Radionuclides in Clinical Chemistry

Phillip L. Howard, M.D.
Associate Professor, Department of Pathology,
The University of Vermont College of Medicine,
Burlington

Thomas D. Trainer, M.D.
Professor, Department of Pathology,
The University of Vermont College of Medicine,
Burlington

With a contribution by
Bruce R. MacPherson, M.D.
Associate Professor, Department of Pathology,
The University of Vermont College of Medicine,
Burlington

Little, Brown and Company Boston

First Edition

Library of Congress Catalog Card No. 80-80587

ISBN 0-316-37470-9

Printed in the United States of America

MV

Series in Laboratory Medicine

Leo P. Cawley, M.D., Series Editor

Clinical Laboratory Statistics, Second Edition
Roy N. Barnett, M.D.

Laboratory Diagnostic Procedures in the Rheumatic Diseases, Second Edition
Alan S. Cohen, M.D.

Renal Functions Tests:
Clinical Laboratory Procedures and Diagnosis
Cristobal G. Duarte, M.D.

Radionuclides in Clinical Chemistry
Phillip L. Howard, M.D., Thomas D. Trainer, M.D.

Practical Blood Transfusion, Second Edition
Douglas W. Huestis, M.D., Joseph R. Bove, M.D.,
Shirley Busch, M.P.H., SBB (ASCP)

Exfoliative Cytopathology, Second Edition
Zuher M. Naib, M.D.

Immunopathology: Clinical Laboratory Concepts and Methods
Robert M. Nakamura, M.D.

Laboratory Management
Jack E. Newell, M.D.

The Diagnosis of Bleeding Disorders, Second Edition
Charles A. Owen, Jr., M.D., Ph.D., E. J. Walter Bowie, M.A., BM., F.A.C.P.,
John H. Thompson, Jr., Ph.D.

Urinary Cytology
Duane N. Tweeddale, M.D.

Serum Protein Abnormalities: Diagnostic and Clinical Aspects
Stephan E. Ritzmann, M.D., Jerry C. Daniels, M.D., Ph.D.

Histopathology of the Bone Marrow
Arkadi M. Rywlin, M.D.

Laboratory Procedures in Clinical Microbiology
John A. Washington II, M.D.

Radionuclides in Clinical Chemistry

Preface

The current issues of clinical chemistry journals are filled with articles on improvements or alterations in existing radioligand procedures. So rapid is the development of new information that even the recent journals themselves cannot keep up with the state of the art. When the idea for this book was formulated, a decision was made not to devote space to specific assay procedures. By publication such information would have been hopelessly out of date. Rather, there exists a core of information about the application of radionuclides in clinical chemistry that might be considered basic or fundamental, or at least to have a half-life exceeding that of specific assay methods. That core material includes some basic physics, instrumentation, counting statistics, assay kinetics, aspects of separation techniques, and antibody production, among other subjects. Even so, as this volume is in press, it is evident that automation will probably be the next major quantum jump in radioligand assays. Several fully automated systems have appeared recently, and others now existing only in prototype form will be on the market within a few months.

Reviews of portions of the manuscript by Jackson J. Clemmons, Ph.D., M.D., and Clinton D. Janney, Ph.D., were exceedingly helpful. Chapter 6 by Bruce R. MacPherson, M.D., introducing his expertise in immunology and antibody production, is gratefully acknowledged. We owe the greatest debt to Mrs. Barbara Gianni, the inexhaustibly patient secretary who typed this manuscript. She was never perturbed by last-minute changes or alterations in already completed material.

P. L. H.
T. D. T.

Contents

1. Basic Physics

Utilization of radionuclides in the clinical laboratory requires neither a detailed study of nuclear physics nor the use of much mathematics. Understanding a few basic concepts, however, is necessary for intelligent equipment use and for performing assays. The various interactions of radiation with matter form the basis for the detection of radioactivity by laboratory instruments. The different modes of decay affect the type of instrumentation used. Highly penetrating gamma rays can be detected by solid state detectors, whereas low-energy beta particles require specialized techniques for detection in the laboratory. A knowledge of radiation penetration into tissues and the ionizations produced forms the foundation for understanding some principles of radiation safety. Most of these physical processes can be described adequately in simple diagrams.

The Atom

The atom consists of a positively charged core or *nucleus* of enormous density surrounded by a cloud of *electrons*.. It can be calculated that its nuclear density exceeds 200,000 tons/mm^3, and that almost the entire mass of the atom is contributed by the nucleus. The remainder of the atom is primarily empty space in which electrons at various energy levels and distances from the nucleus are found. To emphasize the space and mass relationships of a simple hydrogen atom, imagine that a baseball represents the nucleus, and the orbiting electron is a styrofoam ball of the same diameter located about 2 miles from the nucleus. The actual electron orbits are quite hazy and can be conceptualized better as regions of negative charge density around the nucleus. The chemical properties of an atom are derived from the electrons, which in the nonionized state equal the number of protons.

The nucleus consists of positively charged *protons* and uncharged *neutrons*, collectively called *nucleons*. There is increasing evidence that nucleons themselves are composed of even smaller particles called *quarks*. An atom designated by the number and type of nucleons in the nucleus is called a *nuclide*. More than a thousand nuclides exist and a standard format is used to characterize them. The elemental or chemical symbol, such as *C* for carbon, identifies the atom. In the following example the letter X is a general symbol for any element. Total atomic mass (A) is indicated by a superscript on the left. The proton or atomic number (Z) is indicated by a subscript on the left and the neutron number (N) by a subscript on the right.

$${}^{A}_{Z}X_{N}$$

The most abundant form of carbon has an atomic mass of 12 and equal numbers of protons and neutrons. The neutron number can vary without changing an atom's elemental identity. Thus hydrogen nuclides exist with neutron numbers ranging

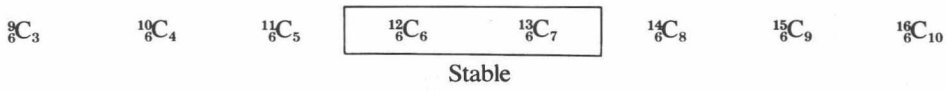

Figure 1-1
Isotopes of carbon.

from 0 to 2. Those nuclides behave chemically like hydrogen since all have one proton and therefore one orbital electron. Groups of nuclides with the same atomic number and different neutron numbers are called *isotopes*. Nuclides also exist with the same neutron number but different atomic numbers. Those nuclides are called *isotones*. Because they have different atomic numbers, the chemical behavior of different isotones is dissimilar.

Nuclear Stability

All possible nuclides do not exist in nature. Many are produced artificially by nuclear reactions. Even more important for our purposes is that some nuclides are unstable. Eight carbon nuclides exist with atomic masses from 9 to 16 (Fig. 1–1). Of those, only the two nuclides with masses of 12 and 13 are stable. The carbon nuclide with a mass number of 14 is a naturally occurring unstable nuclide that is useful in the laboratory. The remaining carbon isotopes are unstable products of nuclear reactions. The exact reasons for the stability of some nuclides and the instability of others are uncertain, but stability appears to be related to the proper balance of several forces within the nucleus.

Holding the nucleus together is a short-range attractive force with equal affinity for both protons and neutrons. Many questions remain unanswered about this nuclear force. Counteracting it, the positive electric charges on protons, when packed into the small volume occupied by the nucleus, create an enormous electrostatic repelling force within the nucleus that tends to separate the protons and split off parts of the nucleus. Stable nuclides are those in which the nuclear attractive force and the electrical repelling force are balanced. Thus, not all combinations of neutrons and protons result in stable nuclei. For nuclides of low atomic number, equal numbers of protons and neutrons seem to give the greatest stability. As the proton number increases, however, stability is maintained only by the presence of a greater number of neutrons, which may be considered to act as a nuclear "glue." Therefore, for stable nuclides of high atomic number, the neutron number is considerably higher than the atomic number.

It is useful to make a chart of the stable nuclides. Figure 1–2 shows that, initially, equal numbers of protons and neutrons result in stability, and the line assumes a 45-degree slope. Eventually the line of stability starts to curve in a direction favoring increased numbers of neutrons. Nuclides with a neutron-proton ratio falling too far on either side of the line of stability are unstable. Spontaneous nuclear changes

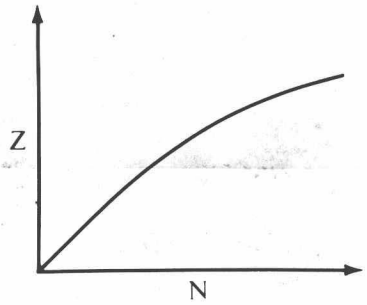

Figure 1-2
The line of nuclear stability. Neutrons (N) are plotted along the abscissa and protons (Z) along the ordinate. Only nuclides with neutron-proton ratios on or near the line are stable. As the number of protons increases, an excess of neutrons over protons is necessary to maintain nuclear stability.

will occur and tend to shift the neutron-proton ratio toward the line of stability. Radioactivity results from those nuclear changes.

Energy, Waves, and Particles

The unit of measure for the energy of radioactive decay is the *electron volt,* that is, the energy gained by an electron as it passes through a potential difference of 1 volt. The electron volt is equivalent to 1.6020×10^{-12} erg. The electron volt is a more convenient unit to use for the energy of subatomic particles than the erg. Larger multiple units of the electron volt are kilo-electron volt (kev) for thousand, mega-electron volt (mev) for million, and billion electron volt (bev) for billion. In the United Kingdom giga-electron volt (gev) is used instead of bev.

Physicists divide the world of the atom into several levels. Many familiar events of everyday life reflect occurrences at the atomic and molecular levels. Most reactions in chemistry and biology are explained at the atomic level. The energies involved in chemical reactions are relatively low and seldom exceed 1,000 ev. Events at the nuclear level account for such processes as nuclear fission, fusion, and radioactivity. The energy levels of nuclear reactions range into millions of electron volts. Finally, subnuclear events involve billions of electron volts and touch on events that may concern the very origin of the universe. In subatomic events the duality of the wave and particulate properties of matter is far more apparent than it is in familiar everyday events. Thus, although light, x-rays, and gamma rays can be shown to behave as electromagnetic waves in some experiments, other experiments reveal that they exhibit particle-like behavior. ("Particles" of light are called *photons.*) Nevertheless, electrons and other subatomic particles clearly demonstrate wavelike properties. The apparent conflict of events in the subatomic world with our direct daily experiences gives those events a

mystical aura. It should be emphasized also that there is no fundamental difference between gamma rays, x-rays, ultraviolet light, visible light, infrared light, and radio and television waves. All are forms of electromagnetic radiations that differ only in wavelength and frequency and, consequently, have different energy levels. The energy of electromagnetic radiation is related to the frequency by

$$E = hf \tag{1–1}$$

where E is the energy, h is Planck's constant, and f is the frequency. Short-wavelength, high-frequency gamma rays have more energy than long-wavelength, low-frequency infrared light. Some arbitrary distinctions are made, in that electromagnetic radiations originating from a nucleus are called gamma rays, whereas those from deexcitation of orbital electrons are called x-rays, or light rays.

The interconvertability of matter and energy is apparent according to the well-known equation

$$E = mc^2 \tag{1–2}$$

where E is energy, m is the mass of the particle, and c is the speed of light. Thus the electron can be said to have a rest mass equivalent to 0.51 mev of energy. Several common interactions involve the transformation of electrons into electromagnetic radiation and the transformation of electromagnetic radiation into electrons.

Radiation

Negatron (Beta Minus) Decay

Electrons ejected from the atomic nucleus are called *beta particles*. Beta particles may carry either a negative or positive electric charge. Negatively charged beta particles, called *negatrons,* are no different from ordinary electrons. Conventional use of the term *beta particle* with no further clarification implies the negatively charged electron. One of the most common beta-decaying nuclides used in the clinical laboratory is tritium, an isotope of hydrogen. Most hydrogen atoms consist of a single proton occupying the position of the nucleus. A second stable isotope of hydrogen, deuterium, has a nucleus composed of one proton and one neutron. However, the nucleus of tritium has two neutrons in addition to the single proton and that arrangement results in an unstable balance of nuclear forces. Since tritium nuclei have excess neutrons, those nuclides are located to the right and below the line of stability (see Fig. 1–2). Tritium will return to the line of stability by a process that reduces the number of neutrons or increases the number of protons in the nucleus. The reaction is

$$n \rightarrow p^+ + e^- + \bar{\nu}_e \tag{1–3}$$

A neutron (n) decays into a proton (p^+), an electron (e^-), and another particle called an *antineutrino* ($\bar{\nu}_e$). The reaction can be shown for tritium

$$^{3}_{1}H_2 \rightarrow {}^{3}_{2}He_1 + e^- + \bar{\nu}_e \qquad (1\text{–}4)$$

The electron and the antineutrino are ejected from the nucleus with variable amounts of kinetic energy, and the ejected electron is a beta particle. The conversion of the neutron into a proton changes the elemental identity of the nuclide into a stable isotope of helium. The atomic weight remains unchanged. The net result of the nuclear conversion is that the nuclide is shifted into the line of stability. Many other nuclides that have excess neutrons also decay by beta emission. For example, carbon-14 decays by beta emission to nitrogen-14.

$$^{14}_{6}C_8 \rightarrow {}^{14}_{7}N_7 + e^- + \bar{\nu}_e \qquad (1\text{–}5)$$

Tritium is 0.018 mev above the stable ground state and with every decay of a tritium atom, 0.018 mev of energy must be dissipated and divided between the electron and the antineutrino. The distribution of energy between those two particles can vary, so either particle can carry away almost all or a variable fraction of that energy. A typical beta spectrum is shown in Fig. 1–3. Only a few electrons carry the full energy released as a result of beta decay. In fact, most electrons will retain about one-third of their maximum possible energy. The energy difference between the beta particle and the maximum energy of tritium is carried by the antineutrino.

The antineutrino cannot be detected by current laboratory instruments. As we will see, the antineutrino is necessary only for conservation of energy and is otherwise of little importance in clinical laboratory work. Both the neutrino and the antineutrino are, however, fascinating particles in their own right. The word *neutrino* is the Italian diminutive meaning "little neutron," because the neutrino has no charge and essentially no rest mass. Several conservation laws require that an antineutrino, and not a neutrino, accompany each emission of a beta particle. The antineutrino is a form of so-called *antimatter*. Antimatter particles are virtually identical to ordinary particles except for their charge or spin. Antimatter particles are designated in the notation of physics by placing a horizontal bar above the usual symbol of the particle. Isaac Asimov has written a book on the history of the neutrino, which the interested reader should consult.

Positron (Beta Plus) Decay

Positrons might be called antielectrons since they are the antimatter particle for electrons. At least 1.02 mev of energy must be available before positron decay can occur. Positron emission is essentially the reverse of electron emission. Nuclides

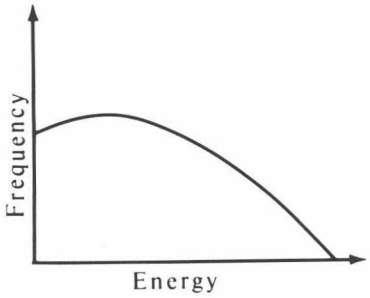

Figure 1-3
Spectrum of typical beta decay. The energy of the beta particles is plotted along the abscissa and the frequency of emissions of beta particles along the ordinate.

with excess protons are found above and to the left of the line of stability (Fig. 1–2), and decay occurs by conversion of a proton (p^+) into a neutron (n), a positron ($\bar{e}^+$), and a neutrino (ν_e).

$$p^+ \rightarrow \bar{e}^+ + \nu_e \tag{1–6}$$

Carbon nuclides of atomic weights 9, 10, and 11 all decay by positron emission. For ^{11}C the reaction is

$$^{11}_{6}C_5 \rightarrow {}^{11}_{5}B_6 + \bar{e}^+ + \nu_e \tag{1–7}$$

The spectrum of positron emission is virtually identical to that of negative beta decay except that the spectral curve is shifted slightly to the right. The shift of the spectrum of positron emission toward slightly higher energies is probably due to the added repulsion of the positron by the positively charged nucleus.

Positrons survive for a relatively short time since they rapidly annihilate an electron of opposite charge. The dual annihilation of a positron and an electron results in the conversion of the mass of both particles into electromagnetic energy called *annihilation radiation*. Annihilation radiation consists of two electromagnetic rays of 0.51 mev that travel away from the point of annihilation in exactly opposite directions. The annihilation reaction is favored for positrons of low kinetic energies. If a positron of higher kinetic energy should annihilate with an electron, the annihilation radiation is slightly greater because of the additional kinetic energy available in excess of the energy of the rest mass of the two particles.

Although nuclides that decay by positron emission currently are not used in the clinical laboratory, positrons and positron annihilation can affect the gamma-ray spectrum. Those effects are discussed in Chap. 2.

Electron Capture

Electron capture is another method by which nuclides with excess protons can return to the line of stability. The basic reaction is

$$p^+ + e^- \rightarrow n + \nu_e \qquad (1\text{–}8)$$

An electron is essentially removed from orbit and brought into the nucleus, where it converts a proton into a neutron and a neutrino. Electron capture is sometimes called K-capture since the lower energy of K-level electrons are captured preferentially. It is also possible for higher energy L- and M-level electrons to undergo electron capture.

When the energy available is less than 1.02 mev, electron capture occurs alone. However, when 1.02 mev of energy is available, positron emission competes with electron capture as a mode of decay, and the same nuclide may decay alternately by both mechanisms. The higher the energy level above 1.02 mev, the more positron emission is favored.

It should be apparent that electron capture alone does not result in detectable radioactivity. Although pure electron capture does occur, it is relatively uncommon. Three processes can give rise to radioactivity subsequent to electron capture.

1. Whenever an electron at one energy level is removed, an electron of higher energy drops to the lower energy state, thus filling the void. The transition of that electron to its new energy level is accompanied by the release of excess energy in the form of electromagnetic radiation. Photons originating from extranuclear deexcitation of electrons in this manner are called x-rays and differ from gamma rays only in terms of their source.
2. Following electron capture, the nucleons also are left in an excited state. When the nucleus returns to the stable ground state, that excess energy is released in the form of gamma rays. Thus both x-rays and gamma rays may be released following electron capture.
3. Finally, electrons also may be ejected as a result of these processes. If an x-ray encounters an orbital electron in its passage from the atom, it can transfer sufficient energy to the electron to eject it from the atom. That occurrence is called the *Auger effect;* the ejected electrons are called *Auger electrons.*

All those phenomena are illustrated in Fig. 1–4.

Isomeric Transition

The release of gamma rays from a nucleus does not in itself result in a change in the neutron-proton ratio of the nucleus. Following electron emission, positron emission, or electron capture, a nucleus is usually left in an excited state for a period of time before it decays by gamma emission. The nuclide is said to be *metastable*

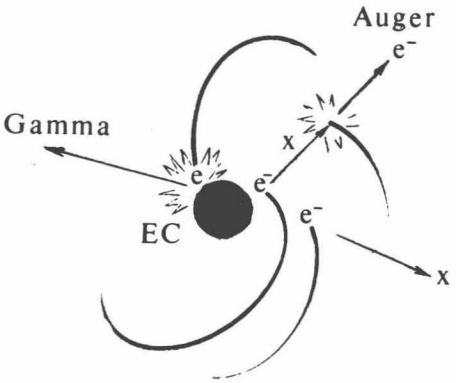

Figure 1-4
Electron capture (EC) is usually accompanied by the release of gamma rays from the excited nucleus. As electrons fall to lower energy levels, x-rays are released. Interactions between the x-rays and orbital electrons can remove those electrons from orbit.

during that period. The emission of the gamma ray that follows allows a transition of the nucleus to a stable state, called *isomeric transition* because no further change occurs in the neutron-proton ratio.

Isomeric transition can also be accomplished by *internal conversion,* i.e., the excited nucleus transfers energy to an electron, which is then ejected from the atom. That electron is called a *conversion electron.* Conversion electrons differ from Auger electrons in that conversion electrons are ejected by a gamma ray rather than an x-ray. Both Auger electrons and conversion electrons should be distinguished from *photoelectrons* and *Compton electrons,* which are also electrons that have been ejected from orbit. The latter two events, however, occur in stable nuclides as a result of extrinsic radiation and will be discussed later.

Gamma ray emission and internal conversion are therefore competing processes in isomeric transition. The *conversion coefficient* indicates the tendency of a nuclide to decay by ejection of conversion electrons compared to its tendency to decay by gamma emission and is expressed by

$$\alpha = \frac{Ne}{N\gamma} \tag{1–9}$$

where α is the conversion coefficient, Ne is the number of conversion electrons, and $N\gamma$ the number of gamma rays emitted over the same period of time.

Alpha Decay

Heavy nuclides with large numbers of neutrons and protons can decay as a result of the ejection of a relatively large particle consisting of two neutrons and two

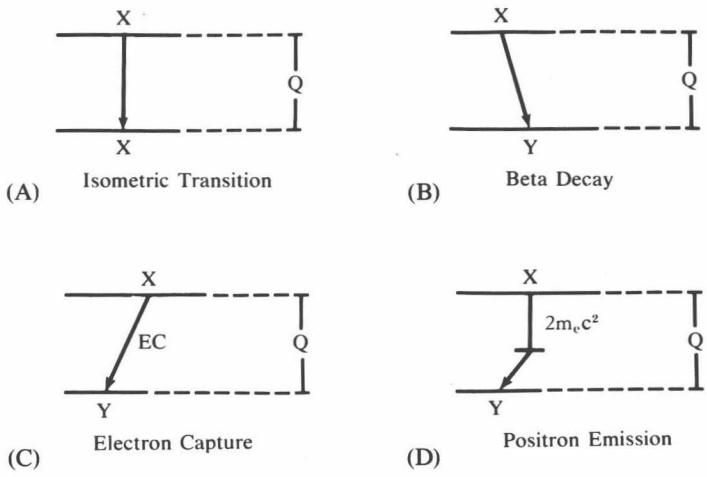

Figure 1-5
Types of radionuclide decay: A. isomeric transition. B. beta decay. C. electron capture. D. positron emission. Q = the quantity of the change in energy between the nuclides.

protons. That particle is essentially the same as the nucleus of a stable isotope of helium, but it is called an *alpha particle*. Alpha particles have relatively low penetrance, but because of their large mass they can do a great deal of biological damage. At the present time, alpha-decaying nuclides are of no importance in the clinical laboratory and will not be discussed further.

Notation of Decay Schemes

Radioactivity results from instability within the atomic nucleus. Nuclides with excess neutrons decay by beta emission. Those with excess protons decay by positron emission or electron capture. In both cases isomeric transitions may occur and give rise to gamma rays. A standard format (Fig. 1–5) is used to indicate the types of radionuclide decay. Horizontal lines represent the energy levels of the nuclide. The bottom horizontal line often, but not always, represents the ground state. The directions of the arrows running between the horizontal lines indicate the different modes of decay based on whether the number of positrons in the nucleus increases or remains the same.

Isomeric transitions do not cause a change in the neutron-proton ratio of the nuclide and are indicated by a vertical arrow (Fig. 1–5A). An increase in nuclear protons, as occurs with beta decay, is indicated by directing the arrow toward the right (Fig. 1–5B). Electron capture, which results in a decreased number of protons in the nucleus, is shown by an arrow labeled EC and directed toward the left (Fig. 1–5C). Positron emission also reduces the number of nuclear protons and is