METHODS IN VIROLOGY

EDITED BY
KARL MARAMOROSCH
AND
HILARY KOPROWSKI

Volume II

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AND

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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

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1 *The Ultracentrifuge*

Roy Markham

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

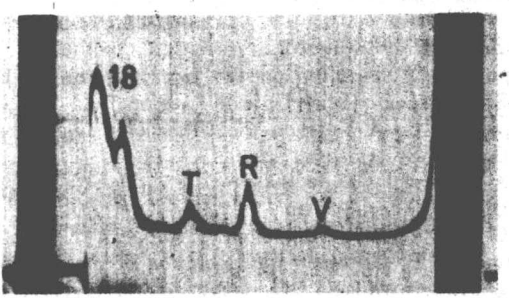

FIG. 1. Untreated sap from pumpkin plants infected with the wild cucumber mosaic virus. The direction of sedimentation is from left to right, and the peaks are, in order, small proteins, the 18 S or fraction 1 protein, empty (top-component) virus particles (T), ribosomes (R), and complete virus particles (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.

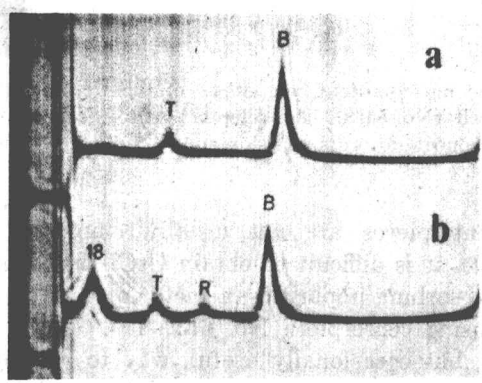

Fig. 2. Sap from plants with wild cucumber mosaic virus, pelleted once in a Model L Spinco, run in a plain cell (No. 1288) and a wedge window cell (No. 1338) to illustrate the use of two cells for comparative purposes. The material in the upper cell (a) was treated with bentonite clay, while that in 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.

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