Practical Physiological Chemistry

HAWK
OSER
SUMMERSON

THIRTEENTH EDITION

McGRAW-HILL
BOOK COMPANY

Practical Physiological Chemistry

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Thirteenth Edition

The Blakiston Division

McGRAW-HILL BOOK COMPANY, INC.

NEW YORK TORONTO LONDON

THIRTEENTH EDITION

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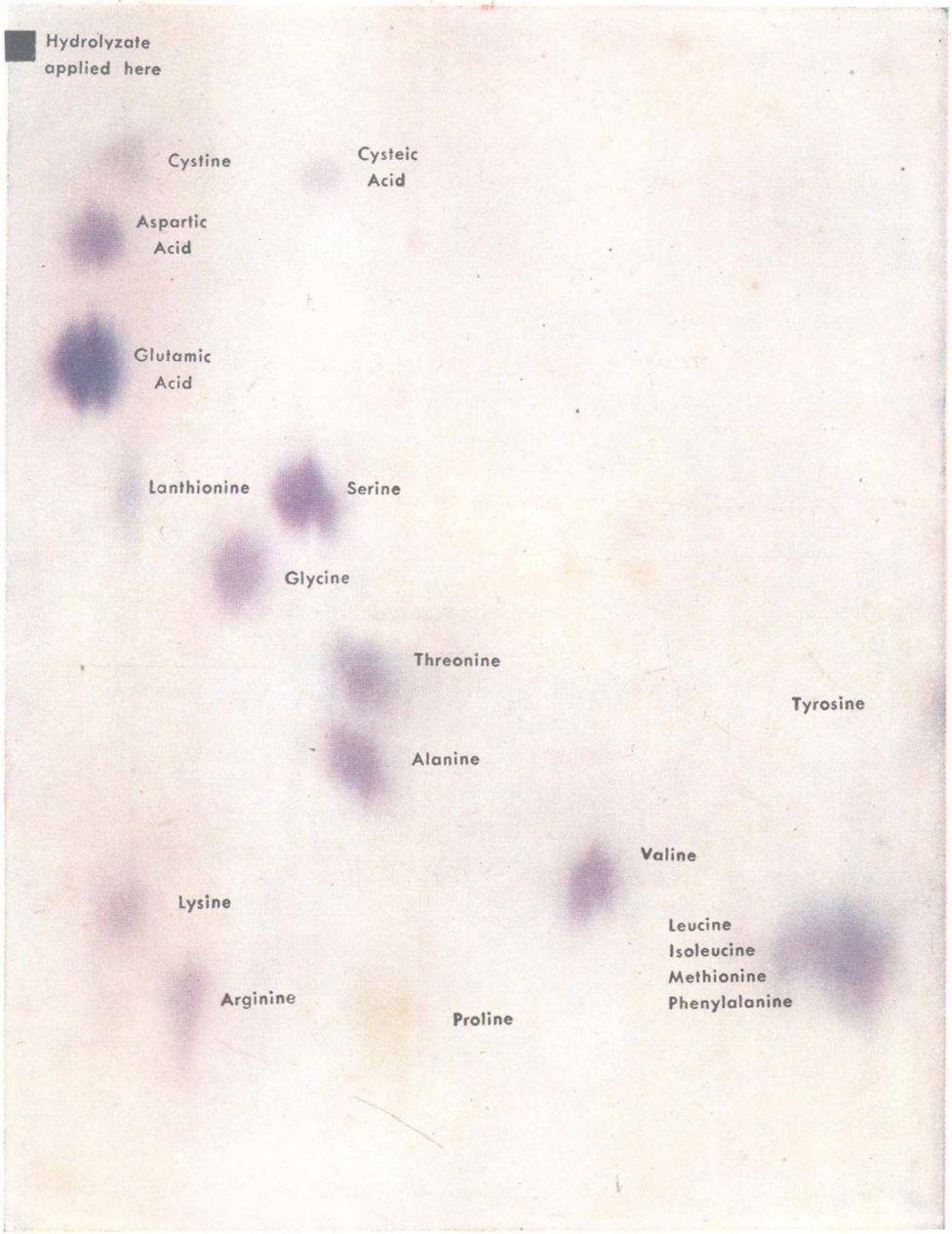
Library of Congress Catalog Card Number: 54-9278

PRINTED IN THE UNITED STATES OF AMERICA

BY THE MAPLE PRESS COMPANY, YORK, PA.



Two-dimensional chromatogram of tips of wool fibers. A drop of the hydrolyzate is placed near the corner of the sheet of paper. This is hung from the long side and the chromatogram developed with s-collidine (1). The paper is dried, hung from the short side, and the chromatogram developed with phenol (2). It is dried again, sprayed with ninhydrin, and heated. Exposure to weather and light has converted some cystine to cysteic acid and some to lanthionine. Otherwise the picture resembles that of pure wool. The clump of unresolved amino acids in the right-hand bottom corner can be separated if benzyl alcohol and butanol are used in place of collidine and phenol, but then the other acids are crowded together. (Courtesy, ENDEAVOUR, The Imperial Chemical Industries, Ltd., London.)

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* Deceased.

Dedicated

To the memory of

LAFAYETTE B. MENDEL,

Guiding Genius of American Biochemistry
who fifty years ago inspired the
First Edition of this work

The Publishers are proud of the long record of continuous authorship of this book under the Blakiston imprint and are grateful for the professional acceptance which has made a Thirteenth Edition possible.

Preface

It is with a mingled sense of pride and humility that the senior author presents this—the Thirteenth Edition of PRACTICAL PHYSIOLOGICAL CHEMISTRY. To have been actively engaged in the authorship of successive editions of a textbook for fifty years is a privilege granted to few in the annals of medical publication. For this good fortune, I am deeply grateful.

Perhaps even more than some of its predecessors, this Edition has undergone extensive revision and expansion. The proportion of the book devoted to textual material, as distinguished from experiments and procedures, has increased to the extent that it now accounts for about one-half of the entire volume. Certain chapters, such as those dealing with Nucleic Acids and Nucleoproteins (Chapter 7), Milk (Chapter 8), Muscular Tissue (Chapter 10), Enzymes and Their Action (Chapter 12), and Isotopes (Chapter 32), have been exhaustively rewritten. To other chapters major additions have been made, as for example Chapter 18 to which a section on Liver Function has been added, and Chapter 23 which now contains a section on Microclinical Chemistry.

Try as its author will, no textbook in such a rapidly developing area of research as biochemistry, can possibly be kept strictly up to the minute; it must inevitably run a losing race with the periodical literature. Nevertheless the authors of this volume and their collaborators have expended every effort in the direction of supplementing established knowledge with current facts, theories, and hypotheses, insofar as this is possible, with full awareness that in some respects, obsolescence commences with the date of publication (if not before). This may be of more concern to the research worker than to the student and for that reason the former should have recourse to original journals such as those cited profusely throughout this book.

An endeavor has been made to keep pace with current advances in the many fields of biochemistry which are benefiting from the availability of advanced techniques and instrumentation. Symbolic of these innovations, which give promise of epoch-making advances, is the replacement of the time-honored Frontispiece of this book depicting absorption spectra of the blood pigments and their derivatives with a new color plate showing a two-dimensional chromatogram of a protein hydrolyzate. For this illustration the authors are greatly indebted to Dr. A. J. P. Martin of the National Institute for Medical Research, London, and to the publishers of ENDEAVOUR, The Imperial Chemical Industries, Ltd., London.

Experimental, analytical, and preparative methods employing these newer techniques and research tools are scattered through various chapters of the book. Only a brief summary can be given here. The student and teacher of biochemistry will find of general interest the new material on electrophoresis; the ultracentrifuge; ion-exchange resins; column and

paper chromatography; countercurrent distribution; the helical structure of proteins; the polypeptide sequence of insulin; the chemistry of the corticosteroids; the synthesis of oxytocin; the chemistry and metabolism of nucleoproteins, nucleic acids and derivatives; the role of muscle proteins and ATP in muscle contraction; the biochemistry of bone and teeth; the kinetics of enzyme action; theories of blood clotting; the intermediary metabolism of carbohydrates, fats, and amino acids, including the role of coenzyme A and "one-carbon fragments"; the cobalamins, thioctic acid, leucovorin, and related factors; isotopes and their use in biochemical research; new material on antibiotics; and much more.

The teacher will find many new experiments suitable for class use, some of which were developed specifically for this Edition, illustrating such topics as paper and column chromatographic separation of amino acids, purine nucleotides, etc.; countercurrent distribution; myosin and actin; glycogen storage and depletion; determination of blood volume with isotope-labeled red blood cells; blood coagulation and prothrombin time; liver function tests; and many others.

The clinician will find new and authoritative sections on: the biochemistry of liver disease; the biochemistry of the bones and teeth, and the use of fluoride in the prevention of dental decay; isotopes and their use in medicine; dextran; cholinesterase; adrenal cortical and pituitary hormones; and the nutritive value of milk. Clinical chemical procedures not found in the previous Editions include the more important liver-function tests (thymol turbidity, cephalin-cholesterol flocculation, bromsulfalein, etc.); determination of blood protein-bound iodine, of blood cholinesterase, and of blood sodium and potassium by flame photometry; and others. A unique and valuable section has also been added on the use of micromethods in clinical chemistry, with detailed procedures and descriptions of apparatus.

Revisions of PRACTICAL PHYSIOLOGICAL CHEMISTRY have never been a one-man job. The success and prestige of the book has been due in no small measure to the fine cooperation shown by many teachers and investigators in the medical and biochemical fields. A list of those who have participated in some degree on one or more editions would read like a combination of WHO'S WHO and WHO WAS WHO in these professions.

In the elaboration of this Edition the authors feel signally honored to have enjoyed the cooperation of many distinguished scientists and educators. To credit them individually with specific contributions would fail to do justice to those whose advice and assistance is reflected in several places, if not generally, throughout the book.

In consequence, deep gratitude is expressed here on behalf of my associated co-authors and myself for major contributions and assistance of the collaborators whose names and affiliations are listed immediately preceding this Preface. Without the splendid and gracious help of these eminent biochemists and physicians it would have been infinitely more difficult to produce what we believe is the finest of a long series of revisions.

Special acknowledgment and thanks are due the following eminent experts and teachers whose generous assistance, both solicited and unsolicited, while not quite as extensive as that of the aforementioned col-

laborators was none the less welcome: Dr. Zoe E. Anderson, Director of the National Dairy Council's Research and Nutrition Service; Dr. A. K. Balls, Professor of Enzyme Chemistry, Purdue University; Dr. Albert L. Chaney, Director of the Albert L. Chaney Chemical Laboratory, Glendale, Cal.; Dr. L. C. Craig, Member, Rockefeller Institute for Medical Research; Dr. Alexander L. Dounce, Assistant Professor of Biochemistry, University of Rochester School of Medicine and Dentistry; Dr. Theodore E. Friedemann, Scientific Director, Medical Nutrition Laboratory, Fitzsimons Army Hospital, Denver, Col.; Dr. Linus Pauling, Professor of Chemistry, California Institute of Technology; Dr. Kurt G. Stern, Adjunct Professor of Biochemistry, Polytechnic Institute of Brooklyn; Dr. Henry Tauber, Associate Professor of Experimental Medicine, School of Public Health, University of North Carolina; Dr. Oscar Touster, Associate Professor of Biochemistry, Vanderbilt University School of Medicine; Dr. Everett C. Cogbill and Mr. Richard M. Rush of the University of Virginia; and Dr. M. A. Derow of Boston University.

Once again it is my pleasure to pay tribute to the skill and untiring efforts of my associates, Dr. Bernard L. Oser, whose more than quarter-century association with this book is well known to its friends, and Dr. William H. Summerson, whose long experience as a teacher and investigator at Cornell Medical College preceding our association, eminently qualified him for the important role he has assumed as a co-author.

For their assistance in matters editorial the authors are particularly indebted to Mrs. Eunice Stevens, editor-in-chief of Blakiston, and her able and conscientious associate editor, Mr. Barney Pisha. Their efforts in standardizing style and typography are reflected in the enhanced appearance of this completely reset edition. In this connection reference may be made to questions of spelling and nomenclature where uniformity of practice in the scientific literature has yet to be achieved. Since the American Chemical Society, more than any other single organization, has been concerned with standardization in this field, the recommendations of that organization have been adopted despite the risk, in some instances, of jarring the sensibilities of a few readers (and occasionally of a co-author). While appreciation is expressed to the publisher's and printer's staffs for their careful efforts in proofreading, the authors assume full responsibility for such errors and oversights as will inevitably be discovered by sharp-eyed readers.

Appropriate acknowledgment has been made through this edition to the numerous authors, publishers and instrument and apparatus companies who so graciously granted permission for the use of illustrations, tables, or other copyrighted material. Appreciation is expressed here for these courtesies and, in the unlikely event that acknowledgment has been omitted in the specific places where such material has been used, the authors claim human frailty and apologize for these oversights.

Finally, the authors are deeply appreciative of the patience and cooperation of the Maple Press whose fine craftsmanship has made possible the production of this attractive volume.

I trust I will be pardoned for concluding this Preface with a short personal note.

The manuscript of the First Edition of this book was drafted high up in the old Hare Laboratory of the Medical School of the University of Pennsylvania, amid the stimulating fragrance of the Department of Anatomy. It served as the basis for a course of instruction even before the edition was in print. Now, a half-century later, and thanks to its friendly reception by the profession, the Thirteenth Edition has rolled off the press.

Among those to whom I was indebted for its genesis, first place must go to the late Professor Lafayette B. Mendel for it was he who generated the spark of inspiration. The value of Dr. Mendel's suggestions and criticism of the manuscript of several editions was great indeed. His wonderful letters, written in long hand with purple ink and crammed full of ideas, references, and suggestions were of incalculable aid. These missives were never less than six pages long and often twice as long. The First Edition was thus, quite naturally, based on the courses as given at Yale University, with minor features as adopted at Columbia University (College of Physicians and Surgeons) where I passed my first teaching assignment (1901–1903) under Professor William J. Gies. Dr. Mendel's interest in the book never flagged. It is therefore fitting and proper that to Dr. Lafayette B. Mendel, guide, philosopher, and friend of many an outstanding biochemist as he was of the senior author, should be dedicated this "Golden Anniversary" Edition of PRACTICAL PHYSIOLOGICAL CHEMISTRY.

PHILIP B. HAWK

April 1954
MIAMI BEACH, FLORIDA

Preface to the First Edition

The plan followed in the presentation of the subject of this volume is rather different, so far as the author is aware, from that set forth in any similar volume. This plan, however, he feels to be a logical one and has followed it with satisfactory results during a period of three years in his own classes at the University of Pennsylvania. The main point in which the plan of the author differs from those previously proposed is in the treatment of the food stuffs and their digestion.

In Chapter IV the "Decomposition Products of Proteids" has been treated although it is impracticable to include the study of this topic in the ordinary course in practical physiological chemistry. For the specimens of the decomposition products, the crystalline forms of which are reproduced by original drawings or by microphotographs, the author is indebted to Dr. Thomas B. Osborne, of New Haven, Conn.

Because of the increasing importance attached to the examination of feces for purposes of diagnosis, the author has devoted a chapter to this subject. He feels that a careful study of this topic deserves to be included in the courses in practical physiological chemistry, of medical schools in particular. The subject of *solid tissues* (Chapters XIII, XIV and XV) has also been somewhat more fully treated than has generally been customary in books of this character.

The author is deeply indebted to Professor Lafayette B. Mendel, of Yale University, for his careful criticism of the manuscript and to Professor John Marshall, of the University of Pennsylvania, for his painstaking revision of the proof. He also wishes to express his gratitude to Dr. David L. Edsall for his criticism of the clinical portion of the volume; to Dr. Otto Folin for suggestions regarding several of his quantitative methods, and to Mr. John T. Thomson for assistance in proof reading.

For the micro-photographs of oxyhaemoglobin and haemin reproduced in Chapter XI the author is indebted to Professor E. T. Reichert, of the University of Pennsylvania, who, in collaboration with Professor A. P. Brown, of the University of Pennsylvania, is making a very extended investigation into the crystalline forms of biochemic substances. The micro-photograph of allantoïn was kindly furnished by Professor Mendel. The author is also indebted for suggestions and assistance received from the lectures and published writings of numerous authors and investigators.

The original drawings of the volume were made by Mr. Louis Schmidt whose eminently satisfactory efforts are highly appreciated by the author.

PHILIP B. HAWK

PHILADELPHIA

Contents

1

Physicochemical Principles

Living matter differs from nonliving in its possession of certain characteristic properties such as growth, reproduction, respiration, and motion. The science of physiological chemistry deals with the application of chemical and physicochemical principles and methods to the study of these phenomena. In the early days of the science this meant the analysis of foods entering the organism and of excreta leaving it; it involved the study of the composition of the various tissues and organs of the body, the blood, the digestive secretions, etc. In this way a great deal of information has been collected concerning the composition of living matter and the fate of the substances that are necessary for continuance of life and growth. Although far from complete these researches have progressed to the point where we now possess a fairly comprehensive picture of the gross changes that take place in protoplasm.

The experimental methods used, however, for the most part involved the destruction of the living cell. In recent years the emphasis has been placed on the mechanisms concerned in the reactions of the living protoplasm itself. Since protoplasm is largely water, this requires a study of the nature of solutions and the complex behavior of mixtures of electrolytes. Since the physical basis of protoplasm is colloidal in character, a study of the peculiar structure and properties of colloidal solutions is also necessary. Some of the more important physicochemical principles that are finding wide and fruitful application in the study of life phenomena are discussed briefly in the following pages.

THE COLLOIDAL STATE

True and Colloidal Solutions. Thomas Graham in 1861 classified all substances into two groups, crystalloids and colloids, depending upon their ability to diffuse through membranes such as parchment. According to Graham, crystalloids readily passed through parchment membranes while colloids did not. We now recognize that matter cannot be classified in this manner since many typical colloids, such as certain proteins, are crystallizable, and practically all crystalloids may, under proper conditions, be brought into the colloidal state.

According to modern concepts, colloidal solutions, instead of being solutions of particular types of matter, are solutions with a characteristic kind of structure. Substances such as glucose or sodium chloride, which form true solutions in water, disintegrate, when dissolved, into individual molecules or ions which are less than 1 mμ (1 millionth mm.) in diameter.

The smallest particle that can be seen with a high-power light microscope has a diameter of about 200 mμ. By means of an electron microscope, particles with a diameter as small as 10 mμ are readily made visible. When the particles of solute are larger than 200 mμ they are said to be in suspension; on standing such particles will gradually separate out. When, however, the solute is dispersed into particles which are intermediate in size between ordinary molecules such as exist in true solutions and the coarse particles found in suspensions, it is said to be in the colloidal state, and solutions containing particles of that size are known as *colloidal solutions* or *sols*. Sols which have become jellylike are called *gels*.

Colloidal solutions, true solutions, and suspensions thus differ from each other fundamentally only in the size of the particles of *solute* (the *disperse phase*) dispersed in the *solvent* (the *dispersion medium*). Because of the dimensions of the disperse phase, colloidal solutions exhibit certain characteristic and unique properties (to be discussed in later sections) which confer upon them their great importance in the structure of living protoplasm. This importance resides in the fact that protoplasm is considered to be a complex system containing many different crystalloidal and colloidal components. Although the structure and properties of this system are too complex to permit exact characterization in the present state of our knowledge, we may gain an insight into these questions by a study of similar, though very much simpler, colloidal systems such as are discussed below.

Preparation of Colloidal Solutions. The relationship between colloidal solutions, true solutions, and suspensions indicated above suggests two general methods by which colloidal solutions may be prepared. These methods are classified as (1) condensation and (2) dispersion methods, depending upon whether the colloidal particles are formed by aggregation of individual molecules or by disintegration of coarse particles of matter.

CONDENSATION METHODS. The principles underlying the preparation of colloidal solutions by condensation methods are similar to those involved in ordinary precipitation reactions. In both processes the solution is permitted to become supersaturated with respect to some particular substance. Such supersaturated solutions, in the presence of suitable condensation nuclei, develop molecular aggregates which continue to increase in size as long as any available material remains in solution. In precipitation reactions this process of growth continues until the particles become visible in a microscope or to the naked eye when they flocculate from solution. By proper regulation of the experimental conditions, which differ for different substances and procedures, the growth of molecular aggregates may be checked when the particles attain the size characteristic of the colloidal state, thus forming colloidal solutions. Whether a particular reaction will lead to the formation of a colloidal solution or a visible precipitate depends, therefore, entirely upon the conditions under which the experiment is carried out. Von Weimarn, who studied this question very extensively, showed that by merely varying the concentrations of $Ba(CNS)_2$ and $MnSO_4$ from N/20,000 to 7 N, the form of the $BaSO_4$ precipitated could be made to vary from large crystals to a colloidal gel. The colloidal state, as indicated in the following scheme, is merely an