METHODS IN VIROLOGY

Volume I

EDITED BY

KARL MARAMOROSCH

HILARY KOPROWSKI

METHODS IN VIROLOGY

EDITED BY

KARL MARAMOROSCH

BOYCE THOMPSON INSTITUTE FOR PLANT RESEARCH
YONKERS, NEW YORK

AND

HILARY KOPROWSKI

THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY
PHILADELPHIA, PENNSYLVANIA

Volume I

ACADEMIC PRESS New York and London 1967

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: 66–30091

PRINTED IN THE UNITED STATES OF AMERICA

List of Contributors

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

BENNETT, C. W., Crops Research Division, United States Department of Agriculture, Salinas, California (393).

BLAŠKOVIČ, D., Institute of Virology, Czechoslovak Academy of Sciences, Mlynska dolina, Bratislava, Czechoslovakia (163).

BOS, L., Institute of Phytopathological Research, Wageningen, The Netherlands (129) (403).

CHAMBERLAIN, ROY W., Arbovirus Vector Laboratory, Communicable Disease Center, Atlanta, Georgia (63).

DICKINSON, A. G., Animal Breeding Research Organization, Edinburgh, Scotland (19).

EISENSTARK, A., Department of Bacteriology, Kansas State University, Manhattan, Kansas (449).

FULTON, R. W., Department of Plant Pathology, University of California, Berkeley, California (237).

HEWITT, W. B., Department of Plant Pathology, University of California, Davis, California (369).

JOHNSON, HARALD NORLIN, Viral and Rickettsial Disease Laboratory, California Department of Public Health, University of California, Berkeley, California (1).

KASSANIS, B., Rothamsted Experimental Station, Harpenden, Herts, England (537).

MACKAY, J. M. K., Animal Disease Research Association, Moredun Institute, Edinburgh, United Kingdom (19).

PHILIP, CORNELIUS B., National Institute of Allergy and Infectious Diseases, Rocky Mountain Laboratory, Hamilton, Montana (105).

PORTERFIELD, J. S., National Institute for Medical Research, Mill Hill, London, England (525).

RASKI, D. J., Department of Nematology, Agriculture Experimental Station, College of Agriculture, University of California, Davis, California (309).

SLYKHUIS, J. T., Plant Research Institute, Canada Department of Agriculture, Ottawa, Canada (347).

SMITH, KENNETH M., The Cell Research Institute, University of Texas, Austin, Texas (411).

STYK, B., Institute of Virology, Czechoslovak Academy of Sciences, Mlynska dolina, Bratislava, Czechoslovakia (163).

SUDIA, W. DANIEL, Arbovirus Vector Laboratory, Communicable Disease Center, Atlanta, Georgia (63).

SWENSON, K. G., Department of Entomology, Oregon State University, Corvallis, Oregon (267).

TEAKLE, D. S., Department of Microbiology, University of Queensland, Brisbane, Queensland, Australia (369).

VAGO, C., Institut National de la Recherche Agronomique, Station des Recherches Cytopathologiques, et Faculte des Sciences, Montpillier, France (567).

YARWOOD, C. E., Department of Plant Pathology, University of California, Berkeley, California (237).

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.

May, 1967

KARL MARAMOROSCH
HILARY KOPROWSKI

Contents of Future Volumes

Volume 2 *Tentative*

Volume 3

* Deceased.

Volume 3 (*Continued*)

Volume 4

Table of Contents

Chapter 8—Plant Virus Transmission by Insects

K. G. SWENSON

Chapter 9—Nematode Transmission

D. J. RASKI AND W. B. HEWITT

Chapter 10—Methods for Experimenting with Mite Transmission of Plant Viruses

J. T. SLYKHUIS

Chapter 15—**Bacteriophage Techniques**

A. EISENSTARK

Chapter 16—**Animal Tissue Culture**

J. S. PORTERFIELD

Chapter 17—**Plant Tissue Culture**

B. KASSANIS

Chapter 18—**Invertebrate Tissue Culture**

C. VAGO

1 *Natural Ecology*

Harald Norlin Johnson

I. The Ecosystem

Prior to 10,000 years ago the natural vegetation of this earth was relatively undisturbed by man. At the present time there is little arable land which does not show the effect of agriculture. The great deciduous forests of the riverine plains of India, China, Europe, and North America have been cut and the land plowed and seeded with domesticated varieties of plants. Man has increased from a rare species to one of the most abundant in the world. Likewise the domesticated animals, birds, and plants have, to a large extent, replaced their wild ancestors. It is this very condition which has brought about an increasing problem of disease in man, domestic animals, and plants. Whenever a species becomes very abundant it is apt to suffer epidemics of disease because it encroaches on other species and so becomes exposed to parasitic organisms with which it has had no prior experience. From what we know of the epidemiology of infections such as influenza, smallpox, mumps, chickenpox, measles, and poliomyelitis, it is difficult to imagine that the viruses which cause these diseases could have been maintained by man when he lived in family or small tribal units and derived his food entirely from wild animals and plants. He undoubtedly suffered sporadic infections with parasites derived from wildlife but there was

little opportunity for these parasites to spread from one family unit to another. Today man is vulnerable to all the parasites of wildlife to which he is susceptible. With the development of cities, man created the basis for disease problems such as bubonic plague, typhus, smallpox, malaria, and probably most of the respiratory and enteroviruses which flourish in him today. Whereas once the water supply was from springs and wells it became necessary to impound water from streams and rivers to supply cities. Such water was drunk without any treatment other than allowing it to settle in clay jars. This is still the practice in some large urban centers in Asia. One can expect that bacteria and viruses infecting wildlife will sooner or later reach man via contaminated water or food and infect him if he is susceptible. Small nocturnal mammals, such as meadow mice, from time to time become very abundant in the wet meadow environment about lakes and streams. Tularemia organisms have been isolated from meadow mice and surface water during die-offs of meadow mice (Jellison *et al.*, 1959). In Russia there have been epidemics of water-borne tularemia in man. The studies of Bolivian hemorrhagic fever have shown that a virus derived from the mouse *Calomys callosus* was the etiologic agent of the epidemic (Johnson, 1965). The virus was excreted in the urine of experimentally infected *Calomys* mice for more than 100 days. The wells in this village were for the most part open pit wells and wild mice were often found in the wells. Infection could also have been contracted from eating food contaminated with rodent urine. There was some person-to-person transmission of the virus. It is conceivable that once introduced into man this virus could start an aberrant cycle of man-to-man transmission with a potentially high fatality rate. Lymphocytic choriomeningitis virus of house mice is another example of a virus which might be found in water supplies. An outbreak of leptospirosis in army troops on an exercise in the mountain jungle of Panama was found to be related to exposure to stream water. Leptospira organisms were isolated from mice, rats, and opossums trapped along the stream (Mackenzie *et al.*, 1966). Bacillary dysentery and cholera are common water-borne diseases. In view of the great variety of bacteria and viruses that can be isolated from the sewage of modern cities it is not hard to believe that sewage contamination of wells and surface water in olden times must have been a major source of disease agents and the means for the maintenance of parasites in the human population.

A secondary effect of the storage of water in jars in the early urban communities was to domesticate mosquitoes. This resulted in urban epidemics of yellow fever, dengue, malaria, filariasis, and a variety of mosquito-borne virus diseases.