1962). *J. Natl. Cancer Inst.* **29**, 355–373.
Svet-Moldavsky, G. J. (1957). *Nature* **80**, 1299–1300.
Svet-Moldavsky, G. J. (1958a). *Acta Virol. (Prague)* **2**, 1–6.
Svet-Moldavsky, G. J. (1958b). *Nature* **182**, 1452–1453.
Svet-Moldavsky, G. J. (1961). *Acta Virol. (Prague)* **5**, 167–177.
Svet-Moldavsky, G. J. (1963). *Acta Unio. Intern. Contra Cancrum* **19**, 299–301.
Svet-Moldavsky, G. J., and Skorikova, A. S. (1960). *Acta Virol. (Prague)* **4**, 47–51.
Svet-Moldavsky, G. J., and Svet-Moldavskaya, I. A. (1964). *Science* **143**, 54–55.
Svoboda, J. (1958). *Folia Biol. (Prague)* **4**, 205–208.
Svoboda, J. (1960a). *Folia Biol. (Prague)* **6**, 21–26.
Svoboda, J. (1960b). *Folia Biol. (Prague)* **6**, 96–100.
Svoboda, J. (1960c). *Nature* **186**, 980–981.
Svoboda, J. (1961). *Folia Biol. (Prague)* **7**, 46–60.
Svoboda, J. (1962a). *Folia Biol. (Prague)* **8**, 133–139.
Svoboda, J. (1962b). *Folia Biol. (Prague)* **8**, 215–220.
Svoboda, J. (1962c). *In* "Mechanisms of Immunological Tolerance" (M. Hašek, A. Lengerová, and M. Vojtíšková, eds.), pp. 199–210. Academic Press, New York.
Svoboda, J. (1962d). *Neoplasma* **9**, 33–37.
Svoboda, J., and Chýle, P. (1963). *Folia Biol. (Prague)* **9**, 329–342.
Svoboda, J., and Klement, V. (1963). *Folia Biol. (Prague)* **9**, 403–411.
Svoboda, J., and Šimkovič, D. (1963). *Acta. Unio Intern. Contra Cancrum* **19**, 302–303.
Svoboda, J., Chýle, P., Šimkovič, D., and Hilgert, I. (1963). *Folia Biol. (Prague)* **9**, 77–81.
Temin, H. M. (1960). *Virology* **10**, 182–197.
Temin, H. M. (1961). *Virology* **13**, 158–163.
Temin, H. M. (1962). *Cold Spring Harbor Symp. Quant. Biol.* **27**, 407–414.
Temin, H. M. (1963a). *Virology* **20**, 235–245.
Temin, H. M. (1963b). *Virology* **20**, 577–582.
Temin, H. M. (1964a). *Proc. Natl. Acad. Sci. U.S.* **52**, 323–329.
Temin, H. M. (1964b). *Virology* **23**, 486–494.
Temin, H. M., and Rubin, H. (1958). *Virology* **6**, 669–688.
Temin, H. M., and Rubin, H. (1959). *Virology* **8**, 209–222.
Thorell, B. (1961). *Acta Pathol. Microbiol. Scand.* Suppl. 144, 215–217.
Thorell, B. (1962). *Proc. Congr. European Soc. Haematol. 8th, Vienna, 1961*, pp. 102–103. Karger, Basel.
Thorell, B. (1963). *Intern. Rev. Exptl. Pathol.* **2**, 47–60.
Thurzo, V., and Smida, J. (1963). *Neoplasma* **10**, 445–447.
Thurzo, V., Smida, J., Smidova-Kovarova, V., and Šimkovič, D. (1963). *Acta Unio Intern. Contra Cancrum* **19**, 304–305.
Ting, R. C. (1964). *Virology* **22**, 568–574.
Trager, G., and Rubin, H. (1964). *Natl. Cancer Inst. Monograph* **17**, 575–585.
Trávníček, M., Buřič, L., Řiman, J., and Šorm, F. (1964). *Neoplasma* **11**, 571–584.
Vigier, P. (1958). *Bull. Cancer* **45**, 460–475.
Vigier, P. (1959). *Virology* **8**, 41–59.
Vigier, P., and Goldé, A. (1959). *Virology* **8**, 60–79.

Vigier, P. and Goldé, A. (1964a). *Compt. Rend. Acad. Sci.* **258**, 389–392.
Vigier, P., and Goldé, A. (1964b). *Virology* **23**, 511–514.
Vogt, P. K. (1963). "Viruses, Nucleic Acids and Cancer," pp. 374–386. Williams & Wilkins, Baltimore, Maryland.
Vogt, P. K. (1964). *National Cancer Inst. Monograph* **17**, 523–541.
Vogt, P. K. (1965). *Virology* **25**, 237–247.
Vogt, P. K., and Luykx, N. (1963). *Virology* **20**, 75–87.
Vogt, P. K., and Rubin, H. (1961). *Virology* **13**, 528–544.
Vogt, P. K., and Rubin, H. (1962). *Cold Spring Harbor Symp. Quant. Biol.* **27**, 395–405.
Vogt, P. K., and Rubin, H. (1963). *Virology* **19**, 92–104.
Vrba, M. (1963). *Acta Virol. (Prague)* **7**, 525–533.
Vrba, M., and Chaytor, D. (1964). *Folia Biol. (Prague)* **10**, 50–53.
Wagle, S. R., Levine, A. S., and Ashmore, J. (1961). *Biochim. Biophys. Acta* **51**, 421–422.
Wagle, S. R., Ashmore, J., and Levine, A. S. (1963). *Cancer Res.* **23**, 1808–1812.
Wallbank, A. M., Sperling, G., Stubb, E. L., and Hubben, K. (1962). *Proc. Soc. Exptl. Biol. Med.* **110**, 809–811.
Waters, N. F., and Burmester, B. R. (1961). *J. Natl. Cancer Inst.* **27**, 655–661.
Waters, N. F., and Fontes, A. K. (1960). *J. Natl. Cancer Inst.* **25**, 351–357.
Waters, N. F., Burmester, B. R., and Walter, W. G. (1958). *J. Natl. Cancer Inst.* **20**, 1245–1256.
Weber, G., Banerjee, G., Levine, A. S., and Ashmore, J. (1961). *J. Natl. Cancer Inst.* **27**, 869–873.
Yamagiwa, S., Ono, T., Ueda, A., and Inoue, M. (1963). *Japan. J. Vet. Res.* **11**, 99–122.
Yamaguchi, J. (1962). *In* "Proceedings of the Fifth International Congress for Electron Microscopy" (S. S. Breese, ed.), Vol. 2, PP-1. Academic Press, New York.
Zeigel, R. F. (1961). *J. Natl. Cancer Inst.* **26**, 1011–1039.
Zilber, L. A. (1961). *J. Natl. Cancer Inst.* **26**, 1295–1309.
Zilber, L. A., and Kryukova, I. N. (1957). *Probl. Virol. (USSR) (English Transl.)* **2**, 247–252.
Zilber, L. A., and Kryukova, I. N. (1958). *Vopr. Virusol.* **3**, 166–170.
Zilber, L. A., and Shevljaghyn, V. (1964). *Nature* **203**, 194–195.

AUTHOR INDEX

Numbers in italics refer to pages on which the complete references are listed.

A

Aach, H. G., 51, 233, 250, 254, 263, *271*
Abercrombie, M., 318, *374*
Ada, G. L., 304, *374*
Adams, E. V., 314, *374*
Afanasiev, M. M., 209, *217*, *218*
Agrawal, H., 12, 17, *22*
Ahlström, C. G., 365, 366, 367, 368, 369, 370, 371, 372, *374*, *375*, *381*
Ainsworth, G. C., 119, *134*
Aitken, T. H. G., 282, 286, *290*, *292*
Albrecht, P., 284, *291*
Alexander, L. J., 155, *158*
Allard, H. A., 64, *93*
Allen, R., 284, *292*
Allington, W. B., 98, 109, 113, 115, 116, 117, *136*
Allison, A. C., 303, 347, *375*
Altman, R. M., 283, *289*
Amano, S., 334, *380*
Ambrose, E. J., 318, *374*
Amies, C. R., 306, *375*
Amos, J., 61, 87, *93*, 102, 104, 105, 108, *134*
Anderer, F. A., 9, *22*, 26, 44, 45, *58*, 225, 234, 235, 251, *271*
Anderson, C. R., 75, *93*, 153, *159*, 280, *292*
Andrese, A. P., 355, *381*
Andrewes, C. H., 296, 297, 309, 356, 363, *375*, *378*
Andrews, J. E., 117, *136*
Anfinsen, C. B., 253, *271*
Anonymous, 102, *134*
Ansevin, A. T., 246, *271*, *274*
Apella, E., 243, *274*
Apple, J. L., 145, *158*
Armstrong, D., 309, 310, 349, 369, 370, *375*, *380*, *383*
Arnoult, J., 320, *379*
Arny, D. C., 75, *94*, 209, *220*
Arthur, D. R., 278, *289*
Arvy, L., 308, 330, 331, *377*
Ashmore, J., 323, 331, *375*, *381*, *385*
Ashworth, L. J., Jr., 109, *134*
Atanasoff, D., 119, 122, *134*
Athow, K. L., 89, *93*, 208, *215*
Atkinson, D. E., 109, 111, *134*, 263, 265, 268, *272*
Atkinson, T. G., 116, 117, *134*
Augustin, R., 10, *22*
Avegno, P., 230, *273*

B

Babos, P., 173, 196, *215*
Badami, R. S., 15, *22*, 75, *93*
Bader, J. P., 347, *375*
Baer, H., 289, *289*
Baggett, J. R., 147, *158*
Bagnall, R. H., 16, 18, *23*, 142, 152, 156, *158*, *160*
Baker, E. W., 100, 101, *134*
Baker, S. L., *375*
Balashov, Yu. S., 279, *289*
Bald, J. G., 88, *95*, 174, 177, 178, 179, 186, 196, 205, 207, 214, *215*, *218*, *220*
Ballard, J. C., 150, *159*
Baltimore, D., 45, *58*
Baluda, M., 316, 326, 328, 329, 353, 356, *375*, *382*
Bancroft, J. B., *215*
Banerjee, G., 323, 331, *375*, *385*
Bang, F. B., 354, 363, *375*
Barnett, H. C., 283, *289*, *290*
Barnett, L., 46, *58*
Barnhart, D., 148, *160*
Barrett, M. K., 306, *375*
Barry, R. D., 322, *375*
Bartels, R., 18, *23*
Barton, D. W., 147, 148, *158*, *161*
Basilio, C., 47, *60*
Basler, E. J., 237, 238, *272*
Bates, B., 321, *378*

Bather, R., 303, 304, 321, *375*
Bautz-Freese, E., 44, *58*
Bawden, F. C., 7, 15, 21, *23*, 27, 35, *58*, 167, 174, 176, 180, 182, 186, 187, 194, 201, 203, 204, 211, *215*, 230, 236, 237, 238, 249, 262, 264, 270, *271*
Baxter, L. W., Jr., 207, *215*
Bayreuther, K., 356, *375*
Beal, J. M., 174, 200, *219*
Beale, H. P., 227, 231, *271*
Beard, D., 297, 298, 299, 300, 301, 303, 304, 305, 306, 307, 308, 309, 311, 324, 325, 326, 329, 330, 331, 334, 353, 354, 360, 361, *375, 376, 377, 378, 380, 381, 383*
Beard, J. W., 295, 296, 297, 298, 299, 300, 301, 303, 304, 305, 306, 307, 308, 309, 311, 320, 324, 325, 326, 328, 329, 330, 331, 352, 353, 354, 356, 360, 361, *375, 376, 377, 378, 379, 380, 381, 383, 384*
Beaudreau, G. S., 298, 299, 306, 308, 309, 325, 326, 328, 329, 330, 331, 334, 356, 360, *375, 376, 380, 381, 384*
Becker, C., 308, 325, 326, 328, 329, 330, 331, 334, 356, 360, *375, 376, 377, 380, 381*
Beemster, A. B. R., 7, 17, *23*, 170, 189, 190, 191, 192, *215*
Bell, W., 109, 113, *136*
Bellamy, R. E., 284, 285, 288, 289, *289*, *291*
Bellingham, R. C., 158, *158*
Belyanchikova, Y. V., 110, *136*
Benda, G. T. A., 167, 169, 177, 185, 186, 188, 190, 196, 205, *215*
Benda, R., 280, 282, 285, *289*, *290*
Benedetti, E. L., 334, *376*
Benjamin, E., 9, *23*, 235, 255, *271*
Bennett, C. W., 163, 166, 170, 171, 172, 175, 180, 184, 187, 188, 189, 190, 191, 192, 193, 194, 197, 198, 201, 202, 206, 209, 210, *215, 216*
Bennington, E. E., 289, *289*
Benson, A. P., 172, *218*
Benzer, S., 55, *58*
Bercks, 5, 11, 12, 13, 16, 17, 18, *23, 24, 216*
Berg, Th. M., 16, *23*
Bergeson, G. B., 89, *93*
Bergman, S., 366, 367, 368, 369, 370, 371, *374, 375, 376*
Bergmann, L., 167, 188, 207, *216*
Bergs, V., 317, 355, 360, *376*
Berkeley, G. H., 204, *216*
Bernfield, M. R., 47, 54, *58*
Bernhard, W., 300, 320, 321, 334, *376, 381*
Berry, W. E., 107, *136*
Bertram, D. S., 279, 283, *289, 292*
Best, R. J., 139, *158*, 191, *216*
Biggs, P. M., 344, 354, 355, *376, 381*
Bigornia, A. E., 128, *136*
Billeter, M. A., 44, 50, *58*, 197, *216*
Bird, J., 194, *216*
Bird, L., 366, *383*
Bird, R. G., 279, *289*
Birk, Y., 212, *220*
Bissonnette, H. L., 109, *137*
Björling, K., 74, *93*
Black, L. M., 20, *23*, 82, 83, 93, *96*, 141, 142, *158, 159*, 170, 184, 202, 203, 214, *216, 218*
Blaskovic, D., 282, 283, 286, *289*
Blyth, J. S. S., 344, 354, *379*
Boardman, N. K., 179, 181, *216, 221*
Bockstahler, H. W., 86, *93*
Bode, O., 5, *24*, 126, *136*
Bodine, E. W., 121, 122, 124, *134, 135*
Bodo, G., 232, *273*
Bonar, R. A., 297, 298, 299, 300, 301, 303, 304, 305, 306, 308, 309, 326, 329, 330, 360, *375, 376*
Bonner, J., 268, *275*
Boorman, J. P. T., 279, *289*
Borges, M. de L. V., 181, *216, 217*
Borsos, T., 306, *376*
Bos, L., 11, 15, *23*
Bower, R. K., 313, 354, *376*
Bowler, E., 199, *220*
Boyden, A., 9, *23*
Boyle, L. M., 168, *216*
Bradish, C. J., 228, 229, 230, 259, *271*
Bradley, R. H. E., 69, 71, *93*, 152, *158*, 169, 170, 204, 205, *216*
Brakke, M. K., 82, *93*, 110, 127, *136*
Brandes, J., 1, 2, 3, 4, 5, 7, 11, 13, 16, 17, 18, *23, 24*, 110, *134*, 181, *216*

Brants, D. H., 184, 185, 188, 192, 207, *216*
Braunitzer, G., 45, *60*
Brčak, J., 66, *93*
Brenner, S., 46, *58*
Bridgmon, G. H., 174, *216*
Brierley, P., 157, *159*
Brinton, C. C., Jr., 246, *274*
Briones, M. L., 128, *134*
Brouwer, D., 212, *220*
Brown, C. J., 354, *376*
Bruehl, G. W., 72, 76, *95*, 109, 125, *134*, 147, 158, *159*
Bruno-Lobo, M., 278, *289*
Bryan, E., 363, *378*
Bryan, W. R., 296, 297, 298, 299, 302, 313, 354, 358, *376, 377, 380*
Buescher, E. L., 286, *292*
Bullock, H. R., 286, *292*
Burdick, D. J., 289, *290*
Burdon, R. H., 44, 50, *58*, 197, *216*
Burgdorfer, W., 278, 280, 282, 286, *290, 291*
Burges, P. R. F., 357, *376*
Buřič, L., 303, 304, *384*
Burk, D., 323, *376*
Burke, D. C., 303, *375*
Burmester, B. R., 311, 330, 331, 332, 334, 352, 353, 354, 360, 361, 363, *376, 377, 378, 379, 381, 385*
Burnett, H. C., 120, *134*
Buthala, D. A., 322, *377*
Buttenschön, H., 142, *159*
Buvat, R. M., 185, *216*

C

Cadman, C. H., 8, *23*, 166, 170, 175, 196, 208, *216*
Caldwell, J., 198, 208, 209, *216*
Calnan, D., 298, 299, 358, *376*
Campbell, D. H., 230, *275*
Campbell, R. N., 91, *93*
Cann, J. R., 229, *275*
Canny, M. J., 164, 189, *216*
Capoor, S. P., 200, *216*
Carr, J. G., 306, 344, 354, 358, 365, *375, 377, 379*
Cartwright, T. E., 246, *274*
Carvalho, A. M. B., 17, *24*
Caspar, D. L. D., 26, 44, *58*, 225, 226, 238, 241, 246, 249, 264, *271, 272, 273*
Castle, W., 325, 331, *376*
Causey, C. E., 286, *290*
Cebra, J. J., 231, *272*
Chamberlain, D. M., 352, 358, 363, *383*
Chamberlain, R. W., 277, 282, 283, 287, *290*
Champe, S. P., 55, *58*
Chaplin, J. F., 145, *158*
Chaudhuri, R. P., 72, *93, 215*
Chaytor, D., 370, *385*
Cheadle, V. I., 163, 164, 189, 193, 197, *217*
Cheo, P. C., 210, *216*, 268, *275*
Cherubin, *290*
Chester, K. S., 255, *272*
Chesterman, F. C., 317, 365, *378, 380*
Chiu, R. J., 110, *134*
Christensen, M., 108, *135*
Christophers, S. R., 278, 279, *290*
Chu, I. H., 287, *290*
Chýle, P., 366, 368, 369, 370, 371, 372, *377, 380, 381, 384*
Cibes, H., 194, *216*
Cinader, B., 253, 261, *272*
Claude, A., 295, 319, 325, 352, *376, 377*
Clayton, E. E., 154, *159*
Clements, A. N., 278, 281, *290*
Clinch, P., 77, *93*
Close, R., 190, *216*
Cochran, L. C., 88, *96*, 122, *134, 137*
Cockerham, G., 143, 151, 153, *159*
Cohen, M., 43, *58*, 171, 205, *216*
Coleman, O. H., 158, *159*
Collett, G. C., 284, *290*
Collins, W. E., 281, *290*
Commoner, B., 236, 237, 238, 239, 240, 241, *272*
Condit, I. J., 119, 120, *134*
Connin, R. V., 111, 113, 115, *134, 135, 136*
Cook, A. A., 150, 151, 153, *159*
Coons, G. H., 86, *93*
Corbett, M. K., 269, *272*
Coriell, L. L., 372, *381*
Cornelius, A., 332, 353, 361, *382, 383*

Cornuet, P., 65, *93, 218*
Costa, A. S., 17, 18, *24,* 63, *93,* 171, 172, 210, *215, 216*
Cottral, G. E., 363, *378*
Couch, H. B., 209, 210, *216*
Counter, F. T., 352, *383*
Cox, J., 334, *380*
Crafts, A. S., 163, 164, 184, 189, 193, 195, 214, *216*
Crawford, E. M., 302, 303, 304, 315, *377*
Crawford, L. V., 228, 229, 230, 259, *271,* 297, 302, 303, 304, 315, *377*
Crick, F. H. C., 44, 46, *58,* 267, *272*
Crittenden, L. B., 344, 354, *377*
Cropley, R., 105, 107, *135*
Crowley, N. C., 207, 208, 209, 210, 211, *216*
Cruickshank, J. G., 322, *375*
Crum, R. A., 149, *159*
Currier, H. B., 163, 164, 189, 193, 197, *217*

D

Dahl, D., 8, *24,* 43, *58,* 264, 265, *273*
Dalton, A. J., 300, 320, *379*
Damsteegt, V. D., 147, *159*
Danes, L., 282, *289, 290*
Daniels, J. B., *290*
Daniels, L. B., 122, *135*
Darcel, C., 295, *377*
Darcy, M. de S., 171, 172, *216*
Darlington, C. D., 278, *290*
Das, C. R., 166, *216*
Daubeny, H. A., 145, *159*
Davidson, T. M. W., 143, *159*
Davidson, T. R., 166, *217*
Davies, C. W., 287, *290*
Day, M. F., 68, 71, 72, *93, 94,* 277, 284, *290*
Dean, J. L., 158, *159*
Dean, L. L., 142, *159*
de Berchoux, C., 150, *159*
de la Sierra, A. 321, *378*
Delevič, B., 11, *23*
Delihas, N., 323, *377*
Del Rosario, M. S. E., 115, *135*
Delwiche, C. L., 237, *272*
de Meesters-Manger Cats, V., 66, *95*
Dennis, H. E., 157, *159*
de Silva, D. M., 63, *93*
Desjardins, P. R., 208, *216*
de Thé, G., 308, 320, 325, 330, 331, 353, *375, 377, 380, 381*
de Vos, N. P., 16, *23*
Dewey, S., 86, *93*
de Zeeuw, D. J., 149, 150, *159*
Dhaliwal, S. S., 354, *377*
Diachun, S., 147, *159*
Dickerson, G., 278, *291*
Diener, T. O., 176, 212, *216*
Dietz, P. M., 237, *272*
Dijkstra, J., 186, *217*
Dintzis, H. M., 232, *273*
Dmochowski, L., 330, 331, 334, *377*
Doljanski, L., 317, 333, *377, 379*
Dorner, R. W., 268, *272*
Dougherty, R. M., 298, 309, 313, 314, 317, 318, 355, 359, *377, 378, 383*
Dounin, M. S., 102, *135*
Dourmashkin, R. R., 300, *378*
Downs, W. G., 286, *290*
Drake, R. J., 211, *221*
Drayton, H. A., 297, 298, *378*
Dreyer, W. J., 254, *274*
Dubbs, A. L., 109, *135*
Duffus, J. E., 63, 72, 73, *93,* 171, 172, *216*
Dulbecco, R., 230, *272*
Dunitz, J. D., 238, *275*
Dunkel, V. C., 313, *379*
Dupont-Mairesse, M., 237, *272*
Duran-Reynals, F., 357, 358, 363, *376, 378*
Durand, D. P., 297, *383*
Durrell, L. W., 124, *134*
Dwurazna, M. M., 212, *221*

E

Eastop, V. F., 68, *94*
Eck, R. V., 179, 183, *221*
Eckert, E. A., 295, 298, 299, 300, 305, 306, 307, 311, 324, 325, 326, 329, 352, 354, 360, 361, *375, 378, 381, 383*
Edelman, G. M., 231, 243, *272*
Edlinger, E., 313, *378*

Edney, E. B., 279, *290*
Egerer, A., 154, *159*
Ehrenberg, B., 367, 368, 370, 371, *374*
Ehrhardt, P., 73, *93*
Eidinoff, M. L., 319, 321, 323, *378, 379, 382*
Eklund, C. M., 282, 284, 286, 289, *290, 292*
Elford, W. J., 296, *378*
Elmer, O. H., 65, *93*
Endemann, W., 152, 154, *159*
Eng, J., 319, *378*
Engelbrecht, A. H. P., 182, *217*
Engelbreth-Holm, J., 295, *378*
Enomoto, K., 366, *383*
Ephrussi, B., 317, *378*
Epstein, M. A., 302, 305, 319, 320, *378*
Erichsen, S., 319, *378*
Ernek, E., 284, 287, *290, 291*
Erwin, D. C., 183, *217*
Esau, K., 163, 164, 170, 172, 180, 181, 182, 189, 193, 197, 198, 202, 204, 209, 214, *215, 217, 218, 219*
Eslick, R. F., 209, *217*
Evans, E., 323, *377*
Everett, T. R., 86, *94*

F

Fahey, J. L., 267, *272*
Fairman, M., 321, *379*
Falkow, S., 262, *274*
Fanshier, L., 332, 353, 361, *382, 383*
Farkas, G. L., 145, *161,* 212, *220*
Fassell, G. M., 265, *272*
Fazekas de St. Groth, S., 259, *272*
Febvre, H., 320, 334, 371, *379, 382*
Fedorov, Yu. V., 287, *290*
Fellows, H., 109, 115, *135, 158*
Ferreira, J. F. D., 181, *216, 217*
Ferris, W., 52, 53, *60*
Ferris, W. R., 244, *275*
Finch, J. T., 225, 226, *272*
Fink, M. A., 310, 317, *379, 381*
Finley, A. M., 109, *135*
Flemings, M. B., 286, *292*
Flint, W. P., 101, 129, *135*
Flock, R. A., 87, *93,* 120, 121, *135*
Foard, M., 354, 363, *375*
Fontes, A. K., 311, 354, 360, *377, 385*
Force, E. E., 321, *379*
Ford, R. E., 190, 205, *217*
Forsky, N., 366, 367, 368, 369, 370, *374, 375*
Foster, R. E., 157, *159*
Foulds, L., 295, 352, *379*
Fraenkel-Conrat, H., 26, 30, 40, 41, 42, 44, 45, 46, 49, 51, *58, 59, 60,* 195, 197, *216, 217, 221,* 234, 238, 264, 269, *272, 275*
Franklin, R. E., 224, 226, 237, 238, 240, 241, 246, 264, 265, *272, 273*
Franklin, R. M., 45, *58,* 297, *379*
Frankova, V., 282, *291*
Fraser, D., 238, *274*
Frederiksen, O., *378*
Fredrickson, T. N., 332, 352, 353, *377, 379, 381*
Freeman, G., 357, *379*
Freese, E., 44, *58*
Freire, P., 358, *378*
Frey, S., 225, 240, *274*
Friedrich-Freksa, H., 227, 228, 246, *272, 275*
Friesen, B., 297, 298, 300, 309, 361, *379*
Fritzsche, F., 125, *136*
Fry, P. R., 147, *161,* 168, 187, 195, *217*
Fuchsová, M., 282, *289*
Fujinami, A., 356, *379*
Fujisawa, M., 366, *383*
Fukushi, T., 80, 81, 82, *93,* 179, 180, 181, *217*
Fulton, F., 262, *272*
Fulton, H. G., 129, *135*
Fulton, R. W., 149, 154, *159, 161,* 169, 210, *217, 220*
Funatsu, G., 46, 49, 51, *58*
Furth, J., 306, 323, *376, 380*
Furumoto, W., 167, 212, *217,* 258, 270, *272, 274*
Futrell, M. C., 109, *134*

G

Gaber, S., 288, *290, 291*
Gallus, H. P. C., 139, *158*
Gally, J. A., 231, 243, *272*
Gamez, R., 92, *93*
Gardner, R. S., 47, *60*

Gates, F. L., *384*
Gauch, H. G., 164, *219*
Gaylord, W. H. I., 320, *379*
Gebhardt, L. P., 284, *290*
Geib, A. F., 288, *291*
Gentry, R. F., 361, *377*
George, J. A., 166, *217*
Gey, G. O., 317, *381*
Gibbs, A. J., 3, *23*
Gibson, B. W., 325, 331, *376*
Gibson, W. W., 99, *135*
Giddings, N. J., 142, *159*
Gierer, A., 26, 35, 37, 39, 44, 45, 52, *58*, *59*, 195, *217*, 264, 270, *272*
Gilden, R. V., 371, 372, *380*
Gilmer, R. M., 210, *217*
Ginoza, W., 45, *58*, 176, *220*, 263, 265, 268, 270, *272*
Girardi, A. J., 371, 372, *380*
Gish, D. T., 45, *60*
Giveon, T., 234, *272*
Glaeser, G., 103, *135*
Goetz, I. E., 326, 329, *375*
Gogel, R. H., 282, *290*
Goheen, A. C., 61, 89, *94*
Gold, A. H., 91, *95*, 210, *217*, 238, *275*
Goldberg, R. J., 228, 249, *272*
Goldé, A., 315, 319, 321, 322, 323, *379*, *384*
Gold'in, M. J., 179, 180, 183, 204, *217*
Goldner, H., 372, *381*
Gonzales, C. G., 87, *94*
Good, R. A., 353, *381*
Goodchild, D. J., 18, *23*
Goodman, H., 267, *272*
Gordon, M. P., 33, *58*
Gordon, R. M., 278, 281, *290*
Goth, R. W., 19, *23*
Gould, D. J., 283, *290*
Gowen, J. W., 33, *58*, 354, *379*
Graafland, W., 207, *216*
Graniti, A., 119, *135*
Grant, M. N., 116, *134*
Grayston, J. T., 287, *290*
Greeley, L., 211, *220*
Green, I., 299, 305, 306, 307, 323, 325, *378*, *379*, *381*
Greenleaf, W. H., 150, *159*
Greenwood, A. W., 344, 354, *379*
Gregson, J. D., 278, 281, *290*
Gresikova, M., 284, *291*
Gresser, I., 286, *292*
Grey, C. E., 330, 331, 334, *377*
Griffiths, R. B., 278, 281, *290*
Griggs, W. H., 87, *94*
Grogan, R. G., 91, *93*, 174, 210, *217*, *218*
Gross, M. A., 311, *377*
Grossfeld, H., 319, *379*
Groupé, V., 298, 313, 314, 352, 354, 355, 356, 359, 360, *374*, *376*, *377*, *379*, *381*, *382*, *383*
Grylls, N. E., 156, *160*
Guindy, E., 288, *290*, *291*
Gupta, P. S., 150, *161*
Gusev, A. J., 368, *379*
Gye, W. E., 306, 356, *379*
Gyles, N. R., 354, *376*
Gyrisco, G. C., 71, *94*

H

Haber, E., 253, *271*, *274*
Haddow, A. J., 287, *290*
Hagedorn, D. J., 148, *160*
Haguenau, F., 295, 300, 320, *379*
Hakkaart, F. A., 16, *23*
Halberstaedter, L., 317, *379*
Halfhill, E., 289, *290*
Hamachi, M., 278, *291*
Hamachige, S., 283, *291*
Hamers-Casterman, C., 196, *217*
Hamlyn, B. M. G., 204, *215*
Hampton, E. G., 319, 323, *379*
Han, S. G., 283, *290*
Hanafusa, H., 297, 298, 307, 309, 310, 333, 336, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 350, 351, 354, 355, 358, 360, *379*, *382*
Hanafusa, T., 297, 298, 307, 310, 333, 336, 338, 339, 340, 341, 342, 346, 347, 350, 351, 354, 355, *379*
Handschuh, D., 44, 45, *58*, 225, 235, 251, *271*
Hannah, A. E., 76, *95*
Hansen, H. P., 3, *23*
Hanson, J. B., 207, *216*
Hardy, J. L., 286, *292*
Harmon, S. A., 157, *159*
Harpaz, I., 212, *220*
Harrington, W. F., 245, *272*
Harris, J. I., 233, *273*
Harris, R. J. C., 295, 315, 356, 357, 358, 365, *379*, *380*
Harris, T. T., 8, *23*
Harrison, B. D., 3, 5, *23*, *24*, 90, *93*, *94*, 166, 167, 170, 176, 186, 203, 211, *215*, *217*
Harrison, E., 151, *161*
Hart, R. G., 57, *58*, 225, 269, *273*
Haruna, I., 45, *58*
Harvey, W. R., 155, *161*
Harwood, R. F., 289, *290*
Haselkorn, R., 44, 50, *59*, 251, 252, 255, *274*
Hatano, S., 356, *379*
Hatton, R. G., 61, 87, *93*, 102, 104, 105, 108, *134*
Hayashi, M., 51, *58*
Hayashi, Y., 179, *217*
Hayes, R. O., *290*
Heaysman, J. E. M., 318, *374*
Heidelberger, M., 227, 228, *273*
Heine, U., 297, 300, 305, 308, 320, 325, 330, 331, 334, 353, *375*, *376*, *377*, *380*
Heinrich, E. C., 155, *161*
Heinze, K., 72, 78, *94*, 130, *135*
Helms, K., 169, *217*
Helmy, I., 288, *291*
Henderson, R. G., 145, 152, *159*
Hendrick, R. D., 86, *94*
Henson, L., 147, *159*
Herold, F., 17, *23*, 181, *217*
Herridge, E. A., 168, *217*
Hertzsch, W., 18, *24*
Hess, A. D., *290*
Hewitt, W. B., 61, 89, *94*, 172, *218*
Hidalgo, F. G., 183, *219*
Hildebrand, E. M., 168, *217*
Hildebrandt, A. C., 29, *60*, 207, *221*
Hilgert, I., 369, *384*
Hill, D. W., 284, *290*
Hills, G. J., 225, 240, *274*
Hirai, A., 168, 178, *217*
Hirai, T., 168, 177, 178, 181, 204, 214, *217*, *220*
Hirumi, H., 83, 84, *95*, 181, 203, *220*
Hitchborn, J. H., 204, *217*, *225*, 262, *273*, *274*
Ho, W. T. H., 119, *135*
Hobart, O. F., Jr., 194, *217*
Hobbs, G. A., 115, *135*
Hoggan, I. A., 64, *94*
Hollings, M., 18, *23*
Holmes, B. E., *382*
Holmes, F. O., 8, 15, *23*, 31, *58*, 140, 145, 146, 154, 155, *159*, *160*, 206, *218*
Holmes, K. C., 224, 238, 264, *272*, *273*
Holmes, N. D., 115, *135*
Holoubek, V., 33, *59*, 140, *160*
Honda, S. I., 214, *218*
Hongladarom, T., 214, *218*
Honma, S., 155, *160*
Hoogstraal, H., 288, *290*, *291*
Hooker, W. J., 172, 183, *218*
Horne, R. W., 3, *23*, 262, *274*
Horne, W. T., 119, 120, *134*
Horsfall, 230, *275*
Horstmann, D. M., 297, *375*
Hotchin, J. E., 316, 328, *382*
Houston, B. R., 76, *94*, 109, *135*, 157, *160*, 172, 210, *217*, *218*
Hu, S. M. K., 287, *290*
Huang, C. H., 283, *291*
Hubbeling, N., 148, 156, *160*
Hubben, K., 297, *385*
Hudson, A., 281, *291*
Hudson, W., 169, *221*, 270, *275*
Hudson, W. R., 43, *58*, 171, 205, *216*
Huebner, R. J., 309, 310, 332, 349, 369, 370, *375*, *380*, *383*
Hughes, T. E., 279, *291*
Hughes, W. F., 361, 363, *383*
Hurlbut, H. S., 283, 285, 287, *291*
Hutchins, L. M., 121, 122, *134*, *135*
Hutchinson, P. B., 79, *94*
Hutson, G. A., 284, 288, *291*
Hutton, E. M., 174, *218*
Hysell, P., 283, *291*

I

Ichikawa, Y., 334, *380*
Iizuka, N., 179, 180, 182, 184, *218*
Imai, T., 320, *381*

Inoue, M., 352, 385
Inouye, T., 146, 160
Irzykiewicz, H., 68, 93
Ishiguro, H., 308, 320, 325, 330, 353, 377, 380
Ishii, M., 236, 275
Ising-Iversen, M., 366, 368, 380
Ives, D. R., 322, 375
Iwakata, S., 333, 334, 380
Izumi, I., 286, 292

J

Jamieson, P. P., 326, 375
Jedlinski, H., 168, 218
Jeener, R., 196, 217, 236, 237, 272, 273, 275
Jennings, D. L., 150, 160
Jensen, C., 371, 372, 380
Jensen, D. D., 83, 86, 87, 94
Jensen, J. H., 29, 32, 59
Jensen, S. G., 197, 198, 218
Jerne, N. K., 230, 273
Jirásek, J., 368, 381
Jørstad I., 135
Johnson, B. L., 148, 160
Johnson, J., 5, 23, 32, 59, 199, 218
Johnson, K. W., 148, 160
Jones, H. A., 142, 149, 161
Jones, L. S., 88, 96, 122, 135, 137
Jones, O. W., 47, 59
Jonkers, A. H., 286, 289
Jonsson, N., 365, 366, 367, 368, 369, 370, 374, 375, 376, 383
Joshi, B. S., 150, 161

K

Kabat, E. A., 230, 273, 306, 323, 376, 380
Kahler, H., 299, 297, 380
Kahn, A., 268, 272
Kahn, R. P., 208, 218
Kaiser, M. N., 288, 290, 291
Kaminski, M., 243, 273
Kantack, E. J., 117, 135
Kardos, E. H., 289, 290
Karrer, H. E., 334, 380
Karush, F., 230, 273
Kashiwagi, Y., 181, 217
Kassanis, B., 3, 16, 19, 21, 23, 24, 32, 59, 76, 77, 78, 94, 167, 173, 184, 185, 186, 188, 196, 206, 207, 215, 218, 255, 273
Kato, R., 366, 368, 375
Kauffmann, F., 8, 21, 24
Kausche, G. A., 31, 33, 59
Kegler, H., 17, 24
Keifer, H. H., 87, 94, 98, 109, 115, 134, 135
Kemp, W. G., 174, 175, 221
Kendall, F. E., 227, 228, 273
Kendrew, J. C., 232, 273
Kennedy, J. S., 68, 94
Kennedy, R. C., 282, 289, 290, 291
Kenzy, S. G., 354, 363, 380
Keogh, E. V., 313, 380
Kerling, L. C. P., 207, 216
Khanna, P. P., 150, 161
Kikumoto, T., 66, 94, 181, 205, 218
Kimura, I., 82, 93, 179, 180, 181, 206, 217, 218
King, C. L., 109, 110, 117, 135
Kingsbury, D. W., 322, 380
Király, Z., 145, 161
Kishore, H., 153, 160
Kissling, R. E., 277, 290
Kitajima, E. W., 17, 18, 24
Kitaoka, M., 286, 292
Kleczkowski, A., 11, 24, 26, 59, 167, 176, 215, 218, 225, 227, 228, 231, 241, 242, 243, 248, 249, 250, 251, 254, 273
Klement, V., 365, 366, 367, 369, 370, 377, 380, 384
Klinkowski, M., 18, 24, 152, 156, 161
Klotz, I. M., 230, 273
Klug, A., 224, 226, 238, 241, 246, 264, 265, 272, 273
Knight, C. A., 7, 8, 23, 24, 29, 43, 44, 45, 50, 58, 59, 60, 197, 216, 233, 234, 250, 262, 264, 265, 268, 269, 273, 306, 380
Knight, R. C., 61, 87, 93, 102, 105, 108, 134
Knutson, H., 117, 135
Köhler, E., 90, 94, 187, 189, 190, 191,
Koelle, G., 152, 160
200, 218
Kohles, G. M., 282, 290

Kohn, A., 317, 381
Kolehmainen, L., 179, 218
Kollmer, G. F., 156, 160
Kolman, J. M., 283, 291
Kontaxis, D. G., 168, 187, 218
Kopkova, E. A., 110, 136
Koprowski, H. K., 371, 372, 380
Koshimizu, Y., 179, 180, 182, 184, 218
Kozuch, O., 284, 291
Kramer, G., 33, 59
Krczal, H., 86, 96
Kristensen, H. R., 108, 135
Kroeger, A. V., 268, 273
Kryukova, I. N., 365, 366, 367, 385
Kundin, W. D., 283, 291
Kunkel, L. O., 29, 59, 80, 81, 94, 140, 160, 200, 218
Kursanov, A. L., 164, 189, 193, 218
Kuwata, T., 357, 380
Kvíčala, B., 77, 94

L

Lafferty, K. J., 260, 261, 262, 272, 273
Lagerlöf, B., 333, 381
Lambertz, P., 185, 218
Lamey, H. A., 86, 94
LaMotte, L. C., Jr., 280, 281, 282, 284, 285, 290, 291
Landa, Z., 368, 381
Landblom, N., 124, 135
Landsteiner, K., 8, 24, 267, 273
Langford, P. L., 331, 377, 380
Lanni, F., 262, 273
Lanni, Y. T., 262, 273
Lapresle, C., 243, 273
Lara, E. F., 130, 135
Larose, R. N., 352, 363, 383
Larson, R. H., 16, 23, 148, 156, 160, 161
Lashbrook, R. V., 185, 218
Latterell, R. L., 208, 216
Lauffer, M. A., 169, 218, 246, 271, 273, 274
Lavand'homme, C., 236, 273
Laver, W. G., 302, 381
Laviolette, F. A., 89, 93, 208, 215
Lavoipierre, M. M. J., 278, 281, 291
Lees, A. H., 105, 135
Legg, J. T., 75, 94
Lehmann, W., 125, 136
Lemoine, P., 236, 273
Lengyel, P., 47, 60
Leung, C. Y., 9, 23, 235, 255, 271
Levan, A., 366, 368, 372, 375, 381
Levine, A. S., 323, 331, 381, 385
Levine, S., 363, 381
Levintow, L., 252, 275
Leyon, H., 179, 181, 218
Li, L. Y., 119, 135
Libich, J., 282, 290
Libíková, H., 278, 291
Lichard, M., 284, 291
Lihnell, D., 102, 135
Limasset, P., 218
Lindner, R. C., 214, 218
List, G. M., 124, 135
Lister, R. M., 8, 23, 175, 216
Littau, V. C., 83, 94, 184, 202, 218
Liu, C., 283, 291
Livingston, L. G., 185, 195, 218
Lloyd, B. J., 296, 297, 380
Lo, W. H. Y., 317, 381
Loebenstein, G., 212, 218
Lofy, M. F., 288, 291, 292
Lojkin, M. E., 227, 231, 271
Longworth, J. F., 156, 160
Loring, H. S., 185, 196, 218
Loughnane, J. B., 77, 93
Love, W. C., 323, 375, 381
Lower, R. L., 154, 161
Lüderitz, O., 9, 24
Lutes, D. D., 154, 160
Luykx, N., 316, 319, 385
Lwoff, A., 262, 274

M

Maat, D. Z., 12, 15, 22, 24
McCaughey, W. F., 52, 60
McClary, C. F., 354, 363, 380
MacLeod, R., 12, 24, 268, 274
McClure, H. E., 286, 292
McCown, J., 286, 292
McGlohon, N. E., 207, 215
McGuire, J. M., 89, 94, 194, 218
McIntyre, G. A., 169, 217
McKinney, H. H., 29, 32, 59, 109, 110, 111, 115, 125, 134, 135, 158, 160, 168, 209, 211, 216, 218, 220

MacKinnon, J. P., 142, *160*, 191, *218*
McLaren, A., 176, *218*
McLarty, H. R., 102, *135*
McLean, D. M., 142, 149, *161*
McNeal, F. H., 109, *135*, 209, *218*
McWhorter, F. P., 183, 198, 200, 204, 208, *218*, *220*
Maizell, J. V., 252, *275*
Malabatis, G., 288, *291*
Malkiel, S., 227, 228, 230, 231, 263, 268, 269, *274*
Malkova, D., 282, 283, *291*
Malmgren, R. A., 317, *381*
Manaker, R. A., 313, 317, *379*, *381*
Mandel, B., 258, 260, 262, *274*
Mandel, H. G., 44, 50, *59*
Mandel, M., 262, *274*
Mann, T. J., 145, *158*
Mannweiler, K., 334, *381*
Manzer, F. E., 206, *218*
Maramorosch, K., 68, 82, 83, 84, 86, *94*, *95*, 181, 203, *220*, 278, *291*
Markert, C. L., 243, *274*
Markham, R., 12, *24*, 88, *95*, 225, 240, 243, 262, 268, *274*
Marmur, J., 262, *274*
Martin, C., 206, 207, *219*
Martin, M. W., 149, 157, *160*
Massee, A. M., 61, 87, *93*, *94*, 99, 102, 103, 105, 108, *134*, *135*
Matsui, C., 66, *94*, 181, 183, 205, *218*
Mattern, C. F. T., 225, *274*
Matthaei, J. H., 47, 51, *59*, *60*
Matthews, R. E. F., 10, *24*, 44, 50, *59*, 79, *94*, 168, 187, 195, 198, *217*, *218*, 243, 244, 262, 263, *274*
Mattingley, P. F., 278, *290*
Matus, A., 44, 50, *59*
Maver, M. E., 298, 299, *376*
Maxfield, H. K., *290*
Medina, A. C., 210, *218*
Melchers, G., 246, *272*
Mellors, R. C., 317, *381*
Mendle, L., 359, *381*
Menke, G. H., 149, *160*
Menon, K. P. V., 170, *218*
Merigan, T. C., 254, *274*
Merrett, M. J., 145, *161*
Merrill, H. M., 82, *94*
Metcalf, C. L., 101, 129, *135*
Metcalf, R. L., 101, 129, *135*
Meyer, R., 183, *217*
Milbrath, J. A., 166, 189, *216*, *218*
Miles, J. A. R., 278, *291*
Milicic, D., 212, *218*
Miller, P. M., 43, *59*
Miller, R. S., 47, *60*
Mills, J. A., 253, *274*
Mills, W., 317, *381*
Mishanec, W., 147, 148, *158*, *161*
Mitchell, J. E., 208, *216*
Mitchell, J. W., 164, 174, 200, *218*, *219*
Mitsuhashi, J., 83, 84, *95*, 181, 203, *220*
Miyake, M., 148, *160*
Miyamoto, Y., 170, *219*
Moloney, J. B., 296, 297, 298, 299, 300, 302, 320, 358, *376*, *379*, *380*
Mommaerts, E. B., 307, 324, 325, *378*, *381*, *383*
Moore, S., 45, *59*, 253, *275*
Morand, J. D., 65, *93*
Morel, G., 206, 207, *219*
Morgan, H. R., 319, 355, 359, *377*, *378*, *381*
Morgunova, T. D., 365, *383*
Morley, F. H. W., 156, *160*
Moses, E., 317, *381*
Moskovets, S. N., 130, *135*
Moss, L. M., 158, *161*
Moyer, J. T., 286, *292*
Müller, G. W., 18, *24*
Mueller, W. C., 76, 79, *94*
Mulligan, T. E., 17, *24*, 76, 87, *94*, *96*, 125, *135*
Mulvania, M., 255, *274*
Mundry, K. W., 26, 32, 34, 35, 37, 39, *58*, *59*, 163, 168, 186, *219*
Munroe J. S., 317, 352, 355, 365, 366, 367, *381*
Murakishi, H. H., 155, *160*
Murphy, J. B., 295, 352, 354, 356, 358, *377*, *381*, *384*
Murphy, P., 77, *93*

N

Nagaich, B. B., 119, *136*

Nagaraj, A. N., 203, 214, *219*
Nakazawa, M., 181, *217*
Namba, R., 170, 204, *217*, *219*
Nault, L. R., 71, *94*
Naylor, A. W., 205, *215*
Nelsen, D., 363, *381*
Nelson, R. L., 289, *290*
Neuzil, P. V., 363, *380*
Newman, R. J., 288, *292*
Newmark, P., 236, 237, 238, *272*, *274*, *275*
Newton, W., 65, *94*
Ng, J. M., 237, *272*
Nichols, W. W., 368, 372, *381*
Nielsen, L. W., 157, *160*, 206, *219*
Niemeyer, L., 126, *136*
Nirenberg, M. W., 47, 51, 54, *58*, *59*, *60*
Nishiumi, Y., 320, *381*
Nixon, H. L., 3, 5, *23*, *24*, 173, 203, 204, *218*, *219*, *225*, *274*
Noble, R. J., 119, *136*
Noguchi, A., 286, *292*
Norman, A., 265, *275*
Norris, D. O., 206, *219*
Norval, I. P., 140, *160*
Nosek, J., 284, *291*
Novikoff, A. B., 308, 330, *381*
Noyes, W. F., 317, *381*
Nozu, K., 45, *58*

O

Oberling, C., 300, 320, 334, *376*
Ochoa, S., 44, 45, 50, *58*, *60*, 197, *216*
Ochs, G., 126, *136*
Oertelis, S., 262, *273*
Ogata, T., 286, *292*
Ohmann-Kreutzberg, G., 125, *136*, 199, *219*
Ohno, S., 329, *375*
Ohtaka, Y., 45, *58*
Okada, Y., 286, *292*
Okano, H., 320, *381*
Okazaki, W., 317, 332, 344, 348, 352, 354, *377*, *379*, *381*, *382*
Okuno, T., 286, *292*
Okuyan, M., 310, 349, 369, 370, *375*, *380*
Olitsky, P. K., 65, *94*
Oliveira, A. R., 18, *24*
Ono, T., 352, *385*
Onove, K., 267, *274*
Orenski, S. W., 83, 84, *95*, 181, 203, *220*
Orlob, G. B., 65, 66, 75, *94*
Orr, C. W. M., 281, *291*
Osborn, H. T., 72, *94*
Ossiannilsson, F., 66, 74, *93*, *94*
Oswald, J. W., 76, *94*, 109, *135*, 210, *217*
Owen, R. D., 262, 263, 267, *274*

P

Pacumbaba, R. P., 128, *136*
Padgett, F., 331, *377*
Paine, J., 75, *94*
Painter, J. C., 329, *381*
Painter, R. H., 99, *135*
Pang, E-Wa, 76, *95*
Parrish, R. G., 232, *273*
Parsons, D. F., 329, 330, *376*, *381*
Paul, H. L., 5, 7, 16, *23*, *24*
Pavlovskii, E. N., 280, 282, 285, *291*
Pavri, K. M., 280, 282, *292*
Pawlitschek, W., 199, *219*
Payne, L. N., 344, 354, 355, *376*, *381*
Peacock, P. R., *375*
Peak, J. W., 156, *160*
Pearce, J. H., 253, *272*
Perez, A., 321, *378*, *382*
Perry, B. A., 142, 149, *161*
Perry, B. T., 304, *374*
Peters, D., 73, *95*
Peterson, R. D. A., 353, *381*
Petraitis, R., 323, *381*
Pfankuch, E., 31, *59*
Philip, C. B., 278, *291*
Phillippe, M. R., 3, 5, *23*
Phillips, D. C., 232, *273*
Piacitelli, J., 270, *275*
Pickels, E. G., 319, *377*
Pikovski, M., 333, *377*
Piraino, F., 332, 352, *379*, *381*
Pirie, A., *382*
Pirie, N. W., 5, 7, *23*, *24*, 230, 236, 237, 238, 249, 262, 264, 270, *271*
Pittman, H. A., 88, *95*
Pittman, U. J., 117, *136*
Pogodina, V. U., 283, *290*
Pollard, M., 359, *381*

Ponsen, M. B., 73, 78, *95*
Pontén, J., 355, 356, 359, *382, 383*
Pop, I., 109, 113, *136*
Pope, D. T., 157, *160*
Porter, K. R., 319, *377*
Posnette A. F. 105, 107, *135*
Pound, G. S., 148, *160, 161*, 186, 209, *220, 221*
Pratt, M. J., 11, *24*
Prentice, I. W., 72, 74, *95*
Pressman, D., 267, *274*
Preston, W. H., Jr., 174, 200, *219*
Price, W. C., 8, *24*, 127, *136*, 169, 205, *218, 219*, 262, 269, *274, 275*
Prince, A. M., 298, 313, 315, 344, 354, 358, 359, 360, *382*
Prince, H., 323, *376*
Procter, C. H., 119, *135*
Proeseler, G., 86, *95*
Provvidenti, R., 142, 147, 148, *158, 160, 161*
Purcell, R. H., 301, 303, 304, *376*
Purchase, H. G., 317, 348, 353, *381, 382*
Purdy, H. A., 255, *274*
Purdy, W. J., 306, *379*

Q

Quak, F., 167, 184, 185, 188, 207, *218*
Qualset, C. O., 147, *161*
Quantz, L., 5, 16, 18, *24*
Querfurth, G., 5, *23*

R

Rada, B., 354, *383*
Raemer, R., 344, 354, *377*
Ragetli, H. W. J., 145, *161*, 174, 175, 203, 212, *221*
Ralph, R. K., 44, 50, *59*
Rappaport, I., 28, 37, 38, *59*, 167, 185, 186, 187, 195, 203, 212, *219*, 227, 228, 230, 231, 233, 251, 252, 255, 256, 257, 258, 259, 261, 262, 263, 265, 266, 267, 269, 270, *274, 275*
Rasa, E. A., 170, 202, *217, 219*
Raski, D. J., 61, 89, *94*
Rasmusson, D. C., 147, *160, 161*
Rauscher, F. J., 298, 356, 359, *379, 382*
Rawlins, T. E., 204, *219*
Razvyazkina, G. M., 110, *136*
Reda, I. M., 302, *382*
Reed, F. C., 314, *383*
Reeve, R. H., 307, 324, *378*
Reeves, W. C., 284, 285, 288, 289, *289, 291, 292*
Rehacek, J., 282, 283, 284, *289, 291*
Reitberger, A., 179, *219*
Reithel, F. J., 243, *274*
Resconich, E. C., 180, 191, 213, *219*
Reyes, T. T., 210, *219*
Rich, A., 238, *275*
Rich, M. A., 321, *382*
Richards, W. R., 76, *95*
Richter, J., 18, *24*
Riek, R. F., 278, 281, *291*
Riker, A. J., 29, *60*, 207, *221*
Řiman, J., 303, 304, 307, *382, 384*
Robb, S. M., 183, *219*
Roberts, D. A., *219*
Roberts, F. M., 67, 68, *96*, 170, 172, *219*
Rochow, W. F., 71, 74, 76, 78, 79 *94, 95*, 205, *219*
Rodenberg, S. D., 236, 237, 238, 239, 240, 241, *272*
Röttger, B., 178, 203, 214, *220*
Roland, G., 203, *219*
Rosenberg, J. T., 286, *292*
Ross, A. F., 190, 205, 211, 212, *217, 219, 220*
Ross, H., 151, 152, *160*
Rothschild, L., 371, *382*
Rott, R., 300, 302, 311, 322, *378, 382*
Rous, P., 295, 352, 353, 354, 355, 356, 358, *381, 382*
Rozendaal, A., 16, *24*
Rubin, H., 297, 298, 300, 301, 306, 307 309, 310, 313, 314, 315, 316, 317, 318, 319, 327, 328, 329, 332, 335, 336, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 353, 354, 355, 356, 358, 360, 361, 362, *379, 380, 382, 383, 384, 385*
Rubio-Huertos, M., 182, 183, 204, *219*
Ruffili, D., 333, *383*
Rush W. A., 284, 289, *291, 292*
Russell, G. E., 3, *23*

B I B L 399C A

Rychlíkova, M., 306, *383*
Ryder, E. J., 210, *219*

S

Sácha, I., 365, *380*
Sadisivan, T. S., 37, *59*
Saelhof, C., 366, *383*
Saldeen, T., 365, *383*
Samuel, G., 88, *95*, 200, *219*
San Juan, M. O., 143, *160*
Sang, J. H., 242, *275*
Santilli, V., 270, *275*
Sarkar, S., 246, *275*
Sarma, P. S., 309, 310, 332, 349, 369, 370, *380, 383*
Scanlon, J., 286, *292*
Schachman, H. K., 245, *272*
Schäfer, W., 300, 311, *378*
Schaller, C. W., 147, *160, 161*
Scharff, M. D., 252, *275*
Scherer, W. F., 286, *292*
Schieber, D. L., 237, *272*
Schlegel, D. E., 168, 179, 204, 207, *217, 218, 219, 220*
Schlumberger, H. D., 234, *271*
Schmelzer, K., 17, *24*, 152, 156, *161*
Schmidt, F., *383*
Schmidt, H. B., 17, 18, *24*, 73, *95*, 125, *136*, 199, *219*
Schmidt-Ruppin, K. H., 365, 366, *383*
Schmutterer, 62, *95*
Schneider, C. L., 86, *93*
Schneider, H., 87, *94*
Schneider, I. R., 164, 166, 171, 174, 189, 190, 195, 198, 199, 200, 203, 205, 206, 214, *219, 220, 221*
Scholtissek, C., 322, *382*
Schramm, G., 38, 40, 44, 45, *58, 59*, 178, 195, 203, 214, *217, 220*, 227, 228, 238, 242, 245, 246, 264, 269, 270, *272, 275*
Schroeder, W. T., 142, 147, 148, *158, 160, 161*
Schulz, J. T., 101, 129, *136*
Schumacher, G., 242, 245, 269, *275*
Schuster, H., 33, 38, 39, 40, 42, 57, *59*
Schuster, M. F., 130, *136*
Scott, F. M., 199, *220*
Scrivani, R. P., 284, 285, 288, 289, *291*
Sears, R. D., 145, *159*
Sehgal, O. P., 41, 52, *59*, 196, *220, 244, 275*
Seinhorst, J. W., 19, *24*
Sela, I., 212, *220*
Sela, M., 253, *271*
Seth, M. L., 128, *136*
Sevoian, M., 352, 358, 363, *383*
Shalla, T. A., 179, 180, 181, 195, 214, *220*
Shanta, P., 170, *218*
Shapiro, V. S., 365, *383*
Sharp, D. G., 295, 296, 297, 298, 299, 300, 305, 306, 307, 308, 309, 324, 325, 329, 352, 361, *375, 378, 381, 383*
Sharpes, P., 317, *381*
Sheffield, F. M. L., 167, 182, 204, *220*
Shenefelt, R. D., 130, *135*
Shepherd, R. J., 210, *220*
Shevljaghyn, V., 371, *385*
Shikata, E., 82, 83, 84, 85, *93, 95*, 179, 180, 181, 203, *217, 220*
Shimizu, M., 9, *23*, 235, 255, *271*
Shimizu, T., 358, 360, *383*
Shinkai, A., 82, *95*
Shipp W., 44, 50, *59*
Shope, R., 278, *289*
Showers, W. B., 86, *94*
Siegel, A., 26, 37, 39, 41, 42, 43, 44, 52, *58, 59*, 163, 167, 169, 171, 176, 187, 196, 205, *216, 219, 220, 244*, 251, 252, 255, 256, 258, 262, 263, 265, 270, *274, 275*
Sill, W. H., Jr., 109, 110, 115, 117, 128, *134, 135, 136*, 158, *158*
Silva, D. M., 8, 18, *24*, 264, 265, *273*
Siminoff, P., 314, *383*
Šimkovič, D., 356, 368, 369, 370, *383, 384*
Simmonds, N. W., 151, *161*
Simon, L., 45, *60*
Simons, J. N., 72, 74, 75, *95*, 158, *161*
Simons, P. J., 300, 309, 313, 314, 317, 356, 357, 358, *377, 378, 380, 383*
Sims, R., 284, *292*
Sinclair, J. B., 149, *161*
Singer, B., 264, *272*
Singer, S. J., 214, *220*, 230, *275*

Singh, G. P., 209, *220*
Singh, H. B., 150, *161*
Singh, K. R. P., 280, *292*
Sinha, R. N., 130, *137*
Sisler, W. W., 146, *161*
Sisson, M. A., 124, *135*
Sjaholm, V., 199, *220*
Sjögren, H. O., 369, *383*
Skorikova, A. S., 368, *384*
Slykhuis, J. T., 62, 76, 87, 88, *95*, 98, 99, 102, 109, 110, 111, 113, 115, 116, 117, 118, 124, 125, 127, *134, 136*
Smida, J., 356, *384*
Smidova, V., 354, 356, *383*
Smidova-Kovarova, V., 356, *384*
Smith, B. D., 99, 106, 107, 108, *136*
Smith, C. E. G., 278, 285, *290, 292*
Smith, F. F., 101, *136*, 157, *159*
Smith, F. H., 198, 200, 204, 208, *220*
Smith, K. M., 66, 72, 77, 83, 88, 90, *95*, 122, 125, 126, 129, *136*, 173, 174, 180, 181, 191, 201, *220*, 243, *274*
Smith, S. H., 179, 207, *220*
Smyth, D. G., 253, *275*
Sobey, 242, *275*
Sokoloff, B., 366, *383*
Sol, H. H., 19, *24*
Solberg, R. A., 177, 178, 186, 205, 207, 214, *215, 220*
Solovyev, V. D., 280, 282, 285, *291*
Solymosy, F., 145, *161*, 212, *220*
Sommer, J. R., 308, 330, 331, *376, 377*
Soost, R. K., 155, *161*
Sooter, C. A., 289, *289*
Sorm, F., 303, 304, *384*
Sorokin, H., 179, *220*
Southam, C. M., 286, *292*, 352, 355, 365, 366, 367, *381*
Spackman, B. H., 45, *59*
Spangler, J. M., 323, *376*
Spasoff, L., 145, *159*
Spencer, J. H., 352, 355, 356, *383*
Spense, L., 286, *290*
Sperling, G., 297, *385*
Speyer, J. F., 47, *60*
Spiegelman, S., 45, 51, *58*
Sprague, G. F., 211, *220*
Stace-Smith, R., 145, *159*
Staehelin, M., 33, *58*
Stamm, D. D., 288, *292*
Stanford, E. H., 157, *160*
Stanley, W. M., 8, *24*, 45, *60*, 227, 231, 274
Stanton, G. J., 284, *290*
Staples, R., 98, 109, 110, 113, 115, 116, 117, 127, *136*
Starlinger, P., 251, 270, *275*
Steere, R. L., 6, *24*, 167, 180, 214, *220*
Stegwee, D., 73, 78, *95*
Stein, W. H., 45, *59*, 253, *275*
Stenback, W. A., 297, *383*
Stenkvist, B., 359, *383*
Stewart, J. A., 359, *377*
Stewart, R. C., 321, *379*
Stim, T., 328, 356, *375*
Stonehill, E. H., 321, *382*
Stormont, C., 267, *275*
Stouffer, R. F., 205, *220*
Stover, R. H., 150, *161*
Stricker, F., 323, *381*
Strickland, A. G. R., 230, *272*
Struckmeyer, B. E., 202, *220*
Strugger, S., 185, *220*
Stubb, E. L., 297, *385*
Stubbe, H., 31, 33, *59*
Stubbs, L. L., 74, *95*
Sturgess, O. W., 170, *220*
Sturm, E., *384*
Sudia, W. D., 277, 282, 283, *290*
Sugiyama, T., 45, *59*
Sukhov, K. S., 32, *59*, 66, *95*
Sulkin, S. E., 284, *292*
Summanwar, A. S., 183, *218*
Sun, W. K. C., 287, *292*
Sunderland, D. W., 145, *161*
Suneson, C. A., 147, *161*, 210, *217*
Suyemoto, W., 283, *290*
Suzuki, N., 181, *217*
Sverak, L., 331, *384*
Svet-Moldavskaya, I. A., 367, 368, *384*
Svet-Moldavsky, G. J., 365, 366, 367, 368, 369, *384*
Svoboda, J., 306, 357, 358, 365, 366, 368, 369, 370, 371, 372, *377, 379, 380, 381, 383, 384, 385*
Swailes, G. E., 115, *135*
Swarbrick, T., 107, *136*
Sylvester, E. S., 68, 69, 71, 78, *95*

T

Takahashi, T., 204, *220*
Takahashi, W. N., 35, *59*, 176, 183, *218, 220*, 236, 237, 238, *272, 275*
Tall, M. G., 262, *275*
Talmage, D. W., 229, *275*
Taniguchi, T., 185, *220*
Tanner, C. E., 243, *273*
Tatchell, R. J., 279, *292*
Teakle, D. S., 90, 91, *95*
Temin, H. M., 313, 315, 317, 318, 321, 323, 336, 337, 339, 348, 350, 356, *378, 382, 384*
Tempelis, C. H., 288, *291, 292*
TenBroeck, C., 82, *94*
Tenenbaum, E., 317, *379*
Thaine, R., 164, 189, 193, *220*
Thiry, L., 43, *59*
Thomas, J. I., 285, *291*
Thomas, L. A., 284, *292*
Thomasine, M., 89, *93*
Thompson, A. E., 154, *161*
Thomson, A. D., 139, *161*, 262, *273*
Thorell, B., 295, 303, 304, 307, 308, 325, 356, *375, 382, 384*
Thornberry, H. H., 3, 5, *23*, 122, *135*, 154, *161*
Thresh, J. M., 88, *95*, 104, 105, 107, 108, *135, 136, 137*, 156, *160*
Thurzo, V., 356, 370, 372, *383, 384*
Timian, R. G., 109, *137*, 146, *161*
Ting, R. C., 346, *384*
Tinsley, T. W., 167, 184, 185, 188, 207, *218*
Tio, M. A., 194, *216*
Toko, H., 125, *134*
Toko, H. V., 72, 76, *95*
Tournier, P., 262, *274*
Trager, G., 336, 351, *384*
Trávníček, M., 303, 304, *384*
Traylor, M. A., 288, *290, 291*
Trevan, D. J., 318, *377*
Trifonow, D., 17, *24*
Trim, A. R., 3, *23*
Troutman, J. L., 149, 152, 154, *159, 161*
Tsuchizaki, T., 206, *221*
Tsugita, A., 8, *24*, 26, 30, 44, 45, 46, 49, 51, *59, 60*, 234, 263, 264, 265, 269, *273, 275*
Turner, H. C., 310, 332, 349, 369, 370, *380, 383*
Tyrrell, D. A., 230, *275*

U

Ueda, A., 352, *385*
Uhl, R., 323, *381*
Uhlig, H., 45, *58*

V

Valentová, N., 369, 370, 372, *383*
van der Want, J. P. H., 7, 11, 15, 17, *23*, 69, *95, 96*
van Heuven, J. C., 19, *24*
van Hoof, H. A., 19, *24*, 69, *96*
Van Kammen, A., 212, *220*
van Regenmortel, M. H. V., 12, 13, 17, *24*, 268, *275*
van Rysselberge, C., 237, *275*
van Slogteren, D. H. M., 16, *23, 24*, 183, 204, *219, 275*
van Slogteren, E., *275*
van Soest, W., 66, *96*
Varma, M. G. R., 280, 282, *292*
Vashisth, K. S., 119, *136*
Veldee, S., 40, *60*, 197, *221*
Vigier, P., 300, 313, 315, 320, 321, 322, 359, *376, 379, 384, 385*
Vogt, M., 230, *272*
Vogt-Köhne, L., 168, 195, *221*
Vogt, P. K., 298, 309, 315, 316, 317, 318, 319, 327, 328, 329, 332, 333, 340, 342, 343, 344, 345, 351, 354, *382, 385*
Von Volk, J., 19, *24*, 86, *96*
Vostrova, N. G., 179, 183, 204, *217*
Vrba, M., 313, 370, *385*

W

Wadsworth, D. F., 109, *137*
Wagle, S. R., 323, *385*
Wahba, A. J., 47, *60*
Walker, A. J., 287, *290*
Walker, J. C., 148, 149, *161*, 174, 175, *216, 217, 221*

Wallace, H. A. H., 130, *137*
Wallace, J. M., 87, *93*, 120, 121, *135*, 211, *221*
Wallbank, A. M., 297, 326, 328, 329, 356, 360, *375*
Wallin, J. R., 109, *137*
Walter, J. M., 150, 155, *161*
Walter, W. G., 311, 330, 334, 360, *377*, *385*
Walters, H. J., 62, 64, 66, *96*, 109, *137*
Wang, S. P., 287, *290*
Wang, T. Y., 237, 238, *272*
Warner, R. C., 44, 50, *58*, 197, *216*
Wartman, K., 262, *275*
Wasuwat, S. L., 148, 149, *161*
Watanake, D. H., 363, *380*
Waters, N. F., 354, 361, *377*, *378*, *385*
Watson, J. D., 267, *272*
Watson, M., 67, 68, 76, 78, 92, *93*, *96*, 139, *158*, *161*, 204, *215*
Watson, R. D., 155, *161*
Watts-Tobin, R. J., 46, *58*
Way, R. D., 210, *217*
Weaver, E. P., 262, *275*
Webb, H. E., 280, 282, *292*
Webb, R. E., 142, 149, *161*
Weber, E., 45, *58*
Weber, G., 323, 331, *375*, *385*
Webster, R. C., 259, *272*
Weibel, J., 17, *23*, 181, *217*
Weier, T. E., 182, *217*
Weierich, A. J., 204, *219*
Weinstein, D., 330, *376*
Weintraub, M., 145, *161*, 174, 175, 203, 204, 212, *216*, *221*
Weissmann, C., 44, 45, 50, *58*, *60*, 197, *216*
Welkie, G. W., 186, *221*
West, A. S., 281, *291*
Westphal, O., 9, *24*
Wetter, C., 1, 4, 5, 13, 16, 19, *23*, *24*, 110, *134*
Wettstein, D. von, 177, 179, 180, 187, 214, *218*, *221*
Wharton, G. W., 100, 101, *134*, *136*
Wheeler, R. E., *290*
Whitcomb, R. F., 83, *96*, 142, *159*
White, C. L., 298, 299, *376*
White, F. H., Jr., 253, *271*
White, N. H., 213, *221*
White, P. R., 188, *221*
Whitman, L., 282, *292*
Wildman, S. G., 26, 28, 38, 43, *58*, *59*, *60*, 163, 167, 168, 169, 171, 176, 177, 185, 186, 187, 188, 195, 203, 205, 212, 214, *216*, *217*, *218*, *219*, 220, *221*, 234, 258, 262, 263, 265, 268, 270, *272*, *275*
Wildy, P., 3, *24*
Wilhelm, R. C., 38, 39, *59*
Wilkinson, J., 208, *221*
Williams, J. W., 228, *272*
Williams, R. C., 44, *58*, 180, *220*, 238, 264, *272*
Wilson, N. S., 88, *96*, 122, *135*, *137*
Wilson, V. E., 142, *159*
Windle, W. R., 366, 367, *381*
Wittmann, H. G., 33, 42, 44, 45, 46, 47, 49, 52, 57, *58*, *59*, *60*, 225, *275*
Wittmann-Liebold, G., 49, *60*
Wolcyrz, S., 142, *159*
Wong, C., 283, *291*
Wood, M. T., 298, 299, *376*
Woodall, J. P., 283, *292*
Woods, M. W., 179, 183, 208, *221*
Woods, R. D., 3, *23*, *24*, 225, *274*
Woollcombe, T. M., 72, 74, *95*
Work, T. H., 287, *292*
Worley, J. F., 166, 174, 189, 195, 198, 199, 200, 201, 203, 205, 206, 212, 214, *218*, *219*, *220*, *221*
Worth, C. B., 286, *289*, *292*
Wright, N. S., 262, *275*
Wroblewska-Mularczykowa, Z., 283, *292*
Wu, J.-H., 29, 37, *59*, *60*, 169, 207, *221*, 270, *274*, *275*
Wyckoff, H., 232, *273*

Y

Yagi, Y., 267, *274*
Yamada, M., 205, *218*, 237, 238, *272*
Yamagiwa, S., 352, *385*
Yamaguchi, A., 183, *218*
Yamaguchi, J., 305, *385*
Yarwood, C. E., 90, *95*, 163, 166, 167, 168, 169, 170, 173, 194, 201, 206, 212, *221*
Yen, C. H., 287, *290*
Yen, D. E., 147, *161*

Yora, K., 206, *221*
Yoshi, M., 286, *292*
Young, D. A., 156, *158*
Young, J., 45, *60*
Young, J. D., 9, *23*, 235, 255, *271*

Z

Zaitlin, M., 41, 43, 52, 53, *58*, *59*, *60*, 163, 171, 175, 179, 181, 196, 203, 205, *216*, *220*, *221*, 244, *275*
Zander, D. V., 354, 363, *380*
Zech, H., 168, 176, 177, 179, 180, 186, 187, 189, 195, 197, 200, 214, *218*, *221*
Zeigel, R. F., 334, *385*
Zilber, L. A., 365, 366, 367, 371, *385*
Zillig, W., 238, 242, 245, 269, *275*
Zillinsky, R. J., 76, *95*
Zimmer, K., 11, 18, *23*, *24*
Zimmerman, M. H., 164, 189, 193, *221*
Zoltowski, Z., 283, *292*

SUBJECT INDEX

A

Aceria tulipae, virus transmission by, 111–119
Agropyron mosaic virus, transmission of, 127
Alfalfa dwarf disease virus, resistance to, 157
Animal viruses, arthropod transmission of, 277–292
Aphids, virus transmission by, 67–79
Arboviruses,
host alternation and, 284–285
transmission of, 277–292
vertebrate infection by, 282–284
Arthropods,
virus infection of, 278–282
virus transmission by, 277–292
Asparagus virus 1, in potato virus Y group, 17
Aster yellows virus, biological transmission of, 80–81, 83, 86
Aucuba mosaic virus, in X-bodies, 182
Avian leukosis virus (strain RPL 12),
antigens of, 309, 310
classification of, 311
epidemiology of, 363
homotransplantation of, 356
incidence of, 361–364
purification of, 301
Avian myeloblastosis virus strain BAI A,
antigens of, 309–311
chemical composition of, 303
classification of, 311
electron microscopy of, 329–331
growth and maturation kinetics of, 328–329
homotransplantation of, 355–356
host-controlled properties of, 305–308
light microscopic studies on, 329
multiplication of, 331, 335
physical properties of, 295–299
purification of, 301
titration of, 324–328
ultrastructure of, 300
Avian tumor viruses, 293–384
elementary particles of, 297–335
multiplication of, 334
principles of *in vivo* infection of, 352–364
synthesis and release of, 312
(*See also* individual viruses)

B

Bagnall's SB 29 virus, in potato virus Y group, 17
Barley stripe mosaic virus,
morphology of, 3
seed transmission of, 209–210, 211
in tobacco rattle virus group, 14
Barley yellow dwarf virus,
aphid transmission of, 74–76, 79
resistance to, 147, 158
Bean mosaic virus,
pathogenicity of, 142
in potato virus Y group, 17
serology of, 7, 11
Bean yellow mosaic virus,
in potato virus Y group, 17
resistance to, 147–148
serology of, 7, 12
Bean virus 2,
pathogenicity of, 142
resistance to, 148
Beet mosaic virus, nuclear activity and movement of, 179
Beet savoy virus, biological transmission of, 86
Beet yellows virus,
aphid transmission of, 74
distribution of, 182
group of viruses, 18, 19
transport of, 193
Bhindi yellow vein mosaic virus, resistance to, 150
Bird ectoparasites, as virus vectors, 287–288
Black currant gall mite, virus transmission by, 103–109

Broad bean mottle virus,
distribution of, 204
in X-bodies, 183
N-Bromosuccinamide, TMV mutagenesis by, 42

C

Cabbage black ringspot virus,
distribution of, 204
in X-bodies, 182
Cacao swollen shoot virus, resistance to, 156
"Cactus virus 7," in potato virus S group, 16
Cactus virus X,
in potato virus X group, 15
serology of, 12
Cadang-cadang disease, transmission of, 127
Carnation latent virus, in potato virus S group, 16
Carnation mosaic virus, introduction of, 174
Cauliflower mosaic virus, in X-bodies, 183
Cherry mottle leaf virus,
transmission of, 126
transport of, 194
Chicken,
avian tumor virus infections of, 352–356
sarcoma viruses, host range of, 356–358
Chrysanthemum virus B, in potato virus B group, 16
Clover yellow mosaic virus, in potato virus X group, 15
Cocksfoot streak virus, in potato virus Y group, 17
Colorado tick fever virus,
ecology of, 286
tick infection by, 280, 282
Cotton "curliness" virus, transmission of, 130
Cowpea mosaic virus, in potato virus Y group, 17
Cucumber mosaic virus,
aphid transmission of, 75
pathogenicity of, 142
resistance to, 148–149, 154
Cucumber viruses,
relationship to TMV, 264
serology of, 8, 12
Curly top virus, transport of, 191
Currant reversion virus, mite transmission of, 102, 132–133
Cymbidium mosaic virus, in potato virus X group, 15, 16

D

Dahlia mosaic virus, intranuclear crystals from, 183
Dandelion yellow mosaic virus, aphid transmission of, 78

E

Eastern equine encephalitis virus,
acute infection by, 283
ecology of, 288
latent infection by, 284
Eelworms, virus transmission by, 88–90
Eriophyes insidiosus, virus transmission by, 122–124
Eriophyidae, plant virus transmission by, 98–100, 102–128
Erythroblastosis virus (strain R),
antigens of, 310
classification of, 311
homotransplantation of, 356
purification of, 301
viral RNA of, 303
Eucharis mosaic virus, in potato virus Y group, 17

F

Fig mosaic virus, mite transmission of, 119–121, 132–133
Filaree red leaf virus, aphid transmission of, 75
5-Fluorouracil, TMV mutagenesis by, 33
"Freesia mosaic virus," in potato virus S group, 16
Fungi, virus transmission by, 90–91

G

Guinea pig, RSV infection of, 367

H

Hamster, RSV infection of, 366
Henbane mosaic virus,
distribution of, 204
in potato virus Y group, 17
in X-bodies, 182
Hop mosaic virus,
aphid transmission of, 75
potato virus S group, 16
Hydrangea ringspot virus,
in potato virus X group, 15
serology of, 11, 12
Hydroxylamine, TMV mutagenesis by, 42

I

Internal cork virus, resistance to, 157

J

Japanese encephalitis virus,
ecology of, 286–287
latent infection by, 284
in mosquito CNS, 285
mosquito infection by, 280, 281

K

Kyasanur forest disease, tick infection by, 280, 282

L

Leaf crumple virus, intranuclear crystals from, 183
Lettuce big vein virus, fungal transmission of, 91
Lettuce mosaic virus,
in potato virus Y group, 17
seed transmission of, 210

M

Mites, virus transmission of, 87–88, 97–137
Monkey, RSV infection of, 367
Mosquito(es),
anopheline, as arbovirus vectors, 287
digestion in, 278–279
feeding apparatus of, 278
viral infective process in, 280, 288
virus exit portal of, 281
Mouse, RSV infection of, 366

N

Necrotic ringspot virus, pollen transmission by, 210
Nitrous acid, TMV mutagenesis by, 35–41

O

Onion yellow dwarf virus, resistance to, 157
O'nyong-nyong fever virus, ecology of, 287

P

Papaya ringspot virus, in potato virus Y group, 17
Passiflora latent virus, in potato virus S group, 16
Pea early browning virus, in tobacco rattle virus group, 14
Pea enation virus mosaic,
aphid vector of, 71–72
resistance to, 148
Pea mosaic virus,
intranuclear crystals from, 183
in potato virus Y group, 17
Pea streak virus, in potato virus S group, 16
Peach mosaic virus, mite transmission of, 87–88, 121–124, 132–133
Peanut rosette virus, resistance to, 150
Petunia ringspot virus, in X-bodies, 182
Pigeon pea sterility virus, transmission of, 128
Plant virus(es),
biological transmission of, 79–87
classification of, 1–24
distribution of, 163–221
patterns of, 203
tissue-limited viruses, 202–203
elongated,
morphology of, 3–7
serology of, 7–12

introduction of, 163–221
by abrasion, 166
by dodder, 170
by roots, 170
by vectors, 169–170
mite transmission of, 87–88, 97–137
pathogenicity of, 139–161
resistance to, 139–161
genetic aspects of, 146–156
natural selection and, 143–146
seed transmission of, 208–211
taxonomy of, 20–22
translocation of, 163–221
continuity of, 185–186
direction of, 165–166
intermediate-distance, 199–202
long-distance, 189–199
nuclear activity and, 177–179
quantitative aspects of, 164
rate of, 186–188
short-distance, 184–185
-vector relationships, 61–96
aphid, 67–79
biological, 79–87
mechanical, 62–64
Pollen, virus transmission by, 210
Poplar mosaic virus, in potato virus S group, 16
Potato aucuba mosaic virus,
aphid transmission of, 77
in potato virus X group, 15
Potato leaf roll virus,
aphid vector of, 72–73
transport of, 191
Potato stem mottle virus, 15
Potato virus A,
aphid transmission of, 77–78
pathogenicity of, 142
resistance to, 152
Potato virus C, pathogenicity of, 139
Potato virus F, resistance to, 156
Potato virus S,
group, 16–17, 19
resistance to, 156
Potato virus X,
distribution of, 181, 205–206
group of viruses, 15–16, 19
introduction of, 172, 174
mechanical transmission of, 63
pathogenicity of, 143
resistance to, 152–153
serology of, 11, 12
transport of, 190–192
Potato virus Y,
aphid transmission of, 75
distribution of, 204
group of viruses, 17–18, 19
introduction of, 174
mite transmission of, 129–130, 132–133
resistance to, 151–153, 158
serology of, 11
transport of, 190–191
Potato yellow dwarf virus, pathogenicity of, 141
Prairie wheat yellow mosaic virus, resistance to, 158
Prune dwarf virus, pollen transmission of, 210

R

Rabbit, RSV infection of, 367
Raspberry mosaic disease, resistance to, 150
Rats, RSV infection of, 366, 367
Red clover vein mosaic virus, in potato virus S group, 16
Rice dwarf virus,
biological transmission of, 81, 83
distribution of, 181
Rice stripe disease, biological transmission of, 82
Rous associated virus (RAV),
assay of, 332–333
light and electron microscopy of, 333–334
physical properties of, 297–300
as RSV helper virus, 338–339, 340–348
tumor transplantability of, 368–369
viral RNA of, 303
Rous sarcoma virus (RSV),
antigens of, 309–312
assay of, 332–333
chemical composition of, 304–305
chicken as host for, 352–358
classification of, 311–312
defectiveness of, 336–352
cytological evidence for, 339–340
significance of, 349–352

electron microscopy of, 319–321
genetic determination of host susceptibility to, 353–354
growth and maturation kinetics of, 315–319
helper-controlled properties of, 343–348
helper-independent properties of, 348–349
helper virus role for, 340–343
homotransplantation of, 355
host-controlled properties of, 306
in vitro studies on, 371–372
infectivity measurement of, 312
light and electron microscopy of, 333–334
in mammalian hosts, 364–372
multiplication of, 321–324
physical properties of, 295–297
purification of, 302
RAV as helper virus of, 338–339
viral RNA of, 303
virus-free tumors and, 358–361
Ryegrass mosaic virus,
mite transmission of, 124–215, 132–133
in potato virus S group, 16

S

St. Louis encephalitis,
in blowfly CNS, 285
ecology of, 288
mosquito transmission of, 282
Sammons *Opuntia* virus, in TMV group, 15
Seeds, virus transmission by, 208–211
Semlicki Forest virus,
in housefly CNS, 285
mosquito transmission of, 279–281
Severe etch virus, in X-bodies, 182
Southern bean mosaic virus,
distribution of, 205
introduction of, 173–174
seed transmission of, 210
transport of, 189, 195, 198, 200
Southern cucumber mosaic virus,
aphid transmission of, 74
Sowbane mosaic virus, introduction of, 172
Soybean mosaic virus, in potato virus Y group, 17
Spinach yellow virus, aphid transmission of, 74
Spotted wilt virus,
nuclear activity and movement of, 179
pathogenicity of, 139
Strawberry crinkle disease, aphid vector of, 74
Subterranean clover stunt virus, resistance to, 156–157
Sugarbeet curly-top virus,
pathogenicity of, 142
resistance to, 157
Sugarbeet mosaic virus, distribution of, 204
Sugarbeet yellows virus,
distribution of, 202
introduction of, 171, 172
morphology of, 34
transport of, 188
in X-bodies, 182
Sugarcane mosaic virus,
in potato virus Y group, 17
resistance to, 158

T

Tensaw virus, ecology of, 287
Tetranychidae, plant virus transmission by, 100–102, 129–130
Tetranychus telarius, virus transmission by, 129–130
Tick-borne encephalitis,
acute infection by, 283–284
ecology of, 286–288
latent infection by, 284
in tick CNS, 285
tick infection by, 280, 282
vertebrate infection by, 282–283
Ticks,
digestion in, 279–280
feeding apparatus of, 278
viral infection process in, 280–281
virus exit portal of, 281–282
Tobacco etch virus,
intranuclear crystals from, 183
in potato virus Y group, 17
resistance to, 150, 155, 156

Tobacco mosaic virus,
A-protein of, 241–243, 245–252
amino acid sequence of, 225
antigenic structure of, 223–275
defective proteins of, 244
distribution of, 180–184, 203–205, 207, 208, 211–212
genome of, 44–45
group of viruses, 15, 19
as infectious particle, 255–262
intranuclear inclusions from, 183
introduction of, 167–168, 171, 175
mechanical transmission of, 62–63
morphology of, 3, 4, 6, 224–226
movement of, 176–179, 184–190
long-distance, 189–191, 195–196
short-distance, 184–190
mutants, *see* Tobacco mosaic virus mutants
pathogenicity of, 140
precipitation of,
depolymerized virus, 236–255
Goldberg theory and, 228–230
whole virus, 227–236
precipitin curve of, 227–230
protein coat of, 268–271
protein structure of, 267
resistance to, 145, 154, 155
serological behavior of, 7, 8, 11, 252–255
strain relationships of, 262–267
valence of, 231
vector-relationships of, 64–67
in X-bodies, 183
X-protein of, 236–244
Tobacco mosaic virus mutants,
amino acid replacements and, 46–55
artificial production of, 25–60
detection of, 26–30
from different strains, 42–43
genetic code and, 45–55
induction of, 31–44
by chemicals, 33, 35–42
direction of, 43–44
by heat, 31–33
by irradiation, 31, 33–35
isolation of, 29–30
spontaneous production of, 28–29
TMV genome of, 44–45
virus-host interactions of, 27–28
Tobacco necrosis virus,
fungal transmission of, 90–91
introduction of, 169, 173
resistance to, 148, 154
transport of, 199, 201
Tobacco rattle virus,
group of viruses, 14, 19
morphology of, 3, 4
Tobacco ringspot virus,
distribution of, 205
eelworm transmission of, 89–90
introduction of, 167, 171
resistance to, 149–150
seed transmission of, 208
transport of, 194, 195
Tobacco streak virus, resistance to, 154
Tomato atypical virus, serology of, 264–265
Tomato black ring virus, eelworm transmission of, 90
Tomato bushy stunt virus, distribution of, 181
Tomato ringspot virus,
distribution of, 182, 204
seed transmission of, 208
transport of, 200
Tomato spotted wilt virus, transport of, 191
Top yellows virus (pea), resistance to, 156
Tulip mosaic virus, in potato virus Y group, 17
Turnip latent virus, transport of, 191–192
Turnip mosaic virus,
pathogenicity of, 142
resistance to, 148
Turnip yellow mosaic virus,
mechanical transmission of, 63, 79
serology of, 10, 12
in X-bodies, 182

V

Vine panaschüre virus, transmission of, 126
Viruses,
animal, *see* Animal viruses
plant, *see* Plant viruses

W

Watermelon mosaic virus,
in potato virus Y group, 17
resistance to, 157
West Nile virus,
in beetle CNS, 285
transmission of, 282
vertebrate infection by, 283
Western equine encephalitis virus,
ecology of, 288, 289
in grasshopper CNS, 285
host alternation and, 285
latent infection by, 284
Western yellows virus, distribution of, 182
Wheat mosaic virus,
morphology of, 3
resistance to, 148
symptoms of and mite infestation of, 130
in tobacco rattle virus group, 14
Wheat mosaic rosette virus, resistance to, 158
Wheat spot mosaic virus, mite transmission of, 117–119, 132–133
Wheat streak mosaic virus,
mite transmission of, 109–117, 132–133
in potato virus S group, 16
resistance to, 158
White clover viruses,
intranuclear inclusions from, 184
in potato virus X group, 15
serology of, 11, 12
Wound tumor virus,
biological transmission of, 82–83
distribution of, 181, 202–203
pathogenicity of, 141–142

X

X-bodies, virus in, 182–183

Y

Yellow bean mosaic virus, intranuclear crystals from, 183
Yellow net virus, aphid transmission of, 78
Yellow net mild yellows virus, aphid transmission of, 78
Yellow vein disease, aphid vector, 73–74

CUMULATIVE SUBJECT INDEX, VOLUMES 1–11

A

Acridine derivatives, use in virus chemotherapy, **3**, 126–128
Actinophage ϕ-17, electron microscopy of, **10**, 139–140
Adenovirus(es), **9**, 157–193
antigens of, **9**, 186–189
biological properties of, **9**, 168–172
classification of, **9**, 158–160
cytopathic effect of, **8**, 267–268, 277
electron microscopy of, **9**, 172–186; **10**, 139–140
groups and classification of, **9**, 283
growth in cell culture, **9**, 165–168
host range of, **9**, 160–165
human disease caused by, **9**, 160–165
plaque assay of, **8**, 367
structure of, **7**, 317
Agropyron mosaic virus, mite transmission of, **11**, 127
Alfalfa mosaic virus, form and composition of, **3**, 11
Allantoic membrane, influenza virus propagation in, **1**, 141–233
Amino acid(s),
analogues, use in cancer chemotherapy, **3**, 108–114
viral, comparison of, **3**, 23, 25
Amniotic fluid, in virus tissue culture, **1**, 238, 239
Animal virus(es),
acridine derivative effect on, **3**, 127–128
amino acid chemotherapy of, **3**, 110–113
antibiotic chemotherapy of, **3**, 128–129
arthropod transmission of, **11**, 277–292
attachment and penetration to cells, **4**, 102–105
cellular susceptibility to, **8**, 258–259
cytopathic effect of, **8**, 245–285
mechanism of, **8**, 246–258
virus classification and, **8**, 259–278
destruction of, phagocytic factors in, **7**, 358–363
development in isolated tissue, **2**, 81–108
dyestuff chemotherapy of, **3**, 124
electron microscopy of, **2**, 213–219
enzyme inactivation of, **1**, 299
form and composition of, **3**, 17–22
inhibition tests on, **3**, 57–59
metal ion chemotherapy of, **3**, 123–124
nucleic acids of, **1**, 325–326
particle counts and titration for, **4**, 111–158
plant growth regulator effect on, **3**, 132
plaque assay of, **8**, 319–378
method of, **8**, 321–327
special uses of, **8**, 356–366
statistical factors affecting, **8**, 350–356
technical factors affecting, **8**, 327
precipitation of, **1**, 297, 298
properties of, **1**, 303–307
purification of, **1**, 296–299
purity tests for, **1**, 299–303
resistance to, **7**, 327–376
body temperature effect on, **7**, 341–352
hormone effect on, **7**, 333–334
nervous regulation of, **7**, 363–374
protective reactions and, **7**, 352–358
synthetic process of, **2**, 96–108
transmission of, vertical, **4**, 12–14
visualization of, **1**, 307–309
vitamin analogue chemotherapy of, **3**, 115–118
(*See also* Vertebrate viruses and individual viruses)
Antibiotics, use in virus chemotherapy, **3**, 128–131
Antigens, differentiation between type- and strain specific, **1**, 159, 160, 162
Antimetabolites, action in relation to incorporation of, **3**, 137–140

Aphid(s),
-plant virus relationship, **11**, 67–79
virus transmission by, **1**, 42, 69, 80; **6**, 99–101, 117–120
Arabis virus, electron microscopy of, **10**, 139
Arboviruses,
classification of, **9**, 286–288
cytopathic effect of, **8**, 275–276
inactivation of, **9**, 135–137
plaque assay of, **8**, 371
serological relationships among, **9**, 127–156
detection methods for, **9**, 132–135
interpretation of, **9**, 137–149
significance of, **9**, 149–152
transmission of, **11**, 277–292
Arthropod viruses, **9**, 195–240
chemical composition of, **9**, 227–229
cross-transmission of, **9**, 219–222
distribution of, **9**, 197–198
inclusion bodies of, **9**, 208–210
interference between, **9**, 236–238
isolation and purification of, **9**, 225–227
latent infections from, **9**, 222–225
morphology, ultrastructure, and replication of, **9**, 210–217
multiplication in arthropods and plants, **9**, 233–236
pathology of, **9**, 198–208
serology of, **9**, 229–231
tissue culture of, **9**, 231–233
transmission of, **9**, 217–219
types of diseases from, **9**, 197
Aster yellows virus,
California strain, **3**, 254–260
cicadellid transmission of, **1**, 70ff.
multiplication in insect vectors, **3**, 225–233, 245
New York strain, **3**, 254–256
strains of, cross protection between, **3**, 251–273
Avian leukosis virus, **3**, 152–155
chemical constitution of, **3**, 163
electron microscopy of, **2**, 217
infectivity and host response of, **3**, 176–182; **11**, 331–333
particle count on, **4**, 136, 144
physical properties of, **3**, 160–163
purification and properties of, **1**, 295; **3**, 156–158
transmission of, **11**, 361–364
Avian myeloblastosis virus BAI strain A, infectivity studies on, **11**, 324–331
Avian tumor viruses, **11**, 293–385
properties of, **11**, 295–312
virus synthesis and release of, **11**, 312–336

B

Bacteria, viral inhibitors from, **2**, 39–41
Bacterial transformation, **3**, 275–307
genetic recombination and, **3**, 293–307
mechanism of, **3**, 282–293
properties of transforming agents, **3**, 278–282
Bacteriophages, **4**, 25–61
acridine effects on, **3**, 126–127
amino acid analogue chemotherapy of, **3**, 108–110
amino acid composition of, **1**, 10
antibiotic therapy of, **3**, 130–131
biochemistry of, **1**, 10–17, 317
biophysical properties of, **1**, 6–9
chemical composition of, **1**, 10–13
chemistry of, **4**, 50–55
DNAs of, **3**, 26
duplication of phage material, **1**, 26–30
dyestuff effect on, **3**, 125
electron microscopy of, **1**, 2–4, 6–8, 15, 17, 22, 23, 27, 28, 31, 32; **2**, 220–223
enzyme content of, **1**, 11, 13, 14
f2-, electron microscopy of, **10**, 140
form and composition of, **3**, 14–17
genetic recombination of, **5**, 124–135
genetics of, **1**, 17–20, 29; **4**, 42–50; **6**, 137–157
growth requirements of, **1**, 15–17
hydration of, **2**, 258
infection by, initial steps in, **4**, 28–34
inhibition tests on, **3**, 59–61
invasion by, **1**, 22–26
λ-, **8**, 44
properties of, **5**, 156–157
structure and composition of, **8**-4-8
life cycle of, **1**, 20–32
Luria-Latarjet experiment on, **5**, 117–124; **8**, 82–85
lysogeny of, **3**, 136–137; **4**, 34–42; **5**, 151–183
mating in reproduction of, **5**, 95–149
maturation chemistry of, **4**, 55–59
mechanism of DNA replication in, **5**, 135–145
metal ion effects on, **3**, 119–121
multiplicity reactivation of, **5**, 108–110
mutants of, **1**, 17–19, 29, 30
nucleic acid(s), **1**, 6, 9–11, 13–16, 28, 32, 316, 325–329
autoradiography of, **10**, 77–81
biophysical characteristics of, **10**, 65–100
electron microscopy of, **10**, 77–81
orientation of, **10**, 89–96
physical properties of, **10**, 68–77
ϕR, electron microscopy of, **10**, 140
ϕR-174
DNA of, **10**, 77
electron microscopy of, **10**, 140
structure of, **7**, 315–316
P1, properties of, **5**, 157–158
P2, properties of, **5**, 158–159
P22, properties of, **5**, 159–160
properties of, **1**, 1–35; **5**, 154–155
prophage and, **5**, 163–165
bacterial chromosome and, **5**, 185–190
protein and nucleic acid synthesis in, **1**, 13–15
purine and pyrimidine chemotherapy of, **3**, 85–90
radiation inactivation of, **5**, 97–110
radiobiological and genetic properties of, **5**, 98–99
radiosensitivity of the capacity, **5**, 110–117
survival of UV radiation, **1**, 7–9, 17, 19, 24–26, 33
T-,
form and composition of, **3**, 14–17
infection of *E. coli* by, **4**, 63–110
penetration in, **4**, 86–102
reversible attachment in, **4**, 67–86
T-even,
electron microscopy of, **10**, 151–155
growth studies on, **8**, 12–14
T2-,
chloramphenical effects on, **8**, 95–100
DNA of, **8**, 36–41, 106–111
effect of DNA-modifying agents on, **8**, 79–95
growth studies on, **8**, 8–19
maturation of, **8**, 52–57
mode of action, **8**, 65–68
nitrous acid effects on, **8**, 90–95
replication of, **8**, 63–122
structure and composition of, **8**, 4–8
UV effects on, **8**, 79–82
X-ray damage to, **8**, 89–90
T3-, amino acid composition of, **2**, 155
T5-, **8**, 41–44
growth studies on, **8**, 15–19
temperature, properties of, **5**, 152–156
thermal stability of, **7**, 349
transduction and, **5**, 182–185
transmission of, vertical, **4**, 14
vegetative,
definition of, **8**, 19–33
virus maturation and, **8**, 1–61
vitamin analogue chemotherapy of, **3**, 114–115
X-ray effect on, **2**, 122–124, 127, 128
(*See also* Colibacteriophages)
Bean, resistance to viral diseases, **2**, 11–13, 20, 28
Beet yellows virus, group of viruses, **11**, 18
Beets, resistance to viral disease, **2**, 14, 33, 34
Bergoldia viruses, classification of, **1**, 130–132
Biting insects, plant virus spread by, **6**, 103
Bittner virus, electron microscopy of, **10**, 157
Bombyx mori, viruses of, **1**, 92ff.
Borrelina viruses, classification of, **1**, 130–131

Bovine encephalomyelitis, ecology of, 5, 48–49
Bovine enteritis, ecology of, 5, 48
Bovine papula stomatitis virus, electron microscopy of, 10, 149–150
Bugs, plant virus spread by, 6, 102
Bushy stunt virus, electron microscopy of, 2, 209–210

C

Cadang-cadang disease, mite transmission of, 11, 127–128
Canine distemper virus, 7, 46–51
cultivation of, 7, 46–47
cytopathic effect of, 8, 264–267
electron microscopy of, 10, 144
immunology of, 7, 47–48
measles virus related to, 7, 38–46, 55
pathogenesis of, 8, 217–218
plaque assay of, 8, 367
strain variation in, 7, 48–49
use for measles immunization, 7, 49–51
Capsule virus diseases (insect), 1, 95, 107, 108
DNA, comparison of, 3, 37
inclusion bodies of, 1, 101–106
morphology and development of, 3, 207–210
purification and isolation of, 1, 98–100
Carnation mottle virus, electron microscopy of, 10, 134
Cells, virus-infected, parasitic patterns in, 3, 8–11
Central nervous system infections, pathogenesis of, 8, 218–230
Centrifugation, density gradient, of plant viruses, 7, 193–224
Cherry mottle leaf virus, mite transmission of, 11, 126–127
Chick embryo,
influenza virus multiplication in, 1, 141–223, 285
poliomyelitis virus in, 1, 267–269
susceptibility and virus production in, 1, 143–144
Chicken tumor I, infective and host response of, 3, 186–187
Chimpanzees, poliomyelitis in, 1, 249–253, 259, 264, 269
Cicadellids, plant virus transmission by, 1, 69–85
Clover club leaf virus, multiplication in insect vectors, 3, 233–235, 245
Cold virus, plaque assay of, 8, 372
Colibacteriophage, 1, 2, 3, 178, 179, 279, 306, 316
inactivation of, 2, 129, 131, 133, 142, 146, 147
Complement fixation, measurement by viruses, 5, 247–287
comparison of antigens in, 5, 281–286
comparison of sera in, 5, 277
contour representation and, 5, 270–277
parameters of, 5, 250–262
Corn stunt virus, multiplication in insect vectors, 3, 237–238, 245
Cotton curliness virus, possible mite transmission of, 11, 130
Cowpox virus,
particle count on, 4, 136
plaque assay of, 8, 367
Coxsackie viruses, 8, 166
classification of, 8, 167–170
clinical manifestations of, 8, 170–182, 192–193
electron microscopy of, 2, 217–218
morbid anatomy of, 8, 182–185
plaque assay of, 8, 372
poliomyelitis virus and, 1, 269–290
purification and properties of, 1, 290
structure of, 7, 280, 311
Curly top virus, cicadellid transmission of, 1, 70ff.
Curly top virus, multiplication in insect vector, 3, 238–241
Cucumber mosaic virus, resistances to, 2, 3–4
Cucumber viruses 3 and 4, form and composition of, 3, 11
Cucumber viruses 3 and 4,
amino acid composition of, 2, 155
form and composition of, 3, 11
purification of, 6, 46–48
Currant reversion virus, mite transmission of, 11, 102–109

D

Deoxyribonucleic acid,
in bacterial transformation, 3, 278–282
comparative study of in viruses, 3, 22–23, 26
as phage precursor, 8, 33–47
replication of in bacteriophages, 5, 135–145
in various viruses, 2, 165
Drosophila hereditary virus, 5, 195–245
assay of, 5, 199–203
extraction of, 5, 197–199
in host, 5, 204–219
mutation of, 5, 219–225
size of, 5, 203–204
temperature effects on, 5, 225–234
Drugs, effect on resistance to viruses, 7, 368–371
Dyestuffs, use in chemotherapy, 3, 124–125

E

Eastern equine encephalomyelitis, plaque assay of, 8, 370
ECHO viruses,
classification of, 8, 168–169
clinical manifestations of, 8, 168–169
plaque assay of, 8, 372
Ectromelia viruses, plaque assay of, 8, 367
Eelworms, as plant virus vectors, 11, 88–90
Electron microscopy, of viruses, 2, 183–239
contrast enhancement in, 8, 287–318
measurement in, 8, 314–316
methods for, 8, 298–309
use of antibodies in, 8, 311–314
use of enzymes in, 8, 309–311
impure, 2, 198–205
pure, 2, 205–223
shadowing in, 2, 190–193
shape and size measurement of, 2, 193–198
Enanthemata and exanthemata, in virus disease, 8, 233–236
Encephalomyelocarditis virus, electron microscopy of, 10, 138
plaque assay of, 8, 372
Enteroviruses, 8, 165–197
cytopathic effect of, 8, 272–275, 277
epidemiology of, 8, 185–189
electron microscopy of, 10, 138
treatment of, 8, 190–191
pathogenesis of, 8, 185
Enzymes,
effect on viruses, 2, 37–39
of viruses, 2, 174–175
relation to viral activity, 3, 32–35
Equine encephalomyelitis virus,
electron microscopy of, 2, 218
purification and properties of, 1, 281–283
Escherichia coli B, bacteriophage infective process of, 4, 63–110

F

Feline pneumonitis,
ecology of, 5, 47
particle count of, 4, 136
Fibroma-myxoma virus transformation, 7, 103–129
DNAase-sensitive TAM and, 7, 118–122
HEAT-TAM experiments on, 7, 113–118
infectious viral nucleic acids of, 7, 124–126
nature of viruses involved in, 7, 107–112
tissue culture of, 7, 109–110
viral recombination in, 7, 122–124
virulence and thermoresistance of, 7, 108–109
Fig mosaic virus, mite transmission of, 11, 119–121
Foot-and-mouth disease virus, 5, 1–37
active immunity to, 5, 27–31
antiserum and, 5, 24–25
biophysical studies on, 5, 31–34
electron microscopy of, 10, 138–139
geographical distribution of, 5, 6–8
immunological types of, 5, 2–10
methods of typing, 5, 4–6
multiplication of, 5, 25–27
pathogenesis of, 8, 234–235
plaque assay of, 8, 372–373

species adaptation of, **5**, 13–20
titration of, **5**, 20–24
"variant" strains of, **5**, 10–13
Fowl orphan virus, plaque assay of, **8**, 373
Fowl plaque virus,
electron microscopy of, **10**, 141
particle count on, **4**, 136
plaque assay of, **8**, 369–370
Fungi,
as plant virus vectors, **11**, 90–91
viral inhibitors from, **2**, 39–41

G

Gamma globulin,
poliovirus and, **1**, 236, 250, 253, 263
prophylactic agent in man, **1**, 254–257
Giant cell pneumonia, from measles virus, **7**, 35–36
Goat pneumonitis, ecology of, **5**, 51
Granulosis virus, electron microscopy of, **10**, 145
Grapevine viruses, soil-borne, **7**, 139
Gross virus, electron microscopy of, **10**, 157
Gypsy moth, see *Porthetria dispar*

H

Hamster virus, ecology of, **5**, 46–47
Hemadsorption virus, plaque assay of, **8**, 369
Hemagglutination,
by influenza virus, **5**, 289–346
tests, of virus growth, **1**, 149, 150, 152–154
Hepatitis, pathogenesis of, **8**, 232, 233
Herpesviruses,
cytopathic effect of, **8**, 262–264, 277
electron microscopy of, **10**, 127–129
plaque assay of, **8**, 367, 368
Host cell, entrance of virus into, **1**, 200–203
Hydrangea ringspot virus, purification of, **6**, 48–49
Hydration, of viruses, **2**, 241–287
5-Hydroxymethylcytosine, in biological materials, significance of, **3**, 35–43

I

Inclusion bodies,
of arthropod virus, **9**, 208–210
polyhedral, **1**, 92–94
proteins of, **1**, 101–106
of virus, **11**, 183–184, 203–204
Infectious canine hepatitis virus, plaque assay of, **8**, 368
Infectious bronchitis virus, plaque assay of, **8**, 373
Infectivity titrations of viruses, **4**, 111–158
Influenza virus(es),
A, amino acid composition of, **2**, 155
adsorption onto entodermal cells, **1**, 195–200
antigenic variation among, **4**, 18–19
biological properties of, **5**, 291–294
complement fixation activity of, **1**, 158–162
complete form of, **2**, 59ff.
criteria of purity of, **5**, 292
cultivation of, **2**, 82–84, 87, 90–92, 95, 98
in respirometer, **2**, 82–84
electron microscopy of, **2**, 215–217; **10**, 141, 162
enzyme activity of, **1**, 154–158
filamentous, **4**, 148–150
growth curves of, **1**, 164–177
growth cycle phases of, **1**, 195–221
hemagglutination by, **1**, 151; **5**, 289–346
adsorption of, **5**, 299–305
enzymatic process in, **5**, 305–321
reaction scheme of, **5**, 320–341
host cell and, **1**, 143–146
human immunity to, **4**, 300–306
hydration of, **2**, 241
immunological groups of, **4**, 285–287
incomplete forms of, **2**, 59–78
origin of, **2**, 76–78
particle counts on, **4**, 146–148
production of, **2**, 69–76
properties of, **2**, 64–69
infectivity of, **1**, 147–151
interference phenomena of, **1**, 181–195

multiplication of in chick embryo allantois, **1**, 141–227
multiplicity of infector of, **4**, 150–152
multiplicity reactivation and genetic recombination of, **1**, 177–181
neutralization studies on, **9**, 6ff.
particle count on, **4**, 136, 138–144
plaque assay of, **8**, 369
properties of, **1**, 162–164, 285–289
purification of, **1**, 285–289
serological change patterns in, **4**, 297–306
serological relationships among, **4**, 279–310
size and shape of, **1**, 146
soluble antigens of, **4**, 281–285
strains of, antigenic analyses of, **4**, 288
temperature effects on, **7**, 351
Insect vectors,
inherited resistance to, **2**, 11
plant virus multiplication in, **3**, 221–249
Insect virus(es),
capsule type, **1**, 98
chemical composition of, **1**, 118–121
classification and nomenclature of, **1**, 130–132
development of, **3**, 199–220
DNAs of, comparison of, **3**, 27
electron microscopy of, **2**, 219–220
inclusion body proteins of, **1**, 101–106
isolation of, **1**, 95–98
morphology and multiplication of, **1**, 109–118
pathology of, **1**, 92–95
physical properties of, **1**, 106–108, 293–295
polyhedral type, **1**, 92–94
purification of, **1**, 98–101, 293–295
transmission methods of, **1**, 126–130
virus-host relationship of, **1**, 123–130
without intracellular inclusions, **3**, 210–211
Insects, kappa-like cells in, **6**, 336–339
Interferon, **10**, 1–38
assay of, **10**, 3–4
mode of action of, **10**, 14–26
effects on cells, **10**, 21–23
in vivo, **8**, 202–206
nonspecific reactions and, **10**, 46–86
production by cells and viruses, **7**, 352–353; **10**, 4–10
properties of, **10**, 10–26
biological, **10**, 12–14
physicochemical, **10**, 10–12
purification of, **10**, 14–15
in recovery from virus infection, **10**
role in naturally occurring infections, **10**, 56–58
role in normal cells, **10**, 33–34
as therapeutic agent, **10**, 34
Ionizing radiation,
effect on proteins and nucleic acids, **2**, 110–120
effect on viruses, **2**, 109–151

K

Kappa particles, in paramecium, **6**, 229–356

L

Leafhoppers,
cross protection in aster yellows viruses by, **3**, 263–269
plant virus transmission by, **1**, 69–139; **6**, 101–102
virus multiplication in nonvector species, **3**, 241–245
(*See also* Cicadellids)
Lima bean, resistance to viral diseases, **2**, 13–14
Liver infections, pathogenesis of, **8**, 230–233
Louping ill virus, plaque assay of, **8**, 370
Lymantria monacha, viruses of, **1**, 92ff.
Lymphocytic choriomeningitis virus, plaque assay of, **8**, 373
Lymphogranuloma venereum, ecology of, **5**, 41–43
Lysogeny, **4**, 34–42; **5**, 151–193
in bacteria,
immunity and, **5**, 173–177
phage production by, **5**, 177–179
prophage interference and, **5**, 179–182
properties of, **5**, 160–163
stability of, **5**, 171–173
establishment of, **5**, 165–171

M

Mealy bugs, plant virus spread by, **6**, 102
Measles virus, **6**, 205–227
  attenuation of, for man, **7**, 31–34
  canine distemper, virus related to, **7**, 38–46, 49–51, 55
  cross reaction of, with canine distemper virus, **6**, 222–223
  cytopathic effects of, **6**, 211–216; **8**, 264–267, 277
  electron microscopy of, **10**, 144
  growth curve of, **6**, 217–218
  immunization against by canine distemper virus, **7**, 49–51
  isolation and identification of, **6**, 206–207
  particle size of, **6**, 219–220
  pathogenic changes induced by, **7**, 35–36
  plaque assay of, **8**, 368
  spontaneous agents in innoculated cultures of, **6**, 207–208
  thermal stability of, **6**, 218–219
  tissue susceptibility in culture of, **6**, 208–210
  vaccine prospects of, **6**, 225
Mengo virus, plaque assay of, **8**, 369, 372
Meningopneumonitis virus,
  ecology of, **5**, 44–46
  particle count on, **4**, 136
Metal ions, use in virus chemotherapy, **3**, 119–124
Mites, plant virus spread by, **6**, 103; **11**, 97–137
Moloney virus, electron microscopy of, **10**, 156
Mouse encephalomyelitis virus, plaque assay of, **8**, 372
Mouse encephalomyocarditis virus, purification and properties of, **1**, 289–290
Mouse pneumonitis, ecology of, **5**, 46
Mumps virus, **8**, 123–164
  antigenic relationships of, **8**, 155–158
  hemagglutination by, **8**, 150–153
  hemolysis by, **8**, 153–155
  infectivity assays of, **8**, 129–131
  interference phenomena in, **8**, 148–149
  particle count on, **4**, 136, 138–144
  pathogenicity of, **8**, 129
  physical and chemical properties of, **1**, 292–293; **8**, 141–148
  propagation of, **8**, 124–141
  purification of, **1**, 292–293; **8**, 141–142
  -red cell interaction, **8**, 150–155
  relation to Newcastle disease and parainfluenza viruses, **8**, 157–158
  stability of, **8**, 146–147
  structure of, **8**, 142–146
  tissue culture of, **8**, 134–141
Myxoma, fibroma-, virus transformation of, **7**, 103–129
Myxoma viruses, plaque assay of, **8**, 367
Myxoviruses,
  classification of, **9**, 284–286
  cytopathic effect of, **8**, 271–272, 277
  electron microscopy of, **10**, 140–144
  plaque assay of, **8**, 369

N

Naniviruses, classification of, **9**, 288–290
Negative staining, virus structure by, **10**, 101–170
Neutralization, of viruses, **9**, 1–125
Newcastle disease virus,
  electron microscopy of, **2**, 217
  particle count in, **4**, 136, 138–144
  pathogenesis of, **8**, 215–217
  plaque assay of, **8**, 369
  purification and properties of, **1**, 291–292
NITAviruses, groups and classification of, **9**, 280–282
Nucleic acids,
  of animal viruses, **1**, 325–326
  of bacteriophages, **1**, 325
  (*See also under* Bacteriophage)
  properties of, **1**, 317–332
  virus, **1**, 315–332; **2**, 165–174
    composition of, **1**, 320–324
    function of, **1**, 329–330
    structure of, **3**, 65–73
Nun moth, see *Lymantria monacha*

O

O'nyong nyong fever, plaque assay of, **8**, 371
Opossum virus, ecology of, **5**, 47–48
Orf virus, electron microscopy of, **10**, 149–150
Ornithosis, ecology of, **5**, 52–53

P

Papilloma-Polyoma virus group, classification of, **9**, 283–284
Parainfluenza virus, plaque assay of, **8**, 369
Paramecium,
  B particle of, **6**, 252–256
  kappa and related particles in, **6**, 229–356
    comparable particle in other organisms, **6**, 334–339
    destruction of, **6**, 327–331
    function of, **6**, 339–350
    infectivity of, **6**, 331–333
    intracellular ecology and, **6**, 321–327
    as killing particle, **6**, 241
    as reproductive particle, **6**, 294–316
  Mu particle,
    action of, **6**, 278–298
    as a reproductive particle, **6**, 333–334
  N particle, mutations of, **6**, 316–321
  P particle of, **6**, 247–252, 282–284
  structure of, **6**, 233–240
Peach mosaic virus, mite transmission of, **11**, 121–124
Pierce's disease of grapes, cicadellid transmission of, **1**, 70
Pigeon pea sterility virus, mite transmission of, **11**, 128
Plant growth regulators, use in virus chemotherapy, **3**, 131–132
Plant virus(es),
  abrasive transmission of, **4**, 262
  acridine derivative effect on, **3**, 128
  amino acid analogue chemotherapy of, **3**, 113–114
  antibiotic chemotherapy of, **3**, 129–130
  attenuated strains of, **4**, 229–230
  cicadellid transmission of, **1**, 69–85
  density gradient centrifugation of, **7**, 193–224
    applications of, **7**, 218–221
    procedure of, **7**, 211–218
    theory of, **7**, 197–210
  distribution of in plants, **11**, 163–221
    intracellular, **11**, 175
    in various plant tissues, **11**, 202–213
  elongated, classification of, **11**, 1–24
    groups of, **11**, 121–122
    morphology of, **11**, 3–7, 12–20
    serology of, **11**, 7–20
    taxonomy and nomenclature of, **11**, 20–22
  form and composition of, **3**, 12–14
  incubation period of, **4**, 224–226
  -infected plants, heat therapy of, **4**, 234–239
  infection, biochemistry of, **6**, 75–91
    anomalous proteins in, **6**, 78–79
    fate of virus particle in, **6**, 83–84
    growth substances in, **6**, 86–87
    nitrogen metabolism in, **6**, 79–83
    nucleic acid in, **6**, 82–83
    organic acids in, **6**, 87–88
    phosphorus disturbances in, **6**, 84–85
    respiration in, **6**, 85–86
  inherited resistance to, **2**, 1–30
  inhibited tests on, **3**, 52–57
  inhibitors and, **2**, 31–57
  inoculation transmission of, **4**, 248–252
    chemical supplements of, **4**, 269–273
  interaction of, in plants, **10**, 219–255
  introduction of into plants, **11**, 163–221
    methods of, **11**, 166–175
  local-lesion transmission of, **4**, 246–248
  metal ion chemotherapy of, **3**, 121–132
  mite transmission of, **11**, 97–137
  multiplication of, **4**, 230–234
    in insect vectors, **3**, 221–249
  pathogenicity and resistance to, genetics of, **11**, 139–161

plant growth regulator effect on, 3, 131–132
pressure transmission of, 4, 264
purification of, 6, 2–73
procedures of, 6, 4–38
purine chemotherapy of, 3, 74–82
pyrimidine chemotherapy of, 3, 82–85
resistance to, 7, 327–376; 10, 232–235
protective reactions and, 7, 352–358
size, shape, and nucleic acid content of, 3, 11
soil-borne, 7, 131–161
control of, 7, 153–157
ecology of, 7, 148–153
list of, 7, 133
as plant pathogens, 7, 132–142
transmission of, 7, 142–148
strains of, *see under* variation
susceptibility to infection of, 4, 222–224
temperature effects on, 4, 221–278
spread and transmission of, 6, 93–135
agricultural practices and, 6, 126–129
by arthropods, 6, 99–126
by contact, 6, 96–99
ecology and, 6, 103–126
mechanical, 4, 243–278
by seeds, 6, 94–96
translocation of in plants, 11, 163–221
cell-to-cell, 11, 184–202
problems in, 11, 164–166
ultracentrifugation of, 4, 241–270
variation in, 7, 163–191
criteria for strain identification of, 7, 165–170
detection of strains of, 7, 178–179
differences in strains of, 7, 171–178
ecology of, 7, 185–186
isolation of strains in, 7, 170–171
mutation frequency in, 7, 171
origin of strains of, 7, 178–185
-vector relationships, 11, 61–96
aphids' role in, 11, 67–79
biological transmission in, 11, 79–87
of TMV, 11, 64–67
vitamin analogue chemotherapy of, 3, 118–119
Plants,
resistance to viruses, 11, 139–161
virus-infected, anomalous proteins in, 6, 75–78
Plaque assay, of animal viruses, 8, 319–378
Pneumonitis, ecology of, 5, 43
Pock viruses, transmission of, 4, 11–12
Poliovirus, 1, 229–275; 8, 166, 167
antibodies and immunity to, 1, 253–254
biosynthesis of, 1, 245–248
in cell cultures, 7, 1–26
chemical properties of, 6, 181–183
clinical manifestations of, 8, 170–182, 192–193
complement fixation tests for, 1, 264–265
cortisone enhancement of, 1, 251–253
Coxsackie virus and, 1, 269–270
crystallization of, 6, 177–180
cultivation in Hela cells, 2, 85
effects on cell, 7, 9–13
electron microscopy of, 2, 218; 6, 170–174; 10, 132
electrophoresis of, 6, 176
gamma globulin prophylaxis of, 1, 254–257
growth curves of, 1, 241–242; 7, 3–4
host-virus relationship of, 1, 249–251
hydrodynamic properties of, 6, 174–176
identification of virus particles in, 6, 165–170
immunologic types of, 1, 229–231
inactivation and denaturation of, 6, 183–190; 10, 171–217
by chemicals, 10, 188–189
by heat, 10, 173–184
by high pressure, 10, 184–186
by ionizing and nonionizing radiation, 10, 200–213
by supersonics, 10, 186–188
infection by, events in, 7, 6–9
intracellular events of, 1, 242–245, 7, 9–21
liberation of, 7, 21–22
maturation of, 7, 21
morbid anatomy of, 8, 182–185
particle count on, 4, 137, 144–145

physical properties of, 6, 170–180
plaque assay of, 8, 371
properties of, 1, 265–267
purification of, 6, 160–165
purified, 7, 4–6
replication of, 7, 14–20
serological epidemiology of, 1, 257–264
serological properties of, 6, 190–199
tissue culture of, 1, 231–241
types 3 and 2 compared, 1, 267–269
ultrafiltration of, 6, 174
Polyhedral insect viruses,
DNAs of, comparison of, 3, 27
of insects, 1, 92–96, 98–100, 107, 108
morphology and development of, 3, 200–207
Polyoma virus, 7, 61–102
antigenic properties of, 7, 82–87
electron microscopy of, 10, 130–132
host response to, 1, 76–82
in vitro tests on, 7, 94–96
infectivity for animals, 7, 99
plaque assay of, 8, 367–368
propagation of, 7, 63–65
purification of, 7, 93–94
relationship to other viruses, 7, 87
resistance to physical and chemical agents, 7, 96–98
size and morphology of, 7, 93
structure of, 7, 317
Porcine enterovirus, plaque assay of, 8, 372
Porthetria dispar, viruses of, 1, 92
Potato, resistance to viral diseases, 2, 15–19
Potato virus S group of viruses, 11, 16–17
Potato virus X,
form and composition of, 3, 11
group of viruses, 11, 15–16
purification of, 6, 49–51
Potato virus Y,
group of viruses, 11, 17–18
mite transmission of, 11, 129–130
Potato yellow dwarf virus,
cicadellid transmission of, 1, 70ff.
electron microscopy of, 2, 211
purification of, 6, 15–52
Pox diseases, pathogens of, 8, 235
Poxviruses,
cytopathic effect of, 8, 260–262, 277
electron microscopy of, 2, 214–215; 7, 11–111
subgroups and classification of, 9, 278–280
Properdin, virus-neutralizing activity of, 7, 334–341
Proteins, of viruses, 2, 154–165
Pseudorabies virus, plaque assay of, 8, 367
Psittacosis, ecology of, 5, 51–52
Psittacosis-lymphogranuloma group viruses, 5, 39–93
antibiotic therapy of, 5, 73–81
antigenic structure of, 5, 63–66
avian diseases from, 5, 54
biochemical aspects of virus growth of, 5, 70–73
chemotherapy of, 5, 73–81
developmental cycle of, 5, 66–70
electron microscopy of, 2, 214–215; 5, 83
host parasite relationships of, 5, 40–41
human diseases from, 5, 41–44, 53–54
immunity to, 5, 81–82
purification of, 5, 83–85
serologic procedures applied to, 5, 55–63
taxonomy of, 5, 85–86
(*See also* individual viruses)
Purine analogues, use in virus chemotherapy, 3, 74–82, 85–87, 90–92
Pyrimidine analogues, use in virus chemotherapy, 3, 82–85, 87–90, 92

R

Rabbit papilloma virus, 3, 151–152
chemical constitution of, 3, 160
electron microscopy of, 2, 218–219
hydration of, 2, 251
infectivity and host response of, 3, 182–186
physical properties of, 3, 158–160
purification and properties of, 1, 283–285; 3, 155–156
Rabies virus, electron microscopy of, 10, 144

Radiation, non-ionizing, effect on viruses, 4, 191–220
Raspberry ringspot virus, electron microscopy of, 10, 139
Red blood cell, 5, 297–299
REO virus, 8, 165–197
  classification of, 8, 169
  clinical manifestations of, 8, 170–182
  electron microscopy of, 10, 129–130
  treatment of, 8, 190–191
Respiratory infection, pathogenesis of, 8, 208–218
Ribonucleic acids, viral, 1, 315ff.; 2, 23
Rice dwarf virus, electron microscopy of, 10, 139
Rice stunt virus,
  cicadellid transmission of, 1, 70
  multiplication of in insect vector, 3, 223–224, 246
Rickettsial viruses, DNAs of, comparison, 3, 28
Rift Valley fever virus, plaque assay of, 8, 360
Rinderpest virus, 7, 52–54
  cultivation of, 7, 52
  cytopathic effect of, 8, 264–267, 277
  electron microscopy of, 10, 144
  immunology of, 7, 53–54
  relation to measles and rinderpest viruses, 7, 52–53, 55
Ringspot viruses, soil-borne, 7, 140–141
Rous sarcoma virus,
  defectiveness of, 11, 336–340, 349
  electron microscopy of, 10, 156
  helper virus for, 11, 340–348
  infectivity studies on, 11, 312–324
  in mammalian hosts, 11, 364
  plaque assay of, 8, 370
Ryegrass mosaic virus, mite transmission of, 11, 124–126

S

Seeds, plant virus transmission by, 6, 94–96
Semliki Forest virus, electron microscopy of, 10, 157
Sendai virus,
  particle count on, 4, 136
  plaque assay of, 8, 369
Serum, virus inhibitor of, 2, 36–37
Sheep pneumonitis, ecology of, 5, 50–51
Sheep psittacosis-lymphogranuloma viruses, ecology of, 5, 49–50
Shope papilloma virus,
  amino acid composition of, 2, 155
  electron microscopy of, 10, 137
  particle count on, 4, 136, 144
Southern bean mosaic virus,
  hydration of, 2, 257
  form and composition, 3, 11
  purification of, 6, 45–46
  structure of, 7, 280–281, 310–311
Sowbane mosaic virus, electron microscopy of, 10, 134–135
Spotted-wilt virus, resistance to, 2, 9–10
Squash mosaic virus, purification of, 6, 52–54
Squirrel virus, 7, 111–112
Sugarbeet yellows virus, electron microscopy of, 10, 146
Sugarcane, resistance to viral disease, 2, 14–15
SV 40 virus, electron microscopy of, 10, 137

T

Talfan and Teschen diseases viruses, plaque assay of, 8, 372
Thrips, plant virus spread by, 6, 102–103
Tipula iridescent virus,
  electron microscopy of, 10, 133
  structure of, 7, 316–317
Tobacco mosaic virus,
  amino acid content of, 2, 155
  anatomy of, 4, 159–190
  antigenic structure of, 11, 223–292
  components of preparations of, 4, 170–172
  distribution in infected plants, 11, 203–204
  electron microscopy of, 2, 207–209, 213; 4, 166–167; 10, 145
  form and composition of, 3, 11, 12
  group of viruses, 7, 134; 11, 15
  homogeneity of preparations of, 4, 169–170
  hydration of, 2, 251

  -infected plants, proteins from, 6, 76–78
  as an infectious particle, 11, 255–271
  internal structure of, 4, 174–176
  length changes in, 4, 165–167
  morphology of, 11, 224
  multiplication of, 4, 231
  organization of, 4, 172–182
  particle size changes in, 4, 167–169
  proteins from infection by, 4, 182–185
  purification of, 6, 54–59
  resistances to, 2, 4–9
  size of, 4, 163–165
  strains, differences in composition of, 2, 160
  structure of, 7, 233–278
    amino acid composition of, 7, 248–249
    chemical composition of, 7, 245–250
    morphology of, 7, 251–264
    protein subunits of, 7, 264–270
    RNA structure of, 7, 270–278
    size of, 7, 242–245
    structure of, 7, 233–278
  subunits of, 4, 176–179
  surface features of, 4, 173–174
  vector relationship of, 11, 64–67
  mutants, *see* Tobacco mosaic virus mutants
Tobacco mosaic virus mutants,
  artificial production of, 11, 25–60
    genetic code and, 11, 45–55
    induction of, 11, 31–44
    isolation technique in, 11, 26–31
    TMV genome and, 11, 45–55
Tobacco necrosis virus,
  electron microscopy of, 10, 139
  form and composition of, 3, 11
  group of viruses, 7, 134–135
  purification of, 6, 59
  structure of, 7, 280
Tobacco rattle virus,
  electron microscopy of, 10, 145–146
  group of viruses, 7, 136–139; 11, 14–15
Tobacco ringspot virus,
  electron microscopy of, 2, 210–211
  purification of, 6, 59–63
  structure of, 7, 280, 311
Tobacco stunt virus, as soil-borne virus, 7, 136
Tomato blackring virus, electron microscopy of, 10, 139
Tomato bushy stunt virus,
  amino acid content of, 2, 155
  form and composition of, 3, 11, 12
  hydration of, 2, 257
  purification of, 6, 63–65
  structure of, 7, 280, 300–303
Tomato ringspot virus, purification of, 6, 65–67
Tumor(s),
  polyoma virus-induced, 7, 61–62
    anatomical types of, 7, 65–76
  viruses, 3, 149–197
    cytopathic effect of, 8, 268–271, 277
    virus infectivity and host response of, 3, 173–191
Turnip crinkle virus, electron microscopy of, 10, 134–135
Turnip yellow mosaic virus,
  electron microscopy of, 2, 211–122; 10, 133–134
  form and composition of, 3, 11
  hydration of, 2, 258
  purification of, 6, 68–69
  structure of, 7, 280–281, 303–308

U

Ultracentrifugation, of plant viruses, 9, 241–270
Ultraviolet irradiation, of viruses, 4, 191–220

V

Vaccinia virus,
  DNAs of, comparison, 3, 28
  electron microscopy of, 2, 214; 10, 146–149
  hydration of, 2, 258
  particle count on, 4, 136
  plaque assay of, 8, 367
  purification and properties of, 1, 280–281
Vertebrate viruses,
  classification of, 9, 271–296
    "fundamental" criteria for, 9, 273–278

subdivision in, 9, 276–277
relation to insect and plant viruses, 9, 291–292
Vesicular exanthema virus, plaque assay of, 8, 371
Vesicular stomatitis virus,
electron microscopy of, 10, 150–151
plaque assay of, 8, 370
Vine Panaschüre virus, mite transmission of, 11, 126
Virology, comparative biochemistry and, 3, 1–48
Viral infection,
immune and nonimmune reaction in, 10, 40–46
interferon in, *see* Interferon
mechanism of recovery from, 10, 39–64
oxygen tension in, 10, 53–54
temperature in, 10, 50–52
Virus diseases,
experimental models of, 8, 206–208
pathogenesis of, factors influencing, 8, 199–244
respiratory, 8, 208–218
-antibody complex,
collision frequency and, 9, 99–100
competitive inhibition and, 9, 106–107
Danysz phenomenon and, 9, 35–36
dissociation of, 9, 80, 101
electrostatic interaction in, 9, 103–104
equilibrium of, 9, 101–103
mathematical models for, 9, 99–106
rate of formation of, 9, 23–24, 74–75
arthropod-borne, *see* Arboviruses
cell attachment and penetration of, 4, 63–110
-cell interaction, 2, 223–226
chemotherapy of, 3, 49–148
acridine derivatives in, 3, 126–128
amino acid analogues in, 3, 114–119
antibiotics in, 3, 128–131
dyestuffs in, 3, 124–125
metal ions in, 3, 119–124
plant growth regulators in, 3, 131–132
purine and pyrimidine analogues in, 3, 74–108
electron microscopy of, 2, 183–239; 8, 287–318
preparative techniques for, 10, 109–123
enzymes of, 2, 174–175
evolution of, factors in, 4, 1–24
form and composition of, 3, 11–22, 61–62
general features of, 10, 103–109
hydration of, 2, 241–287
with icosahedral symmetry, 10, 123–135
immunity to, 4, 17–19
infection, *see* Viral infection
inhibitory substances for, 10, 2–3
interactions of with virus strains, 1, 39–67
ionizing irradiation of, 2, 109–151
inactivation by, 2, 121–126
structure studies using, 2, 127–142
latent, UV reactivation of, 4, 215–217
lipids in, 2, 176
measurement of complement fixation by, 5, 247–287
metabolic equipment of, 3, 29–32
multiplication of, 4, 2–3
neutralization of, 9, 1–125
antigen-antibody union in, 9, 12–30
of biological function, 9, 31–88
mathematical models for, 9, 99–116
non-ionizing radiation of, 4, 191–220
nucleic acids of, 2, 165–174
particle counts of, 4, 117–155
viable and total counts of, 4, 128–155
photoreactivation of, 4, 212–215
protein crystals, hydration of, 2, 249–255
proteins of, composition, 2, 154–165
pure cultures of, 4, 152–153
small,
spherical, structure of, 7, 278–319
structure of, 7, 225–325
structure shown by negative staining, 10, 101–170
quantitative studies on, 10, 157–160
virus-cell interaction in, 10, 160–162
taxonomy and nomenclature of, 4, 21

transmission of, 4, 3–19
tumor, *see* Tumor viruses
vertebrate, *see* Vertebrate viruses
virulence of, 4, 19–20
X-ray diffraction of, 7, 225–233
comparison with electron microscopy of, 7, 311–315
(*See also* Animal virus, Insect virus, Plant virus, individual types)
Vitamin analogues, use in virus chemotherapy, 3, 114–119

W

Wart virus, electron microscopy of, 10, 132
West Nile virus, plaque assay of, 8, 370
Western equine encephalomyelitis virus,
cultivation in fibroblasts, 2, 85–86
plaque assay of, 8, 370
Wheat mosaic virus, group of viruses, 7, 135–136
Wheat spot mosaic virus, mite transmission of, 11, 117–119
Wheat streak mosaic virus, mite transmission of, 11, 109–117
Whiteflies, plant virus spread by, 6, 102
Wild cucumber mosaic virus, purification of, 6, 69–70
Wound tumor disease,
cicadellid transmission of, 1, 70ff.
multiplication in insect vector, 3, 235–237
purification of, 6, 70

Y

Yellow fever virus,
pathogenesis of, 8, 232–233
plaque assay of, 8, 370