CLINICAL CHEMISTRY: Interpretation and Techniques

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PREFACE

Our basic aim in this second edition is to improve the first edition and make it a better teaching manual for those who work, supervise, or teach in clinical chemistry. We have made some changes, enlarged the content and treatment of some chapters, reordered the material, and made many revisions in order to achieve those goals. Methods that have become obsolete are omitted. We have profited by many of the suggestions and thoughtful comments that were made by users and reviewers of the book.

Most of the methods that are described in detail are the ones that we use at the University Hospital, University of Washington. The selection reflects both our experience and prejudice. In a few situations, several methods are given for a single constituent. For example, an enzymatic and a chemical method are described for cholesterol, since both are widely used; this will enable students to become familiar with the principles of both and to function in a wider range of laboratories. In a book of this size, it is impossible to outline the advantages and disadvantages of the various methods and to justify the selection of each method.

In general, the laboratory tests are grouped according to the function or organ system being tested. The chemical principles of the chosen method are discussed, as are the physiologic and biochemical changes for particular constituents that occur in normal and disease states. The material bearing on clinical interpretation serves as a motivating link between the laboratory worker and the physician as their joint effort is directed toward the diagnosis and treatment of disease. In general, work performance in the laboratory is better when technologists have an understanding of the application of their results, because it helps to give them a feeling of participation in the total medical effort rather than of being robots who crank out numbers of unknown significance.

Chapter 2, "Basic Principles," includes a section in which general principles of chemical analysis are presented for the purpose of review and to encourage accurate, precise, and intelligent work in the laboratory. Another section describes common laboratory instruments and techniques and discusses their basic principles. Several new sections have been added, one of which explains the principles of different types of automated analyzers (continuous flow, discrete, centrifugal, thin-film). Only the basic approaches are mentioned since technologists will have to learn the operation of the particular instruments used by their laboratories. The other new sections deal with radiochemistry and laboratory computers. This chapter also includes a section on laboratory quality control and statistics that emphasizes the need for recognition of random, systematic, or procedural errors in the laboratory. It is necessary not only to recognize error but to determine its magnitude in order to assess the validity of test results or to evaluate the usefulness of procedural changes. Finally, a section dealing with laboratory safety is presented to acquaint students with the potential hazards of working in a clinical chemistry laboratory and the means for minimizing the risks.

All of the succeeding chapters have been updated and modified to some extent. In particular, the chapter on enzymes includes a section on enzyme kinetics; the lipid chapter deals more extensively with lipoproteins; the chapters on endocrinology and on immunochemical techniques have been revised extensively; and the toxicology chapter has been expanded, especially in the sections dealing with therapeutic drug monitoring and pharmacokinetics.

A hospital is a complex institution, and there are many links in the chain before a sample is analyzed and the report received by the physician. When a physician requests a blood test, a nurse or clerk transcribes the request upon a laboratory request form, a member of a blood-collecting team usually obtains the specimen that is brought to the laboratory, given an acquisition number, processed and analyzed. After the analysis has been completed, the result is entered upon an appropriate laboratory form, which goes to a nursing station and then to the ordering physician. Computers may or may not be used in the data handling system. The chain is long and complex and subject to error at a number of points, many of which are beyond the control of the laboratory. It is the laboratory's function to perform the test as accurately and as speedily as possible and to see that the results are properly and correctly entered into the system for delivery to the physician. A laboratory result, no matter how accurately and swiftly performed, becomes useless unless it reaches the attention of the attending physician. Conscientious laboratory workers must be prepared to check various aspects of the chain of communication to insure that the results reach their destination without undue delay. Moreover, the specimen itself must be handled adequately and properly from the time of drawing the blood until the analysis is carried out in the laboratory.

In this age of automation and mechanization, there is a tendency to place too much emphasis upon machine capability and too little upon the capabilities, training, and judgment of the technologists and technicians who operate the instruments. Automated instruments can carry out a number of operations in a repetitive fashion and can make possible the performance of a large number of tests, but most of these instruments require adjustment, calibration, adequate maintenance, and constant surveillance to make sure that the results they generate are both precise and accurate. This requires supervision and control by people who understand the instruments and know what they are doing; in the clinical chemistry laboratory this means that the technologists must have a good basic understanding of clinical chemistry and analytical chemistry.

Manual methods are described in detail in the text because the chemical principles are the same in both manual and automated tests; many teaching laboratories require the students to perform tests manually as well as by automation; and a number of laboratories perform some tests manually or use these tests as a back-up for an automated system.

In addition to technical competence, good laboratory workers must have the feeling at all times that they are members of a medical team dealing with sick people. The work that they do is extremely important because the modern health care expert relies heavily upon the results of chemical measurements of constituents in body fluids and tissues. This sense of concern, when accompanied by the initiative to make sure that the results are dependable and that they reach their proper destination, makes the difference between a good and a mediocre laboratory.

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Chapter 1

INTRODUCTION TO CLINICAL CHEMISTRY

The clinical chemistry laboratory is an important contributor to the medical team involved in the diagnosis and treatment of disease because it performs approximately 50% of the total laboratory requests. Physicians rely heavily upon laboratory test results before making decisions.

It is obvious that laboratory results must be reliable for proper decision-making. Good technique and an understanding of the chemical processes are prerequisites for the production of high-quality analytical work. Clinical chemistry, however, involves more than analysis of body fluids and tissues.

Clinical chemistry has been defined as the study of the chemical aspects of human life in health and illness and the application of chemical laboratory methods to diagnosis, control of treatment, and prevention of disease.[1] Thus, clinical chemistry is a fundamental science when it seeks to understand the physiologic and biochemical processes operant both in the normal state and in disease; it is an applied science when analyses are performed on body fluids or tissue specimens to provide useful information for the diagnosis or treatment of disorders. In some countries, the term "clinical biochemistry" is reserved for the fundamental science and "clinical chemistry" for the applied science, but in the United States, the latter term usually covers both categories.

Some elementary biology is presented for student orientation because a knowledge of the working of the human body under healthy, normal conditions is essential for the understanding of changes that may occur in abnormal or pathologic states. An introductory text in biochemistry[2,3] or biology[4] is recommended for those who have not had courses in biochemistry or molecular biology.

CELLS

The basic unit of the body is the cell, the place where most of the body's chemical reactions occur. The cell has an outer membrane that separates it from other cells and from the tissue fluids that bathe them. A mammalian cell is illustrated in Figure 1.1.

Composition

Mammalian cells have a well-defined nucleus that contains the genetic material (DNA) distributed among chromosomes, and many organized structures or compartments (organelles) in the cytoplasm, in which many different functions and chemical reactions take place. Several of these (mitochondria, vacuoles, endoplasmic reticulum) are illustrated in Figure 1.1. The main oxidative

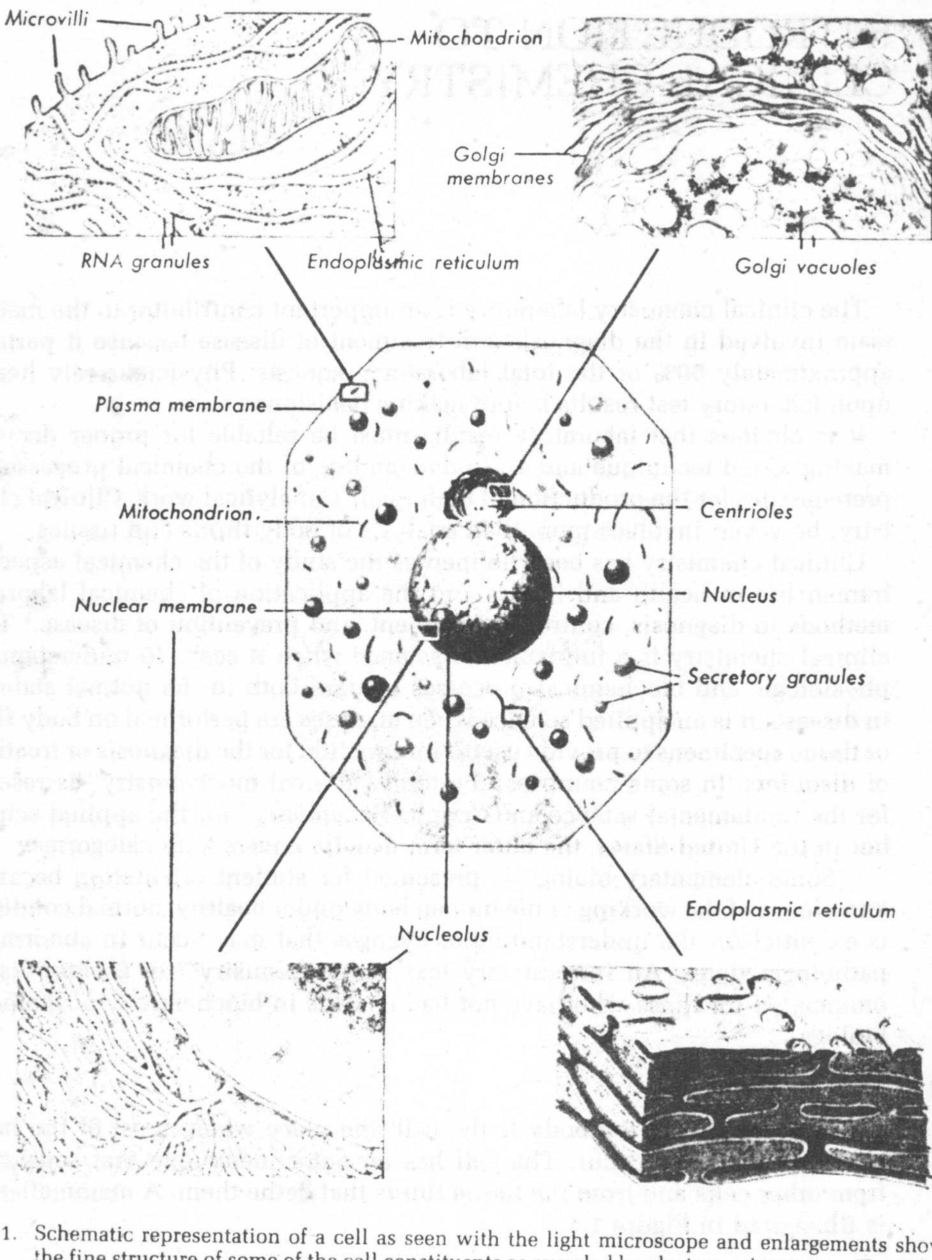

Fig. 1.1. Schematic representation of a cell as seen with the light microscope and enlargements showing the fine structure of some of the cell constituents as revealed by electron microscopy. (From Gray's Anatomy, 29th ed. Philadelphia, Lea & Febiger, 1973.)

reactions for the production of energy occur in the mitochondria; protein synthesis takes place on ribosomes; and many enzymes are located on the endoplasmic reticulum.

The outer cell membrane is composed of a mosaic of a double layer of lipid molecules interspersed with protein molecules and constitutes a means for selective permeability. Some ions and small molecules are able to pass easily into or out of the cell; others require special transport mechanisms for entry or exit while some cannot cross the membrane of the living cell. Special molecules (glycoproteins) on the membrane act as recognition points or receptors for the attachment of particular substances, such as hormones or antibodies, that are present in the circulating blood plasma.

The chemical constituents of cells are simple and complex proteins, nucleoproteins, carbohydrates, lipids, intermediates of these compounds, inorganic salts, and water. The organization within specific cells may vary depending upon the cell's structure and function. The formation, growth, or functioning of cells requires the presence of appropriate raw materials and enzymes and a readily available supply of energy.

Malfunction

Malfunction of a cell may be caused by a variety of factors, such as (1) destruction by trauma or by invasive agents, which include pathogenic microorganisms, viruses, toxins; (2) genetic deficiency of a vital enzyme; (3) insufficient supply of one or more essential nutrients (amino acids, vitamins, or minerals, for example); (4) insufficient blood supply; (5) insufficient oxygen supply; (6) malignancy (uncontrolled tissue growth); (7) accumulation of waste products; (8) failure of a control system; or (9) by a defect in the cellular recognition of certain signals.

ROLE OF THE CLINICAL CHEMISTRY LABORATORY

The major efforts of the clinical chemistry laboratory are devoted to measuring the concentration of various constituents in body fluids (primarily serum but also urine, cerebrospinal fluid, or other fluids). However, it is not easy to predict what is happening at the cellular level when the concentration of a serum constituent is abnormally high or low; additional information is needed. An elevated serum constituent concentration could be caused by excessive intake, excessive body synthesis, excessive breakdown of cells, deficient utilization, deficient excretion, or severe dehydration. The reverse is true for a low concentration. Information derived from the physical examination and the patient's history helps to elucidate the problem, but additional selected tests may be necessary. Gross abnormalities always indicate that something is wrong. It is difficult to make a diagnosis early in a disease, when symptoms are obscure or absent and changes in blood constituent concentrations are minimal. When concentration changes are small, the validity of the test result may be questioned. This is why precision and quality control are so necessary; your contributions are essential for good patient care.

The material in the following chapters provides information on both phases of clinical chemistry: (1) the basic information needed for performing accurate and precise analyses and (2) an explanation of the physiologic and biochemical processes occurring in the body for the major constituents that we analyze. The