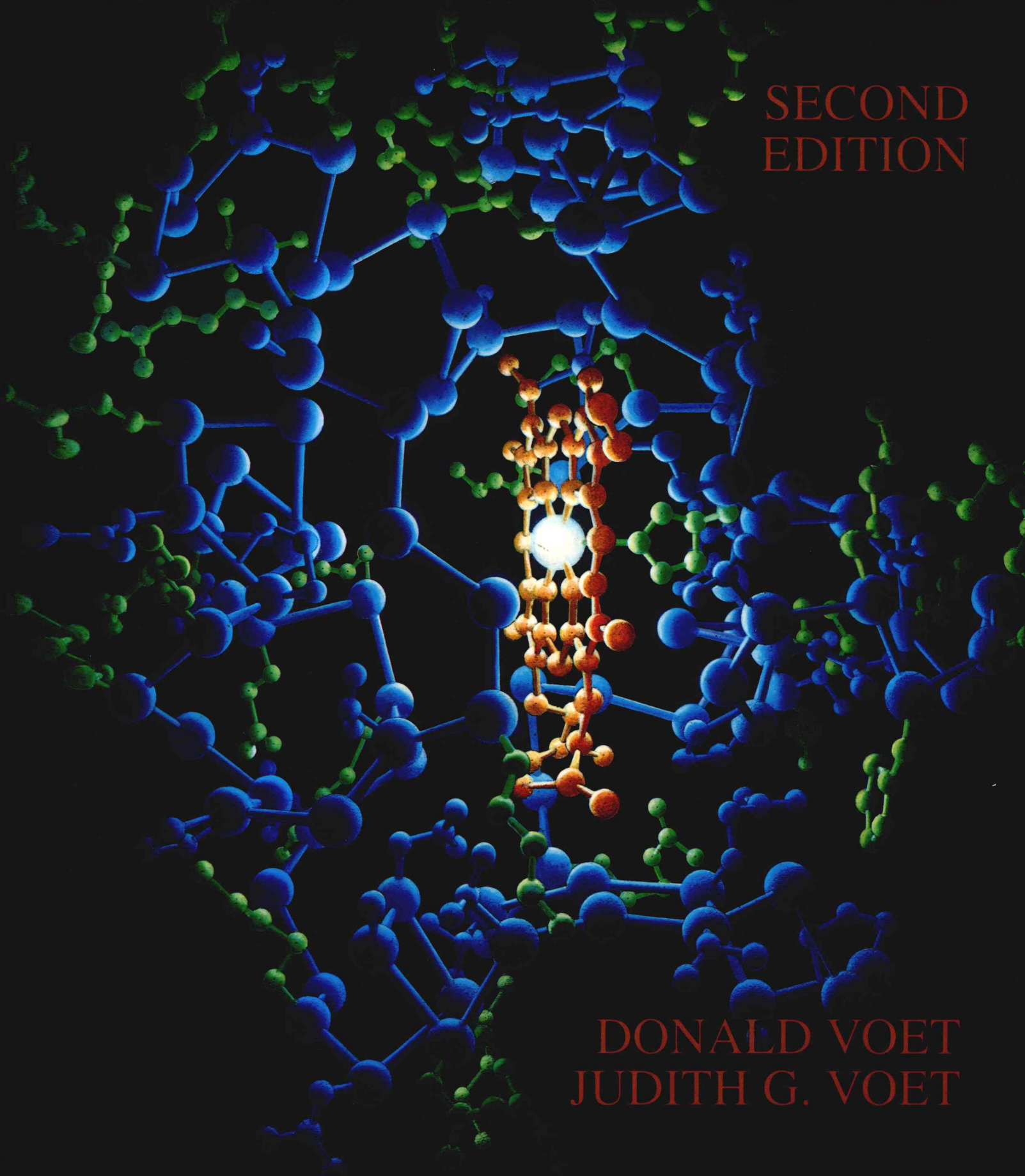
BIOCHEMISTRY

SECOND EDITION



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BIOCHEMISTRY

SECOND EDITION

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BIOCHEMISTRY

To
Our parents, who encouraged us,
Our teachers, who enabled us, and
Our children, who put up with us.

Annual Supplement Subscription Notice

Every year the biochemical literature continues its phenomenal growth. It is therefore increasingly important for the student and teacher alike to keep up with the literature. BIOCHEMISTRY, Second Edition is updated with Annual Supplements prepared by Donald and Judith Voet as a guide for doing so.

Beginning in the Summer of 1996 and each year thereafter an Annual Supplement will be prepared. Each supplement is keyed to BIOCHEMISTRY in that new advances are organized in terms of the textbook sections in which they would logically fit. Since space limitations permit only the most cursory discussions of these topics, the interested reader should consult the pertinent "Additional References" provided at the end of each discussion. The Annual Supplements for the Second Edition of BIOCHEMISTRY will continue until the Third Edition of BIOCHEMISTRY is published.

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Foreword

> *My own scientific career was a descent from higher to lower dimension, led by a desire to understand life. I went from animals to cells, from cells to bacteria, from bacteria to molecules, from molecules to electrons. The story had its irony, for molecules and electrons have no life at all. On my way life ran out between my fingers.*

Albert Szent-Györgi, The Living State, *Academic Press, 1972*

Thirty years ago, concepts in biochemistry were visualized in textbooks, chiefly as a series of organic chemistry formulas in schematic line diagrams. It would not be surprising if students of that time believed that living organisms were indeed composed of lifeless molecules.

A sharp change in biochemistry teaching came in the late 1960s with elucidation of the first three-dimensional structures of large protein molecules such as myoglobin, hemoglobin and the enzyme, lysozyme. In those days, it might require thirty-five man-years to determine, by X-ray crystallography, the structure of a single protein molecule. Thus it would be many years before there were sufficient examples of three-dimensional protein structures to begin to understand how these complex molecular machines acted to control the myriad series of reactions in the living cell.

In 1990, the first edition of this book achieved a grand synthesis of biochemical science, incorporating the flood of new ideas and experiments of the 1970s and 1980s—the development of rapid sequencing of DNA and protein molecules, as well as a proliferation of new three-dimensional structures. With a clear understanding that the one-dimensional linear DNA sequence provided the information for the three-dimensional conformation of protein molecules, the next step was then manipulating the DNA sequence, producing "designer proteins" by gene splicing and site-directed mutagenesis. New techniques for the manipulation of DNA and proteins called for new techniques of visual presentation. The clear and concise writing and the profusion of vivid, colorful illustrations made the first edition a leader in the field of biochemistry publishing.

This second 1995 edition is again a grand synthesis of the continuing flood of new concepts, as well as new structures determined by X-ray crystallography and NMR spectroscopy. The chapter Eukaryotic Gene Expression takes the student to the cutting edge of nucleic acid research by explaining the control of DNA expression in embryogenesis as well as the pathological expression of cancer genes. The expression of DNA is regulated mainly by the control of DNA transcription to RNA. This is a complex process involving protein–protein interactions along with protein binding, and often bending the DNA molecule.

The final chapter, Molecular Physiology, anticipates twenty-first-century molecular medicine by uniting physiological function, cell biology, and anatomy with molecular interactions. Examples are given of the cascade of enzymes in blood clotting, and the cascades of hormones and protein kinases in signalling between cells.

The ambitious Human Genome project aims to sequence the three billion base-pairs of human DNA. There are some 4,000 genetic diseases presently known that are potential targets for gene therapy. The effect of genetic research on the practice of medicine can hardly be overestimated. This book stands as a valuable guide to the science of molecular medicine for the twenty-first century.

IRVING GEIS

New York City
December 1994

Preface

In the five years since the first edition of *Biochemistry* was published, the field of biochemistry has continued its phenomenal growth and at an ever-increasing pace. This expansion of our knowledge has been marked not so much by new paradigms, although there have been plenty of those, but by an enormous enrichment of almost every facet in the field. For example, the number of known protein and nucleic acid structures as determined by X-ray and NMR techniques has increased by over fivefold and, moreover, many of these structures have led to seminal advances in our understanding of a particular subfield. Likewise, the state of knowledge has exploded in such subdisciplines as eukaryotic and prokaryotic molecular biology, metabolic control, protein folding, electron transport, membrane transport, immunology, signal transduction, and so on. Indeed, these advances have affected our everyday lives in that they have changed the way that medicine is practiced, the way that we protect our own health, and the way in which food is produced.

We have reported many of these advances in the second edition of *Biochemistry* and have thereby substantially changed nearly every section in it. We have, nevertheless, largely maintained the pedagogical framework of the first edition. Consequently, the Preface to the First Edition applies equally well to the Second Edition.

The textbook is accompanied by the following ancillary materials:

- A *Solutions Manual,* containing detailed solutions for all of the text's end-of-chapter problems.
- A CD-ROM containing most of the illustrations in the text. With computerized projection equipment, these full-color images can be shown in any prearranged order to provide "slide shows" to accompany lectures. Alternatively, they can be used to print transparencies.
- A diskette containing KINEMAGES, computer animated color images, of selected proteins and nucleic acids that permit the student to manipulate these macromolecules in three dimensions.

In addition, we shall continue our previous practice of annually publishing *Supplements to Biochemistry* that summarize the highlights of the preceding year's biochemical advances. The first of these ~80-page *Supplements* will be available in June 1996 and can be obtained either individually or on a subscription basis.

Finally, we are particularly grateful to the many readers of the First Edition, students and teachers alike, who have taken the trouble to write us with suggestions on how to improve the textbook and to point out errors they have found. We earnestly hope that the readers of the Second Edition will continue this practice.

DONALD VOET
JUDITH G. VOET

Preface

to the First Edition

Biochemistry is a field of enormous fascination and utility, arising, no doubt, from our own self-interest. Human welfare, particularly its medical and nutritional aspects, has been vastly improved by our rapidly growing understanding of biochemistry. Indeed, scarcely a day passes without the report of a biomedical discovery that benefits a significant portion of humanity. Further advances in this expanding field of knowledge will no doubt lead to even more spectacular gains in our ability to understand nature and to control our destinies. It is therefore of utmost importance that individuals embarking on a career in biomedical sciences be well versed in biochemistry.

This textbook is a distillation of our experiences in teaching undergraduate and graduate students at the University of Pennsylvania and Swarthmore College and is intended to provide such students with a thorough grounding in biochemistry. In writing this text we have emphasized several themes. First, biochemistry is a body of knowledge compiled by people through experimentation. In presenting what is known, we therefore stress how we have come to know it. The extra effort the student must make in following such a treatment, we believe, is handsomely repaid since it engenders the critical attitudes required for success in any scientific endeavor. Although science is widely portrayed as an impersonal subject, it is, in fact, a discipline shaped through the often idiosyncratic efforts of individual scientists. We therefore identify some of the major contributors to biochemistry (the majority of whom are still professionally active) and, in many cases, consider the approaches they have taken to solve particular biochemical puzzles. The student should realize, however, that most of the work described could not have been done without the dedicated and often indispensable efforts of numerous coworkers.

The unity of life and its variation through evolution is a second dominant theme running through the text. Certainly one of the most striking characteristics of life on earth is its enormous variety and adaptability. Yet, biochemical research has amply demonstrated that all living things are closely related at the molecular level. As a consequence, the molecular differences among the various species have provided intriguing insights into how organisms have evolved from one another and have helped delineate the functionally significant portions of their molecular machinery.

A third major theme is that biological processes are organized into elaborate and interdependent control networks. Such systems permit organisms to maintain relatively constant internal environments, to respond rapidly to external stimuli, and to grow and differentiate. A fourth theme is that biochemistry has important medical consequences. We therefore frequently illustrate biochemical principles by examples of normal and abnormal human physiology.

We assume that students who use this text have had the equivalent of one year of college chemistry and at least one semester of organic chemistry so that they are familiar with both general chemistry and the basic principles and nomenclature of organic chemistry. We also assume that students have taken a one year college course in general biology in which elementary biochemical concepts were discussed. Students who lack these prerequisites are advised to consult the appropriate introductory textbooks in these subjects.

The text is organized into five parts:

I. **Introduction and Background:** An introductory chapter followed by chapters that review the properties of aqueous solutions and the elements of thermodynamics.

II. **Biomolecules:** A description of the structures and functions of proteins, carbohydrates, and lipids.

III. **Mechanisms of Enzyme Action:** An introduction to the properties, reaction kinetics, and catalytic mechanisms of enzymes.

IV. **Metabolism:** A discussion of how living things synthesize and degrade carbohydrates, lipids, amino acids, and nucleotides with emphasis on energy generation and consumption.

V. **The Expression and Transmission of Genetic Information:** An exposition of nucleic acid structures and both prokaryotic and eukaryotic molecular biology.

This organization permits us to cover the major areas of biochemistry in a logical and coherent fashion. Yet, modern biochemistry is a subject of such enormous scope that to maintain a relatively even depth of coverage throughout the text, we include more material than most one year biochemistry courses will cover in detail. This depth of cover-

age, we feel, is one of the strengths of this book; it permits the instructor to teach a course of his/her own design and yet provide the student with a resource on biochemical subjects not emphasized in the course.

The order in which the subject matter of the text is presented more or less parallels that of most biochemistry courses. However, several aspects of the text's organization deserve comment:

1. We present nucleic acid structures (Chapter 28) as part of molecular biology (Part V) rather than in our discussions of structural biochemistry (Part II) because nucleic acids are not mentioned in any substantive way until Part V. Instructors who, nevertheless, prefer to consider nucleic acid structures in a sequence different from that in the text can easily do so since Chapter 28 requires no familiarity with enzymology or metabolism.
2. We have split our presentation of thermodynamics among two chapters. Basic thermodynamic principles —enthalpy, entropy, free energy, and equilibrium— are discussed in Chapter 3 because these subjects are prerequisite for understanding structural biochemistry, enzyme mechanisms, and kinetics. Metabolic aspects of thermodynamics—the thermodynamics of phosphate compounds and oxidation-reduction reactions—are presented in Chapter 15 since knowledge of these subjects is not required until the chapters that follow.
3. Techniques of protein purification are described in a separate chapter (Chapter 5) that precedes the discussion of protein structure and function. We have chosen this order so that students will not feel that proteins are somehow "pulled out of a hat". Nevertheless, Chapter 5 has been written as a resource chapter to be consulted repeatedly as the need arises.
4. Chapter 9 describes the properties of hemoglobin in detail so as to illustrate concretely the preceding discussions of protein structure and function. This chapter introduces allosteric theory to explain the cooperative nature of hemoglobin oxygen binding. The subsequent extension of allosteric theory to enzymology (Chapter 12) is a relatively simple matter.
5. Concepts of metabolic control are presented in the chapters on glycolysis (Chapter 16) and glycogen metabolism (Chapter 17) through discussions of flux generation, allosteric regulation, substrate cycles, covalent enzyme modification, and cyclic cascades. We feel that these concepts are best understood when studied in metabolic context rather than as independent topics.
6. There is no separate chapter on coenzymes. These substances, we feel, are more logically studied in the context of the enzymatic reactions in which they participate.
7. Glycolysis (Chapter 16), glycogen metabolism (Chapter 17), the citric acid cycle (Chapter 19), and oxidative phosphorylation (Chapter 20) are detailed as models of general metabolic pathways with emphasis placed on many of the catalytic and control mechanisms of the enzymes involved. The principles illustrated in these chapters are reiterated in somewhat less detail in the other chapters of Part IV.
8. Consideration of membrane transport (Chapter 18) precedes that of mitochondrially based metabolic pathways including the citric acid cycle and oxidative phosphorylation. In this manner, the idea of the compartmentalization of biological processes can be easily assimilated.
9. Discussions of both the synthesis and the degradation of lipids has been placed in a single chapter (Chapter 23) as have the analogous discussions of amino acids (Chapter 24) and nucleotides (Chapter 26).
10. Energy metabolism is summarized and integrated in terms of organ specialization in Chapter 25, following the descriptions of carbohydrate, lipid, and amino acid metabolism.
11. The basic principles of both prokaryotic and eukaryotic molecular biology are introduced in sequential chapters on transcription (Chapter 29), translation (Chapter 30), and DNA replication, repair and recombination (Chapter 31). Viruses (Chapter 32) are then considered as paradigms of more complex cellular functions, followed by discussions of newly emerging concepts of eukaryotic gene expression (Chapter 33).
12. Chapter 34, the final chapter, is a series of minichapters that describe the biochemistry of a variety of well-characterized human physiological processes: blood clotting, the immune response, muscle contraction, hormonal communication, and neurotransmission.

The old adage that you learn a subject best by teaching it simply indicates that learning is an active rather than a passive process. The problems we provide at the end of each chapter are therefore designed to make students think rather than to merely regurgitate poorly assimilated and rapidly forgotten information. Few of the problems are trivial and some of them (particularly those marked with an asterisk) are quite difficult. Yet, successfully working out such problems can be one of the most rewarding aspects of the learning process. Only by thinking long and hard for themselves can students make a body of knowledge truly their own. The answers to the problems are worked out in detail in the *Solutions Manual* that accompanies this text. However, this manual can only be an effective learning tool if the student makes a serious effort to solve a problem before looking up its answer.

We have included lists of references at the end of every chapter to provide students with starting points for independent biochemical explorations. The enormity of the biochemical research literature precludes us from giving all

but a few of the most important research reports. Rather, we list what we have found to be the most useful reviews and monographs on the various subjects covered in each chapter.

Biomedical research is advancing at such an astonishing pace that a seminal discovery often leads to the development of a mature subdiscipline within the period of a year or so. Consequently, a textbook on biochemistry can never be truly up to date. In order to alleviate this problem, we shall periodically bring out Supplements to this textbook that review the recent biochemical literature and list some of its most important reviews and research reports. Nevertheless, students should be encouraged to peruse the current biochemical literature for only then will they acquire a feeling for the scope and excitement of modern biochemistry.

Finally, although we have made every effort to make this text error free, we are under no illusions that we have done so. We therefore request that readers provide us with their comments and criticisms.

DONALD VOET
JUDITH G. VOET

Acknowledgments

This textbook is the result of the dedicated effort of many individuals, several of whom deserve special mention:

Lindsay Ardwin, our Copy Editor, put the final polish on our manuscript and eliminated an enormous number of grammatical and typographical errors. Irving Geis provided us with his extraordinary molecular art, generously wrote the Foreword to this textbook, and gave freely of his wise counsel. Laura Ierardi cleverly combined text, figures, and tables in designing each of the textbook's pages. Suzanne Ingrao, our Production Editor, skillfully and patiently managed the production of the textbook. Madelyn Lesure designed the textbook's typography and its cover. Hilary Newman and Stella Kupferberg acquired many of the photographs in this textbook and kept track of all of them. Nedah Rose, our Editor, adroitly directed the entire project and kept our noses to the grindstone. Edward Starr and Ishaya Monokoff coordinated the illustration program. John and Bette Woolsey and their Associates refined and extended the remarkable collection of illustrations that they created for the First Edition of *Biochemistry*.

The atomic coordinates of many of the proteins and nucleic acids that we have drawn for use in this textbook were obtained from the Protein Data Bank at Brookhaven National Laboratory. We created these drawings using the molecular graphics programs RIBBONS by Mike Carson and INSIGHT II from BIOSYM Technologies. Many of the drawings generously contributed by others were made using either these programs or GRASP by Anthony Nicholls, Kim Sharp, and Barry Honig; MIDAS by Thomas Ferrin, Conrad Huang, Laurie Jarvis, and Robert Langridge; MOLSCRIPT by Per Kraulis; or O by Alwyn Jones.

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D.V.
J.G.V.

Brief Contents

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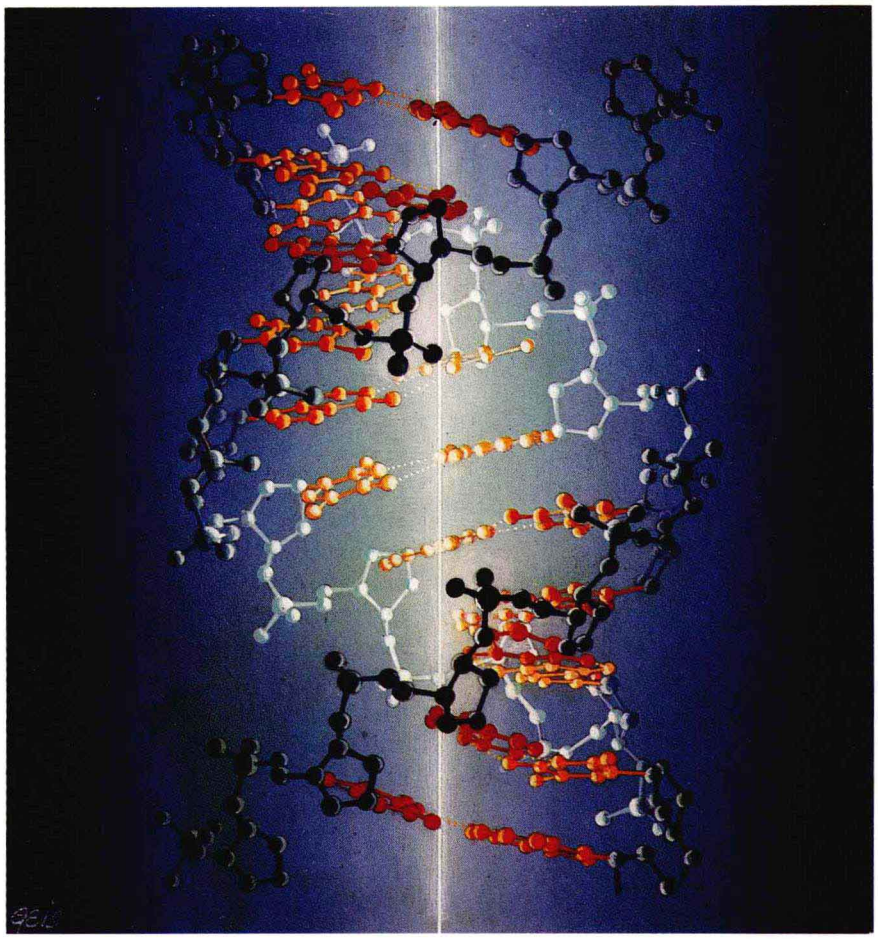
PART

I



INTRODUCTION AND BACKGROUND

"Hot-wire" A-DNA illuminated by its helix axis.