METHODS IN MEDICAL RESEARCH

METHODS IN

Medical Research

Volume 2

JULIUS H. COMROE, Jr., *Editor-in-Chief*

METHODS OF STUDY OF BACTERIAL VIRUSES, *Mark H. Adams, Editor*

PULMONARY FUNCTION TESTS, *Julius H. Comroe, Jr., Editor*

ASSAY OF HORMONAL SECRETIONS, *Eleanor H. Venning Editor*

PREFACE

This series of volumes is devoted to methods and techniques, and there were four main bases for our opinion that such a series might be useful. In the first place, while the results of investigation are constantly subject to critical review, it is less easy to find collected anywhere an appraisal and discussion of the various methods that have been proposed for the solution of some experimental problem. In the second place, it has (especially in physiology) become difficult to secure publication of a paper dealing solely with a technique, or even to include an adequate description of the technique in a paper describing the results obtained. Third, it often happens that a method is modified and improved in continued use, either in the laboratory whence it originated or elsewhere, and such useful modifications find their way into print, if at all, only as brief and scattered indications, and are to a great extent diffused by the uncertain process of personal communication. Fourth, many methods developed during the war have been described only in official reports.

It is pleasant to record that the first volume met with a reception cordial enough to assure us that our confidence in these reasons for launching this series was not misplaced. We hope also that we have learned some lessons, and that this volume and its successors will be still more warmly welcomed. The value of the whole series will increase as a widening field is gradually covered.

Each volume is divided into three to five principal, self-contained sections, each representing, for that volume, one of the broad fields of medical research, such as biochemistry, physiology and pharmacology, microbiology and immunology, and biophysics including radiobiology. Within each of these fields we shall try, year by year, to select narrower topics wherein a restatement of techniques seems timely.

As the topics are selected, we shall try to find experts, like those who have so signally contributed to the first two volumes, willing to act as associate editors for their assigned topic for the year. The responsibilities of the associate editor are by no means light: it is for him to select, within the topic and the space assigned, the methods most worthy of description and the contributors best fitted to describe. Obviously the methods most suitable for description in this form are those which are of wide actual or

potential application and which have not been published in full or have been usefully modified since publication: obviously, too, the inclusion of a method stamps it as being convenient and reliable in the associate editor's expert estimation, though it does not conversely follow that omitted methods are of lesser value. The associate editor may also send each contribution to another experienced investigator for comment and review; this feature of our plan has been found most valuable to all concerned.

As members of the Governing Board, we are very conscious of the lightness of our responsibilities in comparison with those of the associate editors, and, still more, those of Dr. J. H. Comroe, Jr., who to our great satisfaction agreed to assume the further ungrateful task of acting as editor-in-chief for this volume, charged among other things with the duty of distributing space among the sections. He has proved a worthy successor to Dr. V. R. Potter, to whom the first volume owed so much.

Any values which this series may have must be credited to the editors, the contributors and the referees, rather than to us. It remains for us merely to select topics and to try to find equally competent and conscientious editors for the next volume and its successors. To this end we should most gratefully receive any suggestions that readers may care to send us. Volumes 3 and 4 are now in active preparation.

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SECTION I

Methods of Study of Bacterial Viruses

Associate Editor -- MARK H. ADAMS

INTRODUCTION

It is now generally agreed that the bacteriophage principle of d'Herelle is a group of filtrable viruses, parasitic on bacteria and more closely related chemically and physically to the animal viruses than to the plant viruses. Because of the ease with which they and their host may be grown and studied, because of the high precision with which they may be assayed and because a single infected host cell may be readily isolated and observed in the absence of interaction with other cells, the bacterial viruses have become the subjects of widespread research as models of the host-virus relationship. The bacterial viruses have been used as tools in the study of the synthesis of proteins and nucleic acids in infected cells, as a test system in the search for possible chemotherapeutic and antibiotic agents for virus diseases, in the study of the phenomenon of virus interference, in the study of the action of ultraviolet light and x-radiation on viruses and in the study of the mutational patterns of viruses and bacteria. The possibilities inherent in the bacteriophage as a tool in the study of the relationship between virus and host cell have been largely underrated. It is because of the greatly increased interest in recent years in this group of viruses that it was thought worth while to collect in one place the various techniques found most suitable for research in this field.

The author is greatly indebted to Prof. Max Delbrück for a critical reading of the manuscript; many comments and suggestions

of his have been incorporated in the text. The author is also grateful to S. E. Luria, R. Dulbecco, T. F. Anderson and A. H. Doermann for helpful discussions concerning certain portions of the material. However, no one but the author is responsible for any errors or omissions. Some of the photographs showing plaque morphology are included through the kindness of Dr. Delbrück. The author's unpublished experiments which have been included were aided by a grant from the National Foundation for Infantile Paralysis, Inc.

—Mark H. Adams.

BACTERIAL VIRUSES

Host Organisms and Viruses

Bacteriophages have been recorded for the various groups of enteric bacteria such as shigella, salmonella, proteus, aerobacter, vibrio and escherichia. They are also known for bacilli, staphylococci, streptococci, corynebacteria, mycobacteria and actinomycetes. Phagelike agents are not known for protozoa, algae, yeasts or molds. Extensive investigations have been carried out on certain groups of phages, in particular on the staphylococcus phages by Northrop (62), and Krueger, salmonella and coli-dysentery phages by Burnet (13), cholera phages by Asheshov (11), typhoid phages by Craigie (18) and coli phages by Schlesinger (65).

In the United States the most intensively investigated phages are the group of 7 coli-dysentery phages described by Demerec and Fano (29) and studied by Delbrück, Luria, Hershey, Cohen, Beard, Anderson and many others. All methods described here have been tested on this group of phages but are more generally applicable. The work has been reviewed by Delbrück (24, 25).

Media

In work with strain B of Escherichia coli and its phages, the most generally useful medium is nutrient broth which contains 8 g of Difco nutrient broth and 5 g of NaCl/l of water. To this is added 15 g of agar/l to make nutrient agar for plates. For most phage work the agar in the plates should be fairly deep, the requirement being about 30 ml of nutrient agar/Petri dish. The plates should be stored in an incubator at 37 C overnight to dry them before use. Otherwise water of condensation will cause coalescence of plaques and ruin the plates for quantitative use. For preparation of agar slants for routine cultivation of the host organism and its mutants, 2 per cent nutrient agar is most suitable since bacteria from such a slant seem to be more readily dispersed in a uniform suspension.

It is expedient in routine work with strain B of E. coli to add 0.1 ml of 0.4 per cent gentian violet/l of nutrient agar. This added

dye almost completely prevents contamination of plates with gram-positive bacteria from the air. However, in work with other strains of bacteria or with mutants of strain B it is well to test the bacteria for possible susceptibility to the dye before routine use

Soft agar for the agar layer technique contains only 7 g of agar/l and is most conveniently put up in 50 ml amounts in bottles, to be melted and used as needed.

These media are probably suitable for use with any of the enteric bacteria. For more fastidious host organisms special media may have to be devised.

For certain types of work with bacterial viruses it is desirable to grow the host organisms on chemically defined media. In the case of E. coli, such a medium may be very simple, e.g.:

NH_4Cl	1.0 g
$MgSO_4$	0.1 g
KH_2PO_4	1.5 g
Na_2HPO_4	3.5 g
Lactic acid	9.0 g
Water	1000.0 ml

Bring to pH 6.8 with NaOH.

Numerous minor modifications of this medium, usually designated F medium, will be found in the phage literature. This medium may be enriched if desired with various amino acids, growth factors and salts to support growth of more fastidious organisms. E.g., strain B of E. coli will grow abundantly in this medium, but 2 of its phage-resistant mutants will not grow unless certain amino acids are added. Also, most phages of the T group are produced in high titer on strain B of E. coli when grown on F medium, but T5 is not produced unless $CaCl_2$ to a concentration of 0.001N is added. With such a chemically defined medium it is possible to study the effect of the nutritional environment of the host cell on the yield of phage.

With both broth and chemically defined media, maximal growth of E. coli is achieved only with active aeration of the cultures. With an energy source such as lactic acid, aeration is essential for growth. A convenient device for aeration is the Marco air pump.*

*Made by the J. B. Maris Company, Bloomfield, N. J. This company also makes valves and other fittings suitable for low pressure air lines.