CLINICAL CHEMISTRY

Laboratory Manual and Methods

JOSEPH H. BOUTWELL, Jr., Ph.D., M.D.,

Associate Professor of Physiological Chemistry Temple University School of Medicine
Director, Clinical Chemistry Laboratory Temple University Hospital

Illustrated

LONDON
HENRY KIMPTON
134 GREAT PORTLAND STREET, W.1
1961

Printed in the United States of America

Contents

CLINICAL CHEMISTRY

Laboratory Manual and Methods

". . . most human activities advance by virtue of contributions from many different types of individuals, with vastly different endowments, working at different levels. Medical investigation is no exception to this rule."

Medical Research: A Midcentury Survey
Boston, Little, Brown and Company
1955, vol. 1, p. xxxi.

CLINICAL CHEMISTRY

Laboratory Manual and Methods

JOSEPH H. BOUTWELL, Jr., Ph.D., M.D.,

Associate Professor of Physiological Chemistry Temple University School of Medicine
Director, Clinical Chemistry Laboratory Temple University Hospital

Illustrated

LONDON
HENRY KIMPTON
134 GREAT PORTLAND STREET, W.1
1961

Printed in the United States of America

An Appreciation

No author writes a book alone. The content, viewpoint and expression may be uniquely his, but derived from others. It is only right that some of these be mentioned, not that they may share the responsibility for another new book, but that the author may express his appreciation for their inspiration and guidance.

Those who were especially influential upon this present effort include Professor Robert H. Hamilton, Professor and Chairman of the Department of Physiological Chemistry, Temple University School of Medicine, many of whose ideas and approaches in methodology are incorporated herein; and Dr. Howard W. Robinson, Professor of Physiological Chemistry.

The author is especially grateful to Temple University School of Medicine and Hospital for their provision for a climate of opportunity and inspiration in which it was possible to complete this effort.

To the technologists, present and past, staff and students, who contributed by their criticism and suggestions, my thanks are due. There are many others, too numerous to mention specifically.

Special thanks, too, are due to the Publishers, whose assistance is reflected in many details in the text.

J.H.B.

Preface

THE primary purpose of this text is to provide a teaching manual with clear and explicit direction to those engaged in the profession of clinical chemistry, to students and instructors alike. This has required the inclusion of not only "step-by-step" methodology, but also sufficient background and theoretical material so that the work in the laboratory can become not only accurate and precise, but "knowledgeable."

The one general principle which has guided the selection and arrangement of textual material, has been the existence of a clearly demonstrated need by the laboratory worker, whether technologist or pathologist, student or veteran in the field.

The section on *general principles of chemical analysis* will make possible, and encourage, accurate, precise and intelligent work in the clinical chemistry laboratory. A comparatively long section has been devoted to a discussion and description of *primary and secondary standards* and their preparation and use in the laboratory. While modern laboratories are increasingly compelled to rely on commercial assistance (and it is invaluable) in the preparation of pure chemicals, special enzymes, and even pre-mixed reagents, it should be within the capability of a clinical chemistry laboratory worker independently to check the reliability and accuracy of his own results. The material bearing on *clinical interpretation* is included to serve as a motivating link between the laboratory worker and the physician in their joint effort directed toward the diagnosis and treatment of disease. The section on *laboratory control and statistics* has been included to emphasize the need for recognition of random, systematic, and procedural errors in the laboratory. Only when error is not only recognized but measured is correct clinical interpretation possible. Only when error is measured is it possible to evaluate the usefulness of procedural changes.

The sections on *clinical biochemistry* serve to bring to the laboratory worker an appreciation of the factors involved in the evaluation of the clinical status of the patient. Mere technical proficiency is not enough. A section dealing with the *general principles of enzymatic assay* has been included in addition to the methodology of various enzymes. No other analytical task is so beset with traps for the unwary as is that of enzymatic measurements. Mere "cook-book" approaches are not adequate to the problem.

The illustrations of *laboratory calculations* are included to encourage complete mathematical treatment of laboratory data so that the technologist and other workers may be independent of formulæ, especially those adapted to one special procedural technic. The *theory of photometric and gasometric measurement* is developed sufficiently so that commercial instruments may become "servants" and not "masters."

It would be possible to describe methods in so general a way as to require translation into specific volumes of specific reagents before an analysis could be undertaken. It would also be possible (and it is often done) to

describe a method so specifically that the effect of minor variations in technic and glassware used could not be predicted. A mixture of these two approaches combines specific description with sufficient flexibility so that each laboratory worker will be able to adapt the method to local requirements.

A clinical method *begins with the patient*, before the sample is obtained. Although our knowledge of the effect of patient variability on clinical chemistry laboratory data is rather meager, a beginning of this study has been made, and should be made in all laboratories faced with this problem. Technics required for the *collection and preservation* of the sample (blood, urine, feces, etc.) are stressed and possible or probable changes which may occur are outlined.

The analytical methods included have been chosen by a number of varying criteria. They are all in use, or have been in use, in the Temple University Hospital Clinical Chemistry Laboratory under direct supervision by the author for a period of at least several years. They are not *necessarily* the "best" method (most accurate or most precise) known to the author but those included have been subjected to scrutiny as to acceptability of results, and ease and reliability in the hands of student as well as experienced technologists. In some cases, alternate procedures are included, either for teaching purposes or because of special ease of technic or as a reference method.

The methods are arranged alphabetically, within sections. The sections are divided into General Methods, Urine Analysis, Fecal Analysis, Spinal Fluid Analysis, and Toxicology. Each method is presented as follows in outline.

1. *References* to the literature directly bearing on the analytical problem.
2. *General principle* of the method.
3. *Reagents.* An attempt is made to describe the preparation of reagents (*a*) in terms of laboratory preparation (weight and volume) and (*b*) in terms of analytical significance (normality titer).
4. *Procedure.* A step-wise outline of the procedure with details of technic emphasized whenever they are critical.
5. *Calculation.* Since this manual is designed as a teaching aid, derivations of the formulæ required are in many cases outlined. In most cases, an example of an actual laboratory analysis is included.
6. *Notes.* In any case in which experience has shown the necessity, additional clarification of the reasons for a technic or special precaution is included.
7. *Interpretations.* Normal ranges, biochemical and patho-physiological significance of variations are included in this section. To facilitate rapid reference to the significance of the results of a particular test, a special table of *Clinical Significance and Normal Ranges* has been included in the Appendix which abstracts the more complete discussion which is presented with each method.

Reference texts which have been found useful have been included in a short bibliography. This list is not meant as inclusive but only illustrative of the vast material available to the clinical chemistry investigator.

PHILADELPHIA, PENNSYLVANIA JOSEPH H. BOUTWELL, JR.

Contents