ISOTOPIC TRACERS IN BIOLOGY

by Martin D. Kamen

Third Edition

ISOTOPIC TRACERS IN BIOLOGY

An Introduction to Tracer Methodology

by
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PREFACE TO THIRD EDITION

When this book first appeared in 1947*, isotopic tracers were rare—even exotic—in biological laboratories. Hence, the first edition reported and commented on pioneer experiences. Now, the use of tracers is commonplace; so much so that the literature reporting experiments in all branches of biology has proliferated beyond the grasp of any one writer. The text in hand has been revised a number of times to accommodate new and relevant facts, but only one completely new edition has appeared since 1948. A third edition is long overdue.

The objectives of this third edition are the same as those defined in the first edition. The main task in preparing it has been to evaluate new material covering many aspects of a number of fields, some not even mentioned in the first edition. I am tempted to borrow the diplomatic phrase "agonizing re-appraisal" to describe the difficulties inescapable in such a process. Perhaps the most challenging of these was the problem of choosing which among the masses of new work to include. The deciding factor had to be the pedagogic value of the new material, rather than its novelty, in relation to the older material.

Three major changes have been made in this edition. First, the scope of the book has been extended to stable isotopes, as the new title indicates. As a result, the chapters on general methodology have been expanded. New chapters on the elements nitrogen and oxygen have been added. Numerous concrete examples illustrating work with both stable and radioactive tracers have been included; some of these are completely new and others are more detailed than they were in previous editions.

Second, the chapters on nuclear physics and chemistry (I–III) have been rewritten and modernized.

Third, two new chapters have been added. One of these (V) is a short discussion of practical matters which arise when investigators make the transition from book learning to the laboratory. The other chapter (VII) is an extension of an original chapter on biochemical applications. It is included to illustrate one of the most important uses of tracer procedures: their application, with other methods, to solve some old problems in which a kind of impasse had developed.

For the rest, I have adhered to my previous practice of selecting material with which I was personally familiar, whether through actual participation

* M. D. Kamen, "Radioactive Tracers in Biology," 1st ed., 1947; 2nd ed., 1951. Academic Press, New York.

(as in the reminiscences about the discovery of C^{14} given in Chapter X), or through acquaintance with investigators doing the researches described.

As in the second edition, I have included current estimates on tolerance amounts of ingested radioactive isotopes. I wish to emphasize that these estimates are nothing more than educated guesses, the bases for which are indicated either in the text or in the references cited.

I would like to add a note of thanks to my wife, Beka Doherty Kamen, who pulled together and edited the critical first draft. I am also heavily indebted to Mrs. Margot C. Bartsch who shepherded and proofread succeeding drafts, and who typed and edited the final draft. Finally, I must thank the critics of previous editions who made many helpful suggestions in their reviews. I have incorporated a number of these in the present edition.

Many colleagues were most generous in giving permission to use material from their books and articles. Acknowledgments are recorded at appropriate places in the text. I should mention in particular the cooperation I received from Dr. Michel Ter-Pogossian, Associate Professor at the Mallinckrodt Institute of Radiology, who made a number of original drawings and supplied unpublished material which was most helpful in preparation of the section on scintillator detectors in Chapter III.

St. Louis, Missouri
April, 1957

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CHAPTER I

ATOMIC NUCLEI, RADIOACTIVITY, AND THE PRODUCTION OF RADIOACTIVE ISOTOPES

1. INTRODUCTORY REMARKS

The application of tracer procedures to biological problems does not depend on detailed knowledge of the physical background of tracer methodology. However, most research workers and students who work with tracers want basic information about the nature of tracers and the fundamentals of atomic and nuclear physics. The introductory chapters of this book are written with this in mind. The following brief discussion of fact and theory about atoms and nuclei can be elaborated by reference to the bibliography included in the text.

2. GENERAL PROPERTIES OF NUCLEI

A. NUCLEAR STRUCTURE

All elements are made up of small ultimate units or *atoms*. Atoms contain a positively charged *nucleus* (radius $<10^{-12}$ cm.) which, although it comprises a very small fraction of the atomic volume (radius $\sim10^{-8}$ cm.), accounts for practically all the weight of the atom. Most of the atomic volume can be said to be relatively "empty," being occupied by negatively charged *electrons*, which are many thousandfold lighter than the nuclear particles. Nuclear matter, in other words, is enormously more dense than atomic matter; its density approximates 10^8 tons per cubic centimeter. Such fantastic densities imply forces of a type not encountered in common experience. It is necessary to suppose that enormous attractive forces spring into being when matter is compressed to form atomic nuclei.

Present knowledge holds that nuclei consist of particles called *neutrons* and *protons*, for which the collective term is *nucleons*. Nuclei are built up by addition of approximately equal quantities of neutrons and protons, beginning with the lightest nucleus, that of the ordinary hydrogen atom. The nucleus of hydrogen is nothing more than a single proton. Protons and neutrons differ in that protons carry a unit positive electric charge (4.8025×10^{-10} electrostatic unit). Further description of these particles must be deferred for a brief excursion into the theory of nuclear forces.

Experimental evidence available indicates that nuclear forces extend only over very short distances—distances, in fact, much less than those assigned to nuclear dimensions. It is assumed that there is a continuous formation of unstable, short-lived particles smaller than nucleons and that nuclear forces arise in some way from exchange of these particles between the nucleons. It is evident that simple Coulombic (electrostatic) forces cannot be involved because the neutron is not electrically charged. Furthermore, the electrostatic forces in the nucleus would be repulsive rather than attractive, because the protons are all positively charged and would cause the nuclei to fly apart.

From investigations of cosmic-ray particles it is known that nuclear particles of mass intermediate between electrons and protons exist. These are called *mesons*. The first to be discovered was found to have a mass equivalent to 210 electron masses and is now called the μ meson. It was suggested that exchange of these μ mesons between nucleons might account for nuclear forces. This idea was not supported by experiment, however, and was abandoned in favor of the idea that another type of meson, discovered later and called the π meson, is the actual particle involved. Its mass is equivalent to 275 electron masses, and it appears to have many other properties necessary to act as the exchange particle between nucleons. At present, the π meson, which occurs in uncharged, positively charged, and negatively charged forms, is central to most theories of nuclear structure. No adequate theory of nuclear forces has yet been developed.

B. Mass Number and Atomic Number

The number of protons in a nucleus is the *atomic number* of the element and is usually symbolized by Z. Values of Z range from 1 for hydrogen to 101 for the most recently discovered transuranic elements. Z is the integral nuclear positive charge. Its magnitude determines the number of negative electrons required to accord with the observed electrical neutrality of the atom.

The total number of nucleons (neutrons plus protons) in the nucleus is called the *mass number* and is usually symbolized by A. The value of A is always expressed as the whole number nearest the atomic weight of the atom under consideration. (For example, see Section 2-D.) Values for A range from 1 to 255.

The atomic number is usually written as a left subscript and the atomic mass number as a right superscript to the chemical symbol; e.g., ${}_{1}H^{1}$, ${}_{11}Na^{23}$, and ${}_{15}P^{32}$ refer to certain atomic species of the elements hydrogen, sodium, and phosphorus, respectively. Sometimes the atomic number is omitted because it can be inferred from the chemical symbol.

C. Isotopes

Because the chemical properties of the atom are determined by the value of the nuclear charge or atomic number, addition of neutrons to any nuclear complex of protons and neutrons changes the mass by an integral amount but does not change the nuclear charge. Since the nuclear charge determines the number of extranuclear electrons, which, in turn, determines the chemistry of the atom, no change occurs in the chemical behavior of the atom when neutrons are added to the atomic nucleus. Consequently there are nuclei, and hence atoms, which vary in nuclear mass but not in chemical nature. These are called *isotopes*. Some elements have only one stable isotope each ($_4Be^9$, $_9F^{19}$, $_{11}Na^{23}$, $_{15}P^{31}$, etc.); others are mixtures of two or more stable isotopes.

Sulfur may be cited as an example. Four isotopes of sulfur with mass numbers 32, 33, 34, and 36 are known. In the nomenclature described above these would be written $_{16}S^{32}$, $_{16}S^{33}$, $_{16}S^{34}$, and $_{16}S^{36}$. Each of these nuclei contains $A = 16$ protons, and $(A - Z) = 16$, 17, 18, and 20 neutrons, respectively. The ratio of the number of neutrons to the number of protons for stable nuclei is very close to unity. It increases with increasing values of Z until at $_{83}Bi^{209}$ there is a ratio of 126/83 or 1.5.

It is also possible for nuclei with the same mass number but different atomic number (*isobars*) to exist. Examples are $_{48}Cd^{113}$ and $_{49}In^{113}$, $_{18}A^{40}$ and $_{20}Ca^{40}$. Finally, it is also possible that nuclei of identical charge and mass number may exist in slightly different configurations or energy states. Such nuclei are called *isomers* (see p. 15).

The terms *isotope*, *isobar*, and *isomer* refer to particular species of atomic nuclei. The collective term for nuclear species is *nuclide*.[1] Thus H^2, H^1, Li^6, P^{31}, Ca^{40}, and Ca^{42} are all nuclides, but only H^2, H^1 and Ca^{42}, Ca^{40} are isotopes of hydrogen and calcium, respectively.

D. Nuclear Mass

The mass of any nucleus is referred to the mass of the oxygen isotope of weight 16 which is defined as having a mass exactly equal to 16. Thus, the hydrogen nucleus, or proton, has a mass of 1.00758 compared to O^{16}. Its mass number, A, is 1, according to the definition in Section 2-B.

The scale used in describing nuclear mass is called the *physical atomic weight scale*. This is not identical with the *chemical atomic weight scale*, which is used to express atomic weights in chemistry. In the chemical scale, the standard weight is that of the natural form of oxygen, which contains small amounts of the rare isotopes O^{17} and O^{18} and is assigned a mass of exactly 16.0000 . . . , although it actually weighs a bit more than

[1] Kohman, T. P., *Am. J. Phys.* **15**, 356 (1947).

the standard O^{16} atom. The chemical unit is larger than the physical unit by 1.000272 ($\pm$0.000005). The uncertainty in the sixth figure arises from fluctuations in the isotopic content at this degree of precision.

The isotopic masses usually encountered in the literature are based on the physical scale. They are not nuclear but atomic masses and include the masses of the extranuclear electrons in the neutral atoms.

E. Other Fundamental Nuclear Properties

In addition to charge and mass, the nucleus has properties analogous to those associated with electrons in atomic physics: spin, mechanical moment, magnetic moment, and electric moment. All nuclei are also subject to one of two types of statistics, depending on the quantum mechanical description employed. This, in turn, depends on whether a nucleus has an odd or even number of constituent particles, i.e., an odd or even mass number. The existence of these properties, however, is not relevant to tracer methodology and need not be considered further.

The nuclei of major importance for this discussion are the *neutron* (n); the *proton* (p); the *deuteron* (d), which is the heavy hydrogen nucleus ($_1H^2$); and the *alpha* particle (α), which is the helium nucleus ($_2He^4$). The *negative electron* or *negative beta particle* (β^-), and the *positive electron* or *positron* (β^+), although they do not exist in the nucleus, are produced by it in certain nuclear transformations. In addition, the list of nuclear entities include *gamma radiations* (γ), which are high-energy photons (electromagnetic radiation quanta), and *neutrinos*, which are hypothetical particles postulated to occur in those nuclear transformations involving β-ray emissions (see p. 11).

3. SYSTEMATICS OF NUCLEI

A. Introduction

Derived from Einstein's theory of special relativity is the concept of equivalence of mass, M, and energy, E, expressed in the relation $E = Mc^2$, where c is the velocity of light. This relation is fundamental for nuclear physics and has been verified convincingly in nuclear studies. According to this principle, the disappearance of mass is accompanied by the liberation of very large amounts of energy. Before discussing the energy magnitudes involved, it is convenient to introduce at this point the concept of the *electron volt* (ev.) as a unit of energy.

A unit electric charge moving through a potential difference of one international volt acquires a kinetic energy which is spoken of as one "electron volt equivalent." The heat energy to which this corresponds can be calculated in the following manner. Suppose one mole (6.02×10^{23}) of electrons is confined in a space between parallel plates of an electrical condenser

charged to 1 volt. The electrons fall into the positively charged plate, their kinetic energy being dissipated as heat. This heat energy in joules is the total charge in coulombs multiplied by the voltage difference across the condenser. One mole of electrons is equivalent to a *faraday*, which is approximately 96,500 coulombs. Hence $96,500 \times 1 = 96,500$ joules of heat appear. One gram-calorie (g.-cal.) corresponds to 4.18 joules, so that 96,500/4.18 or 23,000 g.-cal./mole of heat represents 1 ev. of kinetic energy per atom. Each electron gains a kinetic energy corresponding to heat motion communicated by $23,000/6.02 \times 10^{23}$ g.-cal. Thus, 1 ev. is the equivalent of 1.602×10^{-12} erg. Since heats of chemical reactions usually vary from a few kilocalories to a few hundred kilocalories per mole, it can be seen that the range of chemical energies is included in the range 0 to 10 ev.

The magnitude of the energies involved in nuclear interactions can be shown by application of the mass-energy relation. It is obvious that these energies will be enormous in comparison with ordinary chemical reactions, because, in the latter, no detectable mass loss is observed. In nuclear transmutations, on the other hand, there are very appreciable changes in total mass. It is found that one absolute mass unit (m.u.), $\frac{1}{16}$ of O^{16}, corresponds to 931 millions of electron volts (Mev.).[2] In other words, disappearance of 1 m.u. would liberate approximately 2.1×10^{13} g.-cal. of heat per mole of nuclei involved. The heat liberated in the burning of one mole of sugar to CO_2 and water is only 7.2×10^5 g-cal.

The simplest nuclear reaction is the combination of a neutron and a proton to form a deuteron, i.e.,

$$n + p \rightarrow d + \gamma \qquad (1)$$

This reaction is exothermic (releases energy), 2.18 Mev. of energy as electromagnetic (γ) radiation being emitted on fusion of a neutron and the proton. The γ-ray energy representing the difference in mass between the reactants (free neutron and proton) and product nucleus (the deuteron) is called the "binding energy." This quantity is analogous to the heat of chemical reactions. If the deuteron is to be disintegrated into a neutron and proton, energy is required and the reaction is endothermic. The mass of the neutron can be calculated from data of reaction 1 in the following way. The relation between the mass of the neutron, M_n, the mass

[2] The energy equivalent to 1 atomic mass unit (a.m.u.) can be found readily from the Einstein equivalence relation. M, the weight of 1 a.m.u. in grams, is the weight of $\frac{1}{16}$ of the O^{16} atom divided by Avogadro's number, which is the number of atoms in a gram-atomic weight. Thus, $M = 1/6.02 \times 10^{23} = 1.661 \times 10^{-24}$ g. Multiplying this of light (c^2) gives $1.661 \times 10^{-24} \times (2.998 \times 10^{10})^2$ g. cm.^{2} erg. Since 1 ev. $= 1.602 \times 10^{-12}$ erg, 1 a.m.u. =

of the proton, M_p, the mass of the deuteron, M_d, and the binding energy, ΔE, follows immediately from reaction 1 as

$$M_n = M_d - M_p + \Delta E \tag{2}$$

ΔE converted to mass units is 2.18/931, or 0.00234 m.u. When the known masses are substituted for the deuterium atom and the hydrogen atom,

$$M_n = 2.01473 - 1.00813 + 0.00234 = 1.00894 \text{ m.u.} \tag{2a}$$

This type of calculation can be applied to any nucleus, stable or unstable, provided the binding energy for the reaction whereby such a nucleus is formed is known. In most tables, the masses of the neutral atoms, rather than the masses of the nuclei, are given. Thus, the mass of the extranuclear electrons is included. Atomic mass instead of nuclear mass can be used in these calculations, because the electronic masses cancel out whenever stable isotopes are involved. For example, in reaction 1 the one extranuclear electron from deuterium ($_1H^2$) cancels the electron from protium ($_1H^1$).

It also possible to calculate nuclear masses of unstable isotopes from a knowledge of the maximum energy involved in the disintegration. As an example, C^{14} emits a negative electron (β^- particle) with a maximum kinetic energy of 0.15 Mev. This process forms the residual nucleus N^{14}. Thus,

$$C^{14} \rightarrow N^{14} + \beta^- + 0.15 \text{ Mev.} \tag{3}$$

The mass of N^{14} is 14.00754, so the mass of C^{14} is 14.00754 + 0.00016 or 14.00770. Here, again, it should be noted that the atomic mass is used in place of the nuclear mass. This is because the residual nucleus has its positive charge (atomic number) increased by one unit when a negative electron leaves a radioactive nucleus. Thus, another electron is required in the atomic orbit. As far as the over-all mass balance is concerned, all that happens is that an electron leaves the nucleus and joins the product atom. Hence, no change in total number of electrons is involved.

This is not true when a positive β-ray emitter is involved, because one less electron is required for the product atom. One negative electron with a rest mass equivalent to 0.00055 m.u. goes off with the initially emitted positive electron and is lost from the orbital electrons. The masses of the positive and negative electrons are equal; two electron masses should be added to the product nucleus to attain mass balance when atomic masses are used. In the disintegration of $_7N^{13}$ a positron is emitted and $_6C^{13}$ with atomic mass 13.00761 is formed. The maximum energy of the radiations emitted gives the heat of reaction, ΔE, as 1.198 Mev., which is 0.00129 m.u. Hence, the mass of $_7N^{13}$ can be calculated as follows:

$$_7N^{13} \rightarrow {}_6C^{13} + \Delta E$$

$$m_{N^{13}} = 13.00761 + 0.001$$