

METHODS IN VIROLOGY

EDITED BY

KARL MARAMOROSCH

AND

HILARY KOPROWSKI

Volume II

METHODS IN VIROLOGY

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KARL MARAMOROSCH

BOYCE THOMPSON INSTITUTE FOR PLANT RESEARCH
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AND

HILARY KOPROWSKI

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Preface

Tanto sa Ciascuno Quanto Opera*
St. Francis

Virology is a scientific discipline which operates far beyond the narrow confinement of its goals. Hence, descriptions of methods used to study viruses are scattered throughout articles dealing with all imaginable branches of the life sciences. The search for a particular technique may occupy as much of the scientist's time as the completion of experiments based on that technique.

It was to correct this unfortunate situation that the idea of "Methods in Virology" was first conceived. The editors felt that, in view of the steadily increasing interest in the field of virology, publication of a comprehensive and authoritative treatise on methods used in the study of human, animal, plant, insect, and bacterial viruses would be welcomed by their colleagues. This work will enable virologists, graduate students, and prospective students of virology to appreciate the diversity and scope of the methods currently being used to study viruses, and, most important, to evaluate the advantages, limitations, and pitfalls of these methods.

The contributors were chosen on the basis of their outstanding knowledge of a given method, either as creators of new techniques, or as recognized authorities in their specialized fields. Other than clarity of expression and limitations on the length of presentations, no restrictions were imposed on the contributors. For example, the form of presentation of each chapter was the prerogative of its author. Some chapters follow the time-proven outline of recipes found in cookbooks, others are written in a highly original—even controversial—and sophisticated style.

It was the editors' intent to provide readers interested in one particular technique with a self-contained chapter describing this technique. As a result of this decision, it was sometimes impossible to avoid overlap of information in some chapters. The editors felt that completeness of description warranted this occasional duplication.

The first four volumes of "Methods in Virology" will be published in rapid succession. As new methods of study of viruses develop, their descriptions will be included in future volumes.

* Everybody knows as much as he works.

The editors wish to take this opportunity to thank their Board of Advisors—F. C. Bawden, Sven Gard, George K. Hirst, S. E. Luria, André Lwoff, Roy Markham, K. F. Meyer, George E. Palade, C. Vago, and Robley C. Williams—for invaluable assistance provided in the preparation of this work. They are confident that these efforts were not made in vain, since they will provide virologists everywhere with new and valuable tools to facilitate their quests for new discoveries.

October, 1967

KARL MARAMOROSCH
HILARY KOPROWSKI

Contents of Other Volumes

Volume I

Natural Ecology

Harald Norlin Johnson

Virus Hosts and Genetic Studies

A. G. Dickinson and J. M. K. Mackay

Methods for the Study of Mosquitoes as

Virus Hosts and Vectors

Roy W. Chamberlain and W. Daniel

Sudia

Methods of Studying Ticks and Mites

as Virus Hosts and Vectors

Cornelius B. Philip

Methods of Studying Plants as Virus

Hosts

L. Bos

Laboratory Methods of Virus Transmis-

sion in Multicellular Organisms

D. Blaškovič and B. Styk

Mechanical Transmission of Plant Vi-

rus

C. E. Yarwood and R. W. Fulton

Plant Virus Transmission by Insects

K. G. Swenson

Nematode Transmission

D. J. Raski and W. B. Hewitt

Methods for Experimenting with Mite

Transmission of Plant Viruses

J. T. Slykhuis

Fungus Transmission of Plant Viruses

D. S. Teakle

Plant Viruses: Transmission by Dodder

C. W. Bennett

Graft Transmission of Plant Viruses

L. Bos

Insect Pathogenic Viruses

Kenneth M. Smith

Bacteriophage Techniques

A. Eisenstark

Animal Tissue Culture

J. S. Porterfield

Plant Tissue Culture

B. Kassanis

Invertebrate Tissue Culture

C. Vago

AUTHOR INDEX—SUBJECT INDEX

Volume III

Analysis of Protein Constituents of Vi-

rus

H. Fraenkel-Conrat and R. R.

Rueckert

Analysis of Lipid Components of Viruses

David Kritchinsky and Irwin L.

Shapiro

RNA Virus RNA Polymerase: Detec-

tion, Purification, and Properties

J. T. August and Lillian Eoyang

Immunological Techniques for Animal

Viruses

Jordi Casals

Serological Techniques (Plant Viruses)

R. E. F. Matthews

The Plaque Assay of Animal Viruses

Peter D. Cooper

Transformation Assays

M. G. P. Stoker and I. A. Macpherson

Methods for Selecting RNA Bacterio-

phage

Mamoru Watanabe and J. T. August

Structural Studies of Viruses

J. T. Finch and K. C. Holmes

Microscopic Techniques

Rex S. Spendlove

Volume III (Continued)

Electron Microscopy of Isolated Virus
Particles and Their Components

Robert W. Horne

The Application of Thin Sectioning

C. Morgan and H. M. Rose

Autoradiographic Methods for Electron
Microscopy

Nicole Granboulan

AUTHOR INDEX—SUBJECT INDEX

Volume IV

Techniques for the Study of Interferons
in Animal Virus-Cell Systems

Robert R. Wagner, Thomas J. Smith,

and Allan H. Levy

Methods for the Study of Viral Inhibi-
tors

Felix E. Wassermann

Methods of Inactivation by Ultraviolet
Radiation

A. Kleczkowski

Inactivation of Viruses by Ionizing Ra-
diation and by Heat

William Ginoza

Methods for Testing Antiviral Agents

František Link

Techniques for Studying Defective Bac-
teriophages

Allan M. Campbell

Methods for the Study of Defective
Viruses

Hidesaburo Hanafusa

Cell Cultures and Pure Animal Virus in
Quantity

Howard L. Bachrach and Sydney S.
Breese, Jr.

Methods for Containment of Animal
Pathogens at the Palm Island

Animal Disease Laboratory

Jerry J. Callis and George E. Cottral

Methods of Storage and Preservation of
Animal Viruses

Thomas G. Ward

Methods of Preservation and Storage of
Plant Viruses

Harold H. McKinney and Gustave
Silber

Contamination of Cell Cultures by My-
coplasma (PPLO)

Arthur Brown and Julius E. Officer

Methods for the Study of Colicine and
Colicinogeny

Haruo Ozeki

Methods of Virus Classification

C. H. Andrewes

Experimental Design and Statistical
Methods of Assay

A. Kleczkowski

Methods in Human Virus Vaccine Prep-
aration

Louis Potash

AUTHOR INDEX—SUBJECT INDEX

Table of Contents

<i>List of Contributors</i>	v
<i>Preface</i>	vii
<i>Contents of Other Volumes</i>	xv

Chapter 1—The Ultracentrifuge

ROY MARKHAM

I. General Operation	3
II. Observational Methods	6
III. The Calculation of Sedimentation Coefficients	8
IV. The Partial Specific Volume	17
V. The Calculation of Molecular Weights	21
VI. The Vinograd Density-Gradient Method	22
VII. Virus Polymerization	25
Appendix A: A Note on the Interpretation of Diagonal Schlieren and Other Optical Patterns	27
Appendix B: A Note on the Ultraviolet Light-Absorption Optical System	31
Appendix C: Notes on the Operation of the Spinco Model E Ultracentrifuge	34
Appendix D: Photographic Techniques	35
Appendix E: Centrifugation in Very Dilute Solutions	37
General References	39
References	39

Chapter 2—Equilibrium Ultracentrifugation

H. M. MAZZONE

I. Introduction	41
II. Basic Principles	43
III. Experimental Considerations	49
IV. Procedures Concerned with Decreasing the Equilibrium Time	59
V. Analysis of Data	63
VI. Applications of Equilibrium Procedures in Virology	78
References	86

Chapter 3—Density-Gradient Centrifugation

MYRON K. BRAKKE

I. Introduction	93
II. Materials for Gradients	95
III. Formation of Gradients	99
IV. Addition of Sample	102
V. Centrifugation	102
VI. Analysis of Results	106
VII. Determining Sedimentation Coefficients and Buoyant Density	111
VIII. Determining Concentrations	115
IX. Determining Purities	116
X. Correlation of Activity with Particles	116
References	117

Chapter 4—Miscellaneous Problems in Virus Purification

MYRON K. BRAKKE

I. Introduction	119
II. Selection of Source	120
III. Conditions Affecting Virus Concentration	121
IV. Interfering Materials	122
V. Methods of Virus Release	123
VI. Stability and Aggregation	124
VII. Selective Denaturation and Precipitation of Host Components	129
VIII. How Pure Is Pure?	133
References	135

Chapter 5—New Centrifugal Methods for Virus Isolation

N. G. ANDERSON AND G. B. CLINE

I. Introduction	137
II. Centrifugal Separation Methods	138
III. Experimental Studies	157
IV. Conclusions	177
References	177

Chapter 6—Chromatography and Membrane Separation

LENNART PHILIPSON

I. Introduction	179
II. Exclusion Chromatography	181
III. Adsorption Chromatography	201
IV. Membrane Separation Methods	213
V. Chromatographic Equipment	217
References	230

Chapter 7—Water—Organic Solvent Phase Systems

LENNART PHILIPSON

I. The Effect of Organic Solvents on Viruses	236
II. Removal of Nonviral Components	237
III. Other Applications of Organic Solvents in Virus Purification	242
References	243

Chapter 8—Virus Concentration by Ultrafiltration

KARL STROHMAIER

I. Introduction	245
II. Principle of the Method	246
III. Construction of an Apparatus	256
IV. Results	267
References	274

Chapter 9—Diffusion

ROY MARKHAM

I. Introduction	275
II. Free Diffusion in One Dimension	278
III. The Method of Moments	282
IV. Measurement of Diffusion Coefficients by Rayleigh Optics	287
V. Diffusion in the Ultracentrifuge	292
VI. Diffusion across a Porous Membrane	299
References	302

Chapter 10—Two-Phase Separation of Viruses

PER-ÅKE ALBERTSSON

I. Introduction	303
II. Polymer Phase Systems	304
III. Distribution of Virus Particles	308
IV. Purification and Concentration of Viruses	311
V. Countercurrent Distribution	317
References	320

Chapter 11—Purification of Virus by Adsorption on Cells and Elution

FELIX E. WASSERMANN

Text	323
References	324

Chapter 12—Molecular Sieve Methods

G. K. ACKERS AND R. L. STEERE

I. Introduction	325
II. Principal Uses of Molecular Sieve Columns	326
III. Molecular Sieve Media	331
IV. Virus Purification Methods	337
V. Determination of Molecular Size and Weight	340
VI. Methods for Study of Interacting Components	350
References	364

Chapter 13—Filtration Techniques

VERNON P. PERRY AND MONROE M. VINCENT

I. Introduction	367
II. Types of Filters	371
III. Applications	384
IV. Limitations	387
V. Summary	389
References	389

Chapter 14—Electrophoresis of Viruses

A. POLSON AND B. RUSSELL

I. Apparatus and Techniques	391
II. Partial Purification of Viruses for Zone Electrophoresis	405
III. Zone Electrophoresis of Animal Viruses	406
IV. Zone Electrophoresis of Plant Viruses	415
V. Gel Electrophoresis	418
References	425

Chapter 15—Labeling of Viruses with Isotopes

CLAUDIA HENRY

I. Introduction	427
II. General Considerations	428
III. Applications	446
References	460

Chapter 16—Separation of Viruses into Components

R. K. RALPH AND P. L. BERGQUIST

I. General Introduction	464
II. Isolation of Viral Nucleic Acids	464

III. Isolation of Viral Proteins from Purified Viruses	485
IV. Isolation of Other Viral Components	509
V. Chemical Treatment for Isolating Soluble Viral Proteins	512
VI. Purification and Fractionation of Viral Nucleic Acids	516
VII. Isolation and Demonstration of Double-Stranded Viral RNA	533
VIII. Addendum	537
References	538

Chapter 17—Methods of Degrading Nucleic Acids and Separating the Components

T. H. LIN AND R. F. MAES

I. Introduction	547
II. Degradation of Nucleic Acids	548
III. Separation of Nucleic Acid Components	561
IV. Oligonucleotide Patterns	587
References	604

Chapter 18—Assay of Infectivity of Nucleic Acids

S. SARKAR

I. Introduction	607
II. Absolute and Relative Measure of Infectivity	608
III. General Procedure for Assay	609
IV. Appendix	633
Acknowledgments	640
References	640
<i>Author Index</i>	645
<i>Subject Index</i>	662

I *The Ultracentrifuge*

Roy Markham

I. General Operation	3
A. Rotors, Cells, Etc.	3
B. Speeds	5
C. Temperature	5
D. Composition of Solution	5
II. Observational Methods	6
A. Diagonal Schlieren Optics	6
B. Rayleigh Optics	7
C. Ultraviolet Optics	8
D. Direct Recording of Ultraviolet Absorption	8
III. The Calculation of Sedimentation Coefficients	8
A. Measurement of the Boundary Position: The Scale ...	9
B. The Logarithmic Graph Paper	11
C. The Protractor	13
D. Application to Ultraviolet Optics	15
E. Correction to Standard Conditions	15
F. Correction for Concentration	17
G. Estimation of Appropriate Speed for a Run	17
IV. The Partial Specific Volume	17
Pycnometric Measurement of \bar{v}	18
V. The Calculation of Molecular Weights	21
VI. The Vinograd Density-Gradient Method	22
A. The Cell	23
B. Procedure	23
VII. Virus Polymerization	25
Appendix A: A Note on the Interpretation of Diagonal Schlieren and Other Optical Patterns	27
Appendix B: A Note on the Ultraviolet Light-Absorption Optical System	31
A. Ultraviolet Light Source	32
B. The Filter System	32
C. Light Source Alignment	34
Appendix C: Notes on the Operation of the Spinco Model E Ultracentrifuge	34
Appendix D: Photographic Techniques	35
A. Materials	35
B. Processing	36
Appendix E: Centrifugation in Very Dilute Solutions ...	37
General References	39
References	39

Next to the electron microscope, the analytical ultracentrifuge is probably the most useful apparatus for examining virus preparations, though this is not always fully appreciated. There has always been a mental block among biologists with respect to this machine, largely because the early machines, which in any case required a considerable knowledge of physical chemistry, were surrounded with a mystique that was not often dispelled by the possessors of such apparatus. Many biologists feel that the use of the ultracentrifuge is complex and beyond their abilities. The object of this article is to dispel this feeling and to indicate the potential of this extremely powerful tool for the virologist, and also to indicate how it can be used to determine the actual masses of virus particles. There is, however, no doubt that the latter function of the ultracentrifuge is not as generally useful to the virologist as the ability to give rapid semiquantitative analyses of virus-containing solutions.

This ability of the ultracentrifuge to study crude mixtures (Fig. 1) has only recently been fully exploited, largely because the early machines were complex, dangerous, and cumbersome, as well as rare. As a consequence, they were reserved for very special investigations, and only the most highly purified virus suspensions were examined by this means. It is ironic that the use of this instrument on crude specimens could have advanced virology extremely rapidly if at the time someone had thought of using it for this purpose.

Nowadays, however, the analytical ultracentrifuge is fairly common in biological laboratories, and in order to derive the greatest benefit from it it is necessary only to realize that it can be very useful and that

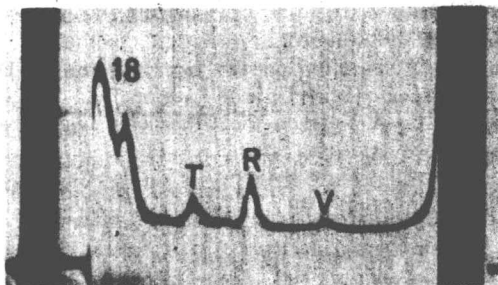


FIG. 1. Untreated sap from pumpkin plants infected with the wild cucumber mosaic virus. The direction of sedimentation is from left to right, and the peaks are, in order, small proteins, the 18 S or fraction 1 protein, empty (top-component) virus particles (T), ribosomes (R), and complete virus particles (V). Notice how easily one can recognize individual components and also get a rough idea of relative concentrations on such a crude preparation. (Schlieren angle 60° ; speed 35,600 rpm.)

it is relatively easy to use. The Beckman-Spinco Model E ultracentrifuge is considerably easier to learn to use than is an automobile. To date in this laboratory at least 30 research workers and assistants have regularly used the ultracentrifuge by themselves. Naturally, no piece of apparatus is completely foolproof, and since an ultracentrifuge is an expensive and potentially dangerous instrument, it should be used with due care. However, it is not true that only physical chemists have the necessary training to use it successfully.

The discussion in Section I refers in particular to the Spinco Model E ultracentrifuge, and cell numbers, etc., refer to accessories supplied for this particular instrument.

I. General Operation

A. ROTORS, CELLS, ETC.

The most generally useful rotor is the AN-D used with 12-mm cells. Cells with a centerpiece made of Kel-F (Nos. 1288 and 1338) are suitable for use up to 44,700 rpm and will cover almost all normal usage; gaskets are not required other than for the filling hole. This type of rotor will take two cells at once for schlieren observation, one cell (No. 1338) having a prismatic window (Fig. 2). Rotors taking several cells are also available, but they may prove cumbersome, while those rotors taking cells with long light-path centerpieces will probably not prove of so much use in virus work.

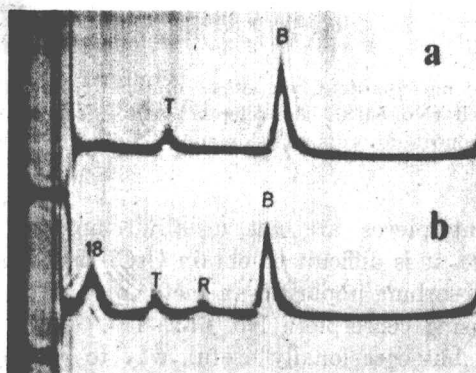


FIG. 2. Sap from plants with wild cucumber mosaic virus, pelleted once in a Model L Spinco, run in a plain cell (No. 1288) and a wedge window cell (No. 1338) to illustrate the use of two cells for comparative purposes. The material in the upper cell (a) was treated with bentonite clay, while that in the lower (b) was not. Note that the bentonite removes the ribosomes and small proteins. The 18 S, top component (T), ribosome (R), and bottom component (B) peaks are labeled.