

METHODS IN ENZYMOLOGY

Volume XXI
NUCLEIC ACIDS
Part D

Methods in Enzymology

Volume XXI

Nucleic Acids

Part D

EDITED BY

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Preface

Since the publication of the previous two volumes of "Methods in Enzymology" dealing with nucleic acids, this field of research has seen continued and rapid development. In order to maintain a comprehensive coverage of pertinent methodology for workers in the biological sciences, this compilation is extended in the form of two additional volumes: "Nucleic Acids and Protein Synthesis," Volume 20, Part C, and "Nucleic Acids," Volume 21, Part D.

Part D deals with the resolution of nucleic acids and their involvement in repair, restriction, recombination, and replication. Portions of this volume deal with the transcription control of DNA by repressors and initiators as well as the methods currently employed for specific localization genes at the molecular and biological levels of enzyme investigation.

We wish to express our appreciation to the numerous authors who contributed so ably and generously and to the very capable staff of Academic Press for their valuable assistance and cooperation.

We dedicate this volume to Drs. Maurice R. Atkinson and Daniel F. Bradley.

LAWRENCE GROSSMAN
KIVIE MOLDAVE

METHODS IN ENZYMOLOGY

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- I. Preparation and Assay of Enzymes
- II. Preparation and Assay of Enzymes
- III. Preparation and Assay of Substrates
- IV. Special Techniques for the Enzymologist
- V. Preparation and Assay of Enzymes
- VI. Preparation and Assay of Enzymes (*Continued*)
Preparation and Assay of Substrates
Special Techniques
- VII. Cumulative Subject Index

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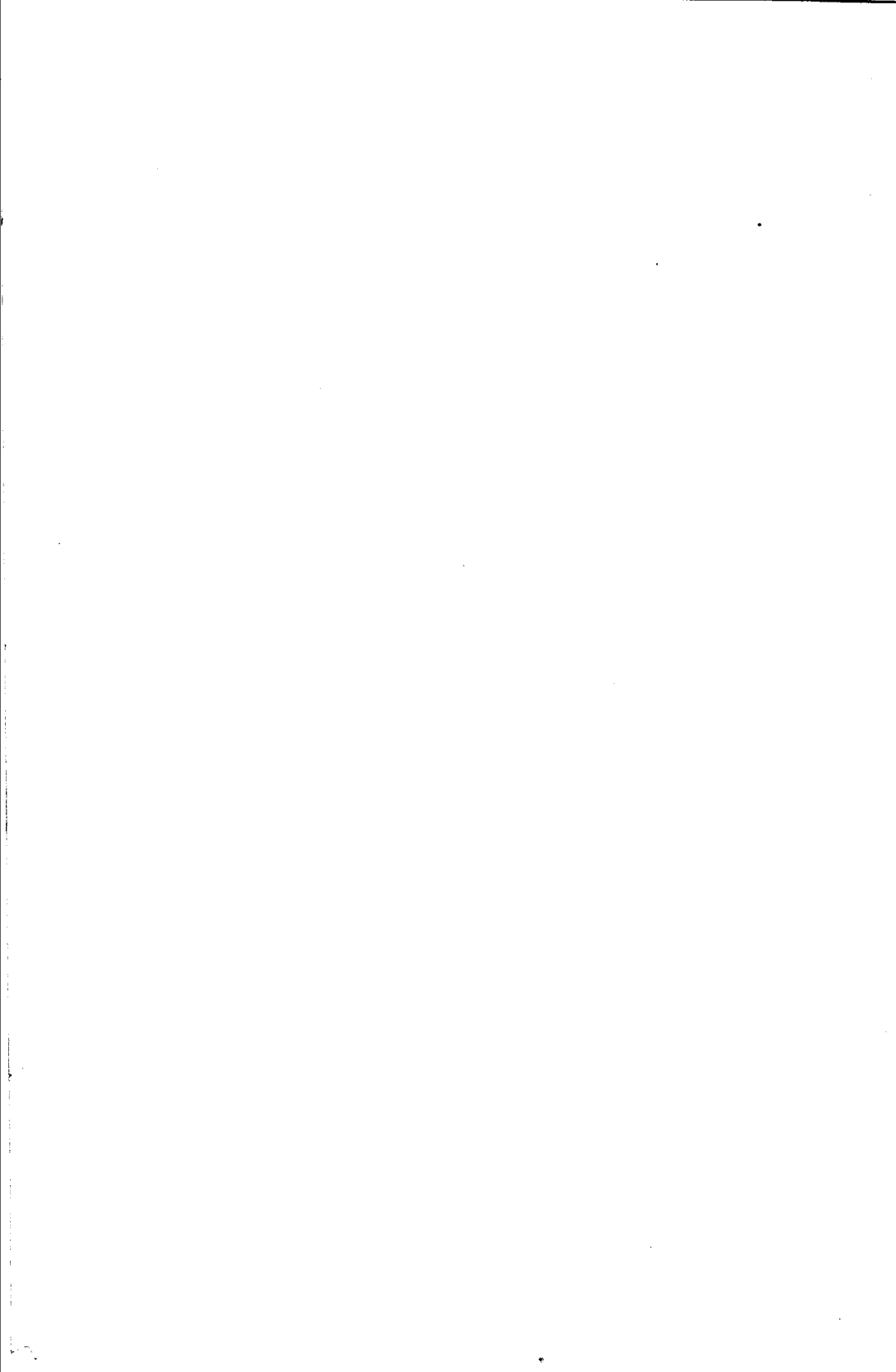
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Section I
Techniques for Structural Analysis



[1] Nucleic Acid Structure Analysis by Polarographic Techniques

By EMIL PALEČEK

Principles of Polarography

Polarography is an electrochemical method that deals with the relations between the potential of a mercury indicator electrode in an electrolytic cell and the current that flows through it.^{1,2} The principle of classical direct-current polarography is shown in Fig. 1. The mercury indicator electrode most frequently used in polarography is the dropping electrode (*P*); this consists of a capillary (*C*), one end of which is connected to the reservoir of mercury (*M*) and the other immersed in the solution being investigated. Because of the hydrostatic pressure of the mercury column, mercury flows through the capillary, forming droplets at the capillary tip. Thus the surface of the polarizable indicator electrode is continually renewed; the measured currents are accurately reproducible and are independent of the previous history of the experiment. As the nonpolarizable reference, electrode (*R*) serves either as a pool of mercury on the bottom of the vessel (*V*) or as a separate electrode (e.g., a calomel one) with a surface many times larger than that of the indicator electrode.

A gradually increasing (or decreasing) voltage is applied to the electrodes from an external source (*G*), and at the same time the current is measured. As long as the potential does not reach a value at which electron transfer between the electrode and a substance in the solution starts, no substantial current appears (the so-called charging or capacity currents observed under these conditions are due to the fact that each drop of mercury falling from the capillary must be charged to a potential corresponding to the applied voltage; these currents do not, as a rule, exceed 10^{-7} A). When the potential reaches a value at which some substance in the solution is reduced or oxidized (such a substance is called a depolarizer), a sharp current increase occurs (Fig. 1b). The increase continues until a potential is reached when all the depolarizer in the neighborhood of the electrode is exhausted and the current is limited by the transport rate of the depolarizer. A polarographic step³ appears, the height (*H*) of which depends on the

¹ L. Meites, "Polarographic Techniques." Wiley (Interscience), New York, 1965.

² J. Heyrovský and J. Kůta, "Principles of Polarography." Publ. House Czechoslovak Acad. Sci., Prague, 1965.

³ The term "step" refers to the curve obtained by means of direct-current polarography and normal pulse polarography; derivative pulse-polarographic curves are called "waves."