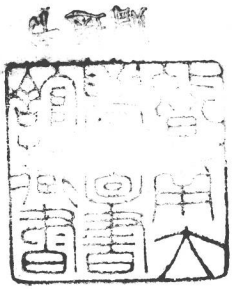
RADIOACTIVE TRACERS IN BIOLOGY

An Introduction to Tracer Methodology

BY

MARTIN D. KAMEN

Associate Professor of Chemistry
Chemist to the Edward Mallinckrodt Institute of Radiology
Washington University, St. Louis, Missouri



1947

ACADEMIC PRESS INC., PUBLISHERS
NEW YORK, N. Y.

Published in the United States of America

THE MURRAY PRINTING COMPANY
CAMBRIDGE, MASSACHUSETTS

Organic and Biological Chemistry

A SERIES OF MONOGRAPHS

EDITED BY

LOUIS F. FIESER AND MARY FIESER
Harvard University, Cambridge, Mass.

VOLUME I

RADIOACTIVE TRACERS IN BIOLOGY

BY MARTIN D. KAMEN

1947

ACADEMIC PRESS INC., PUBLISHERS
NEW YORK, N. Y.

ACKNOWLEDGMENTS

It is a pleasant task to note the invaluable aid of numerous colleagues who have cooperated most ungrudgingly in offering suggestions and comments on various portions of the text. Professor J. W. Kennedy and Professor A. C. Wahl of Washington University have been particularly helpful in expunging errors which crept into the preliminary chapters. Others who have scrutinized the text critically are Professor S. Spiegelman, Professor S. Velick, and Dr. M. B. Allen of Washington University, Professor S. S. Cohen of the University of Pennsylvania, and Dr. S. Granick of the Rockefeller Institute for Medical Research. For aid in the preparation of drawings and tables thanks are due Mr. Howard Gest, Mr. Harry Huth, and Miss E. Ley. Secretarial assistance on the part of Mrs. Howard Gest, Mrs. R. Feinman, Miss L. Liebich and Miss G. Adams is gratefully recorded. A special citation is in order for Miss Beka Doherty whose suggestions on matters of style did much to improve the clarity of the text. The manuscript was read in proof by Professor W. A. Hynes of Fordham University. Permission to use certain original diagrams and photographs was graciously accorded by the following: Professor C. G. Montgomery, Professor J. W. Irvine, Jr., Professor M. S. Livingston, Dr. A. F. Reid, Dr. F. D. Moore, and Dr. S. M. Seidlin.

The tedium invariably associated with the production of a book was quite obviated by the pleasant surroundings and extensive facilities of the Biological Station, Cold Spring Harbor, Long Island, placed at the writer's disposal by Professor M. Demerec during the summer of 1946.

Much of the text is based on researches carried out during the writer's association with the University of California faculty, notably Professor H. A. Barker, Professor W. Z. Hassid and, in particular, the late Professor Samuel Ruben whose ability and vitality were most salient factors in the development of many techniques as well as successful prosecution of many experiments described.

In conclusion, it is a privilege to acknowledge the indebtedness of the writer to the Director of the Mallinckrodt Institute of Radiology, Professor Sherwood Moore, whose interest and encouragement made the writing of this book possible.

PREFACE

Modern alchemy, conceived in Rutherford's experiments during the second decade of this century, has been born with the emergence of the uranium chain-reacting pile after a gestation period of nearly twenty-five years. The fantastic developments of this quarter century, which might be described aptly as the Era of the Conquest of Avogadro's Number, have a not dissimilar parallelism with the famous Rabelaisian account of the conception and birth of the giant Gargantua. It will be remembered that the arrival of this celebrated infant was expected as a matter of course consequent on the connubial exercises of his progenitors, Grangousier and Gargamille, but not even the most frenzied imaginations escaped amazement at the manner of delivery. The baby issued as a giant from the ear of his mother. A similar astonishment must have gripped even the most case-hardened nuclear scientists on the occasion of the realization of nuclear transmutation on a macroscopic scale.

The impact of these developments on the biological sciences has excited much comment as evidenced in a spate of review articles, Sunday supplements, and other outlets to the reading public so that it appears unnecessary to engage in further discussion in these pages. It is sufficient to note that now there are available radioactive isotopes for practically all elements in amounts apparently adequate for any conceivable purpose in tracer research. This situation coupled with the recent development of commercial methods for isolation and bulk production of the rare stable isotopes of the important biological elements makes it possible to present the biologist with a complete armamentarium of tracer isotopes for use in prosecuting researches into a staggering variety of biological problems.

The rapidity of developments in the science of nuclear transmutation and the inevitable lag in communication of the new knowledge to workers not immediately and intimately involved in such developments appears to make desirable the appearance of a textual guide or manual. This need is particularly acute in the biological fields of application. In the writer's opinion such a text should have three objectives:

(1) To provide an introduction or review of those concepts in nuclear physics an understanding of which is basic to the intelligent application of tracer methods in biology.

(2) To present a systematic and critical survey of existing tracer methods.

(3) To indicate potentialities and limitations of these methods as applied to biological problems.

These objectives should be reached with minimal detail, maximal clarity and condensation sufficient to keep the text to a reasonable size. The major emphasis in subject matter must be on the biochemical and physiological aspects of tracer methodology because it is at the biochemical and physiological levels that closest contact is made.

In view of these considerations, the writer has chosen to organize the text in the following manner. An introductory section (Chapters 1 to 3) deals with the fundamentals of nuclear phenomena and radiochemistry. This material serves as a logical basis for a chapter on the basic principles and procedures in radioactive tracer assay (Chapter 4). This chapter has occasioned the writer some agony in selection of material because in no phase of the science is there more rapid change than in assay instrumentation. An attempt to write a strictly up-to-date account of assay procedures has been abandoned because of the prolix text which would result. For the purposes of this book, it has appeared more desirable and more in keeping with the objectives stated to confine discussion to principles essentially invariant to kaleidoscopic shiftings and variations in techniques which are inevitable and which render obsolete almost every detailed procedure frozen into a text or guide book. However, a number of techniques adequate in past researches are presented. The manipulations described are intended to provide a basis for systematic acquisition of the skills required in researches with radioactive isotopes.

In the section beginning with Chapter 5 and extending through Chapter 10 there is given an initial general survey reenforced by individual chapters on those radioactive isotopes of most importance. Included are descriptions of special assay methods as further elaboration on the general remarks in Chapter 4. In the hope of achieving maximal clarity the writer has adopted the practice of presenting actual experimental protocols with accompanying analyses. This procedure dependent as it is on data available only from researches involving participation of the writer results of necessity in an apparent but unintended immodesty. Throughout the text numerous examples introduced to illustrate a particular aspect of tracer methodology are interpolated. The researches cited are chosen because of the chance acquaintance of the writer with such material and not because of any inherent superiority to other researches. It should be remarked that a large portion of the tracer work accomplished to date has been carried out using stable isotopes and little or none of this work has been mentioned because of the limitation of subject matter to researches carried out with radioactive isotopes. This

omission as well as others occasioned by space limitations should be filled in by the industrious reader using the references appended to each chapter. These reference lists are not complete but serve to provide a basis for compilation of a complete bibliography. The literature up to June of 1946 is included for the most part.

In the final section (Chapters 11 *et seq.*) there are presented discussions of isotopes which may be classified as of secondary importance. A final chapter contains a survey of special topics dealing especially with medical applications. An extensive description of these applications does not appear to be indicated at this time owing to the relatively undeveloped state of medical tracer research.

In conclusion it should be remarked that this book is not a compendium for the expert. It is an introduction to tracer methodology as applied to biological research. It is addressed to biochemists, physiologists and medical biologists whose previous contact with nuclear physics and chemistry is minimal and who wish to acquire familiarity with radioactive tracer methods. The writer hopes sincerely and humbly that the text is an aid in appreciating the peaceful applications of nuclear energy and that the avowed purposes of the book are served within the limits set forth.

TABLE OF CONTENTS

Errata

Page 6: Line 14, last word "ton" should read "one"
Line 15, last two words "mus bee" should read "must be"

Page 11: Fig. 1, omit "log of" in legend for ordinate

Page 12: Fig. 2, omit "log of" in legend for ordinate; in both Figs. 1 and 2, ordinates are plotted on log scale

Page 90: Line 6, "3.7×10^{-10} particles/sec." should read "3.7×10^{10} particles/sec."

Page 116: Line 24, "Relation (m)" should read "Relation (l)"

Page 118: Line 3, "$\chi_2=(A_1/A_f)-1\cdot\chi_1$" should read "$\chi_2=(A_1/A_f-1)\cdot\chi_1$"

Page 176: Last line, "(p. 000)" should read "(p. 167)"

Page 218: Footnote (22), "have been repeated" should read "have been reported"

Page 230: Table XIX, Column 3, line 9, "CS^{133}" should read "Cs^{133}"

CHAPTER I

Atomic Nuclei and Radioactivity

1. Introductory Remarks

The intelligent application of tracer procedures to biological problems requires a certain irreducible minimum of knowledge concerning nuclei and nuclear reactions as well as some grasp of general atomic physics. Accordingly, the introductory chapters of this book are given over to a discussion of the physical background of tracer methodology. The degree of elaboration of this material is best left optional with the reader who, by consulting the bibliography included at various strategic places in the text, should be able to probe more deeply into the subjects discussed briefly in the following sections.

2. General Properties of Atomic Nuclei

A. Nuclear Terminology

A coherent discussion of the general properties of atomic nuclei requires definition of the terminology and concepts of nuclear physics. In the following discussion, such a review is given briefly.

Nuclear Mass. The mass of any nucleus is referred to the mass of the oxygen nucleus of weight 16 which is defined as having a mass exactly equal to 16.000. . . . The *mass number*, symbolized by A, is the integral number nearest the actual mass. Thus the hydrogen nucleus or *proton* is found to have a mass of 1.00758 compared to O^{16}. Its mass number A is, therefore, 1. In nuclear nomenclature the mass number is usually written as the right superscript to the chemical symbol for the atom (*i.e.*, H^1, Na^{23}, P^{32}, *etc.*).

Nuclear Charge. The electrical charge carried by the nucleus is positive and equal numerically to the *atomic number*, usually symbolized by Z. The atomic number is the number of extranuclear electrons in the neutral atom because, to satisfy the requirement of electrical neutrality, there must be as many of these as there are positive charges on the nucleus. The atomic number is usually written as a left subscript to the chemical symbol (*i.e.*, ${}_1H$, ${}_{11}Na$, ${}_{15}P$, *etc.*). The nucleus is completely identified if both the mass number, A, and the atomic number, Z, are indicated; thus ${}_1H^1$, ${}_{11}Na^{23}$, ${}_{15}P^{31}$, refer to certain kinds of nuclei for the elements hydrogen, sodium and phosphorus, respectively. In fact, the chemical symbol is superfluous in this nomenclature but is retained for convenience in writing nuclear reactions (Chapter II).

Isotopes. According to modern concepts, the nuclei of atoms are built up by combinations of protons and neutrons. The *neutron* has a mass number of 1 but is uncharged. It may be considered the uncharged analogue of the proton. 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 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.

On this basis the mass number, A, is the total number of particles in the nucleus. The difference $A - Z$ is the number of neutrons. Sulfur may be cited as an example. Four isotopes of sulfur with mass numbers 32, 33, 34 and 36 are known. In the nomenclature discussed 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 $_{20}Ca^{40}$, *etc.* 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.*

Other Fundamental Nuclear Properties. In addition to charge and mass, the nucleus has properties analogous to those associated with electrons in atomic physics — namely, 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 ($_1H^2$); 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^+). In addition, the list of nuclear entities includes *gamma radiations* (γ), which are high energy photons (electromagnetic radiation quanta).

B. *Systematics of Nuclei*

Introduction. Derived from the 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.[1] This relation is fundamental for nuclear physics and has been verified convincingly in nuclear studies. According to this principle, disappearance of mass is accompanied by 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 are confined in a space between parallel plates of an electrical condenser charged to one 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 $\frac{96{,}500}{4.18}$ or 23,000 g.-cal. of heat represents one electron-volt of kinetic energy. Each electron gains a kinetic energy corresponding to heat motion communicated by $23{,}000/6.02 \times 10^{23}$ g.-cal. 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 $0-10$ EV.

The magnitude of the energies involved in nuclear interactions can be shown by application of the mass energy relation. It can be expected 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. $\left(\frac{1}{16}\text{ of } 0^{16}\right)$, corresponds to 931 millions of electron volts (MEV).[2] In

[1] Einstein, A., *Bull. Am. Math. Soc.* **41**, 223 (1935).

[2] A simple method for making this calculation follows: 1 mass unit = $m_H C^2 = \frac{1}{16} M_0 C^2$, where M_0 is the standard mass of one 0^{16} nucleus which is exactly 16 units. The

other words, disappearance of one 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 proton to form a deuteron, *i.e.*,

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

This reaction is exoergic (releases energy), 2.17 *MEV* of energy as electromagnetic (γ) radiation being emitted on fusion of a neutron and proton. This γ ray energy represents the difference in mass between the reactants (free neutron and proton) and product nucleus (the deuteron). It 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 on reaction (1) in the following way. The relation between the mass of the neutron M_n, the mass of the proton M_p, the mass of the deuteron M_d, and the binding energy ΔE follows immediately from (1) as

(2) $$M_n = M_d - M_p + \Delta E.$$

ΔE converted to m.u. is $\frac{2.17}{931}$ or 0.00233 m.u. Substituting the known masses for the deuteron and the proton

(2a) $$M_n = 2.01473 - 1.00813 + 0.00233 = 1.00893 \text{ m.u.}$$

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 be 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

at. wt. divided by Avogadro's number gives M_0 in g., *i.e.*, $\frac{16}{6.02 \times 10^{23}}$ or 2.64×10^{-23} g. The constant, C^2, is equal to 8.99×10^{20} cm.^{2}/sec.^{2}. Hence, 1 m.u. $= \frac{8.99 \times 10^{20}}{16} \times 2.64 \times 10^{-23}$ or 1.49×10^{-3} ergs. There are 4.2×10^7 ergs/g.-cal., so that, converting to g.-at., one g. at m.u. is the equivalent of $\frac{1.49 \times 10^{-3} \times 6.02 \times 10^{23}}{4.2 \times 10^7}$ or 2.1×10^{13} g.-cal. Since it has been shown that one *EV* is equivalent to 2.3×10^4 cal./g. at., one mass unit/g. at. is $\frac{2.1 \times 10^{13}}{2.3 \times 10^4}$ which is 0.93×10^9 *EV* or 931 *MEV*. An accurate calculation leading to the value 931.05 ± 0.15 *MEV* can be found in the review article by Bethe, H. A., *Rev. Mod. Phys.* **9**, 2 (1937).

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 is 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,

(3) $$C^{14} \rightarrow N^{14} + \beta^- + 0.15\ MEV.$$

The mass of N^{14} is 14.00750, so that the mass of C^{14} is 14.00750 + 0.00014, or 14.00764. 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 overall 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, which goes off with the initially emitted positive electron, 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 evolved, ΔE, as 1.198 *MEV* which is 0.00129 m.u. Hence, the mass of $_7N^{13}$ can be calculated as follows:

(4) $$_7N^{13} \rightarrow {}_6C^{13} + \Delta E + 2\beta$$

(4a) $$m_{N^{13}} = 13.00761 + 0.00129 + 0.0011 = 13.01000$$

In all of these calculations it is assumed that the product nucleus is formed in its lowest (most stable) energy state. If this is not the case, γ-radiation corresponding to the transition from the upper energy to the lowest energy state will contribute energy, and hence mass, which must be added to the mass value obtained in the above manner.

Isotope Classification and Nuclear Forces. It may be assumed that non-radioactive isotopes represent stable combinations of neutrons and protons. Thus, the nucleus of carbon must contain, in addition to 6 protons, no more than 6 or 7 neutrons. These combinations correspond to the two stable carbon nuclei found in nature, namely, $_6C^{12}$ and $_6C^{13}$. Eight neutrons cause formation of an unstable configuration ($_6C^{14}$) of

14 particles. The stable configuration for 14 particles is one consisting of 7 protons and 7 neutrons ($_7N^{14}$). By changing a neutron into a proton $_6C^{14}$ is transformed to $_7N^{14}$. This requires emission of a negative β-particle, thus:

(5) $$n \rightarrow p + \beta^-.$$

Suppose 5 neutrons are associated with 6 protons to form $_6C^{11}$. This nucleus represents an unstable configuration of 11 particles, the stable configuration being the naturally-occurring non-radioactive isotope of boron, $_5B^{11}$, which consists of 5 protons and 6 neutrons. A proton is transformed into a neutron with consequent positive electron emission to effect the necessary change in composition. Hence, $_6C^{11}$ disintegrates by positive electron emission to $_5B^{11}$; thus the process

(6) $$p \rightarrow n + \beta^+$$

occurs in the $_6C^{11}$ nucleus. This transformation requires at least one *MEV* of energy since two electron masses ($= 2 \times .00055$ m.u.) must be supplied in this transformation consequent on the loss of a positive and negative electron in the formation of the product atom. When the relative instability of a configuration with excess protons is not sufficient to supply this energy, an alternative process called "K-capture" can take place. The reader will note that essentially the same nuclear composition can be obtained in one of two ways, for example, by adding a negative electron to a nucleus or by removing a positron. A nucleus which should emit a positive electron but is lacking in the necessary energy can reduce its positive charge by capturing an orbital electron from the nearest inner electron shell — the K shell. This K-capture process can take place whether sufficient energy for positron emission is available or not. The prediction of relative probability of K-capture or positron emission is one of the interesting problems in nuclear physics.

Thus, for carbon, an increase in the number of neutrons beyond 7 or a decrease below 6 results in unstable nuclei. One of these, C^{14}, transforms to N^{14}; the other, C^{11}, transforms to B^{11}. Likewise, there is a C^{10} which is even more unstable than C^{11} and transforms to B^{10}. It is found in this way that throughout the whole periodic system there is, for any given number of protons, a restricted number of neutrons which will form a stable combination. The binding energy of a single neutron and proton as in $_1H^2$ has been found to be approximately 2 *MEV* or about 1 *MEV*/particle. Throughout most of the periodic table, however, the binding energy per particle, whether neutron or proton, is considerably higher and essentially constant at 7–8 *MEV*. The only forces known from previous physical experience are those operative between the charged components, *i.e.*, the protons. Since these are Coulomb forces they should