CLINICAL CHEMISTRY:

Interpretation and Techniques

Alex Kaplan, Ph.D.
Head, Clinical Chemistry Division
Professor, Department of Laboratory Medicine
University of Washington, Seattle, Washington

and

LaVerne L. Szabo, M.S.
Assistant Professor, Clinical Chemistry and
Medical Technology Divisions
Department of Laboratory Medicine
University of Washington,
Seattle, Washington

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PREFACE

The primary purpose of this text is to provide a teaching manual with clear and explicit directions to those who work, supervise, or teach in clinical chemistry and to provide sufficient background and theoretical material so that the work in the laboratory becomes understandable as well as accurate and precise.

In general, the 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.

A section on general principles of chemical analysis is included for the purpose of review and to encourage accurate, precise, and intelligent work in the clinical chemistry laboratory. This section also deals with primary and secondary standards and their preparation and use in the laboratory. Although some laboratories are compelled to rely upon commercial assistance in the preparation of pure chemicals, special enzymes, and even premixed reagents, it should be within the capability of clinical chemistry workers to check independently the reliability and accuracy of their own results.

A section on laboratory quality control and statistics has been included to emphasize 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 mag-

nitude in order to assess the validity of test results or to evaluate the usefulness of procedural changes. The theory of photometric and gasometric measurements is presented so that there is adequate understanding of those commonly used instruments.

A hospital is a complex institution, and there are many links in the chain before a sample of a patient's fluid is correctly analyzed and the report is received by the person who ordered it. When a physician requests a particular blood test on a specific patient, a nurse or clerk transcribes the request upon a laboratory request form. A member of a blood-collecting team usually obtains a blood specimen from the patient, whereupon it is brought to the laboratory, given an acquisition number, and is processed and analyzed. After the measurement of a particular constituent has been completed, the result must be 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. For this reason a section on the collection and preservation of samples is included.

A general section on automation is included without going into the specifics of the various automated instruments. Laboratory needs vary so much and the instrumentation field is changing so rapidly that it would be impossible to describe one or two instruments that would be adequate for all laboratories. Only the basic approaches are mentioned, since laboratory workers will have to learn the operation of the particular instruments that are in use in the laboratories in which they are employed. The chemical and analytical principles are essentially the same as for methods performed manually.

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 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 requirement means that the technologists must have a good basic understanding of clinical chemistry and analytical chemistry.

Usually a relatively heavy workload is required to justify economically the purchase of an automated system, although sometimes an improvement in service may be the deciding factor. In any event, many small hospitals and small laboratories have elected to forego the use of automation. When automated instruments

are present, there always has to be a backup system (usually by manual methodology) to be used in case of failure of the instruments or, upon occasion, to carry out individual or emergency tests in a hurry. For these reasons, emphasis in this book is placed upon the performance of analyses manually and understanding the chemical principles involved. The need for understanding quality control and the precision limits of an analytical method applies equally well to both manual and automated methodology.

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 follow through, 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.

Seattle, Washington

Alex Kaplan, Ph.D.
LaVerne L. Szabo, M.S.

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A. K.
L. S.

CONTENTS

Chapter 1

INTRODUCTION TO CLINICAL CHEMISTRY

The International Federation of Clinical Chemistry has tentatively proposed that "clinical chemistry encompasses 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 various body fluids or tissue specimens in order to provide information of clinical value in the diagnosis and treatment of various disorders. In some countries the term *clinical biochemistry* is reserved for the fundamental science and *clinical chemistry* for the applied, but in the United States the latter term is used indiscriminately for both categories.

Because a knowledge of the working of the human body under healthy or normal conditions is essential for the understanding of changes that may occur in abnormal or pathologic states, an abbreviated overview of some of the major components involved in metabolism and growth is provided in this chapter. A discussion of the common tests performed in a clinical chemistry laboratory and the technical details of their performance will follow in later chapters.

CELLS

The basic unit of life is the cell. The cell has an outer membrane which separates it from the external environment in the case of single-celled organisms and from other cells for multicelled organisms. A mammalian cell is illustrated in Figure 1.1.

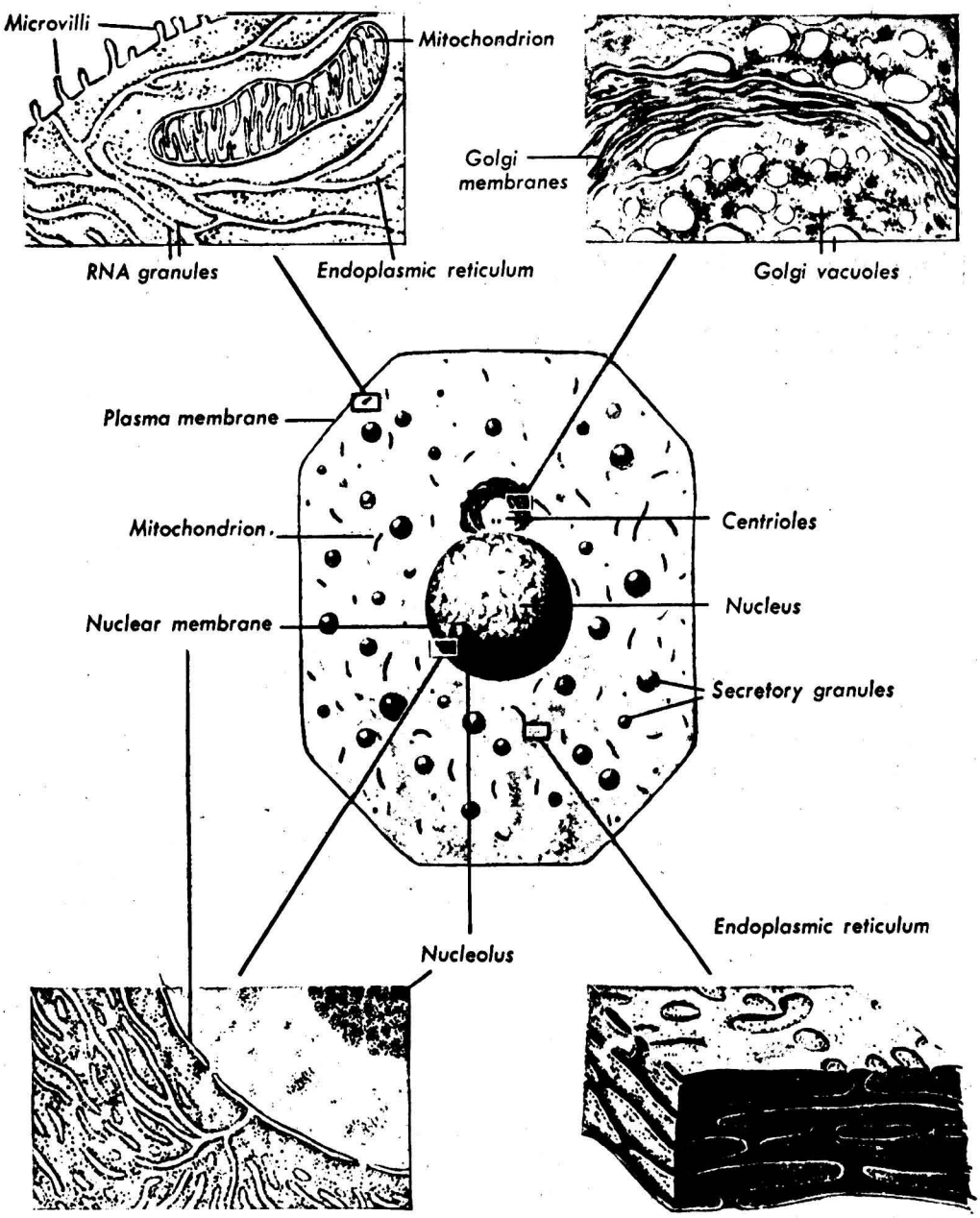


Fig. 1.1. Schematic representation of a cell as seen with the light microscope and enlargements showing the fine structure of some of the cell constituents as revealed by electron microscopy. (From Gray's Anatomy, 29th ed. Philadelphia, Lea & Febiger, 1973.)

The cells from multicelled creatures have certain characteristics in common: a well-defined nucleus which contains the genetic material distributed among chromosomes, and many organized structures or compartments (organelles) in the cytoplasm, separated by membranes, in which many different functions and chemical reactions take place. Several of these (mitochondria, vacuoles, endoplasmic reticulum) are illustrated in Figure 1.1. For example, the main oxidative reactions for the production of energy occur in the mitochondria. The outer cell membrane is usually composed of a double layer of lipids surrounding a protein layer and constitutes a means for selective permeability; some types of molecules are able to pass easily into or out of the cell, but passage to others is restricted.

The chemical constituents of cells are 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 growth of cells or the formation of new ones requires the presence of appropriate raw materials and enzymes and a readily available supply of energy for building the cells and for carrying out their functions. Some of the main features in the utilization of these cellular components will be considered below in order to provide a better understanding of the chemical processes essential for life and growth.

MACROMOLECULES

Protoplasm is characterized by the presence of very large molecules *(macromolecules)* which are *polymers* or chains of much simpler and smaller molecules linked together. The main classes of these biopolymers are *nucleic acids, proteins,* and *polysaccharides*. These polymers are formed by the successive linkage or *condensation* of smaller molecules accompanied by the splitting out of water. Although primarily linear upon formation, a polymeric chain may be folded, spiral-shaped, or globular, depending upon the composition of and charges in the component units of the chain. This variation holds particularly true for the proteins which may assume unique shapes or structures because of the interaction between the various amino acids composing the chain. The macromolecules are utilized in the structure of cells or for the performance of specific chemical functions.

Nucleoproteins and Nucleic Acids

All living cells contain *nucleoproteins*, which combine two different types of polymers, nucleic acid and protein. The protein portion is usually present as an outer coat partially covering the nucleic acid core. The *cell nucleus* is composed primarily of nucleoproteins, but there is some nucleoprotein in the *cytoplasm* as well. The *chromatin material* of cells contains DNA, the storage place for hereditary information, linked to a basic protein. The diffuse chromatin becomes organized into chromosomes immediately before cell division.

The nucleic acids are polymers of three primary constituents: (1) a base (purine or pyrimidine), (2) a pentose sugar (5-carbon sugar, either deoxyribose or ribose), and (3) phosphoric acid. The condensation of purine or pyrimidine base with one molecule of pentose followed by condensation of the attached pentose with one molecule of phosphate yields a *nucleotide*, the fundamental

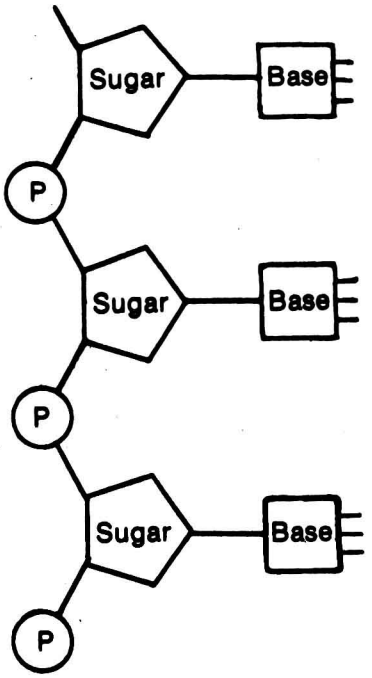


Fig. 1.2. Schematic representation of a portion of a DNA chain, showing the linkage of the nucleotide groups. Each nucleotide group (base-sugar-phosphate) is connected to the one above it by condensation of its phosphate with a hydroxyl group of the upper sugar (deoxyribose). The base may be adenine, guanine, cytosine, or thymine. P signifies phosphate, and *sugar* stands for deoxyribose.

component or building block of nucleic acids. The backbone of the nucleic acid polymeric chain consists of the successive linkage of a phosphate group to a sugar group to a phosphate group, and so on as the chain assumes a spiral shape. Figure 1.2 illustrates the general configuration, showing how the pentose sugar is bound to each of two phosphate groups as well as being the point of attachment for one base. Nucleic acids containing the pentose, *deoxyribose*, are *deoxyribonucleic acids* and are abbreviated *DNA*. Those containing ribose are called *ribonucleic acids* or *RNA*. The two purine bases found in all nucleic acids, both in DNA and RNA, are *adenine* and *guanine*. The two pyrimidine bases of DNA are *thymine* and *cytosine*; those of RNA are *uracil* and *cytosine*. DNA and RNA are synthesized in the cells from simple precursors or from preformed purines and pyrimidines that are derived from ingested nucleoproteins in the diet.

It is not the purpose of this book to go into the complex biochemistry involved in the formation and degradation of DNA and RNA nor to discuss the genetic code and its transmission. Suffice it to say that the genetic information directing the synthesis of all proteins formed in the body is contained in the DNA molecules in code form. The code depends upon the particular sequence of bases in the DNA molecule. Each gene, which is only a portion of the double strand of DNA, contains the information for the synthesis of a particular

protein, many of which are enzymes or catalysts. There are estimates that each human cell contains the information for synthesizing as many as seven million different proteins, although far fewer are actually built. The partial protein coat of the DNA is one of the devices for controlling which of the various coded messages for protein shall be read, since only those uncoated portions of the DNA strand are available for transcription.

The information contained in the DNA is transcribed to an RNA molecule, using the DNA as a template. The message is transported to the cytoplasm where the synthetic machinery of the *ribosomes* (large, complicated organelles) translates the message and synthesizes the particular protein called for by the code.

A change in one or more of the bases in the long DNA polymer comprising a gene causes the formation of an altered protein. These changes in the coded message are called *mutations*, most of which result in varying degrees of impaired function and some of which may even be lethal. Thus, many of the genetic diseases encountered in the population arise from a combination of genes that carry the message for the formation of altered proteins, proteins that fail to do their job and that consequently handicap the recipient individual.

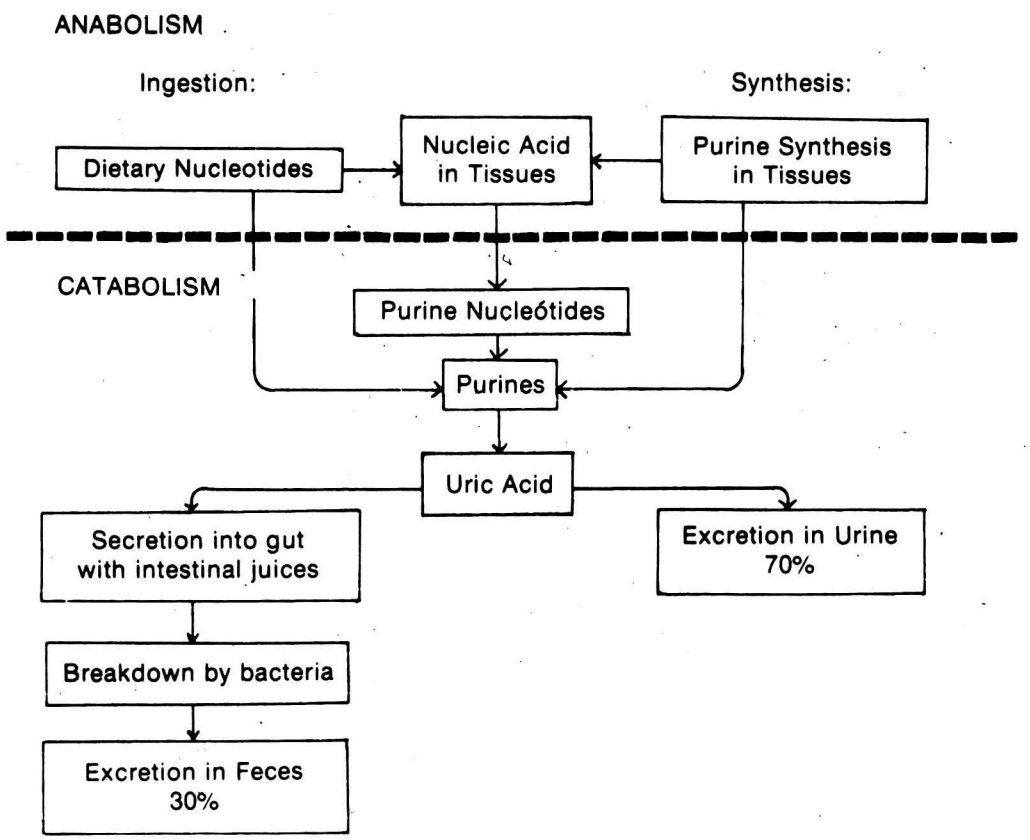


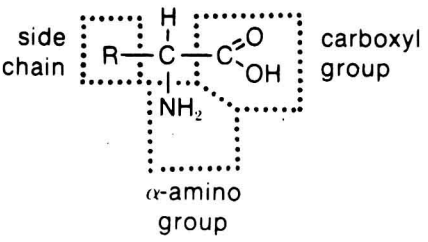
Fig. 1.3. Schematic representation of uric acid formation and excretion.

Other "goofs of nature," such as a mistake in proper transcription of the code for a particular protein, or interchanging part of one DNA chain for another, can also produce disastrous effects in a newborn individual.

Degradation of DNA and RNA takes place with the death of cells and the disintegration of cell nuclei. The phosphate esters are hydrolyzed and reutilized by the body. The sugar moieties can be utilized for energy purposes. The pyrimidines are degraded by enzymes that open the ring structure, an event which leaves the open chain susceptible to further breakdown. The purines in humans, however, are converted by enzymes to the compound *uric acid*, regardless of whether the original nucleotide contained adenine or guanine. There is no enzyme in the human body that can break or open the purine ring, so the waste product, uric acid, is excreted into the urine. As will be discussed later, abnormalities arising from either increased synthesis or decreased excretion of uric acid lead to the formation of a painful disease known as *gout*. The formation of uric acid is shown diagrammatically in Figure 1.3. The measurement of the concentration of uric acid in serum is a commonly performed clinical chemistry test.

Proteins

The second great class of polymers, the proteins, are made up of *polypeptide chains* of *amino acids* linked together by *peptide bonds*. There are some 20 naturally occurring amino acids, of which 12 are synthesized by the body and the remaining 8 must be provided in the diet of humans. The structure of a typical amino acid appears in Figure 1.4. The features common to all amino acids are a terminal carboxyl group (–COOH) and an adjacent amino group ($-NH_2$), although the rest of the structure may vary. Amino acids are linked together by an enzymatic reaction which condenses the $-NH_2$ group of one amino acid with the carboxyl group of another amino acid, as shown in Figure 1.5. A protein may consist of one or more interwined polypeptide chains, some of which may contain 100 or more amino acids. For example, hemoglobin, the



Amino Acid	Side Chain, R
Alanine	CH_3-
Leucine	$(CH_3)_2CHCH_2-$
Tyrosine	$HO-C_6H_4-CH_2-$
Glutamic	$HOOCCH_2CH_2-$

Fig. 1.4. Illustration of the general structure of amino acids. All amino acids contain the terminal carboxyl and adjacent amino group. Some of the common side chains are shown.