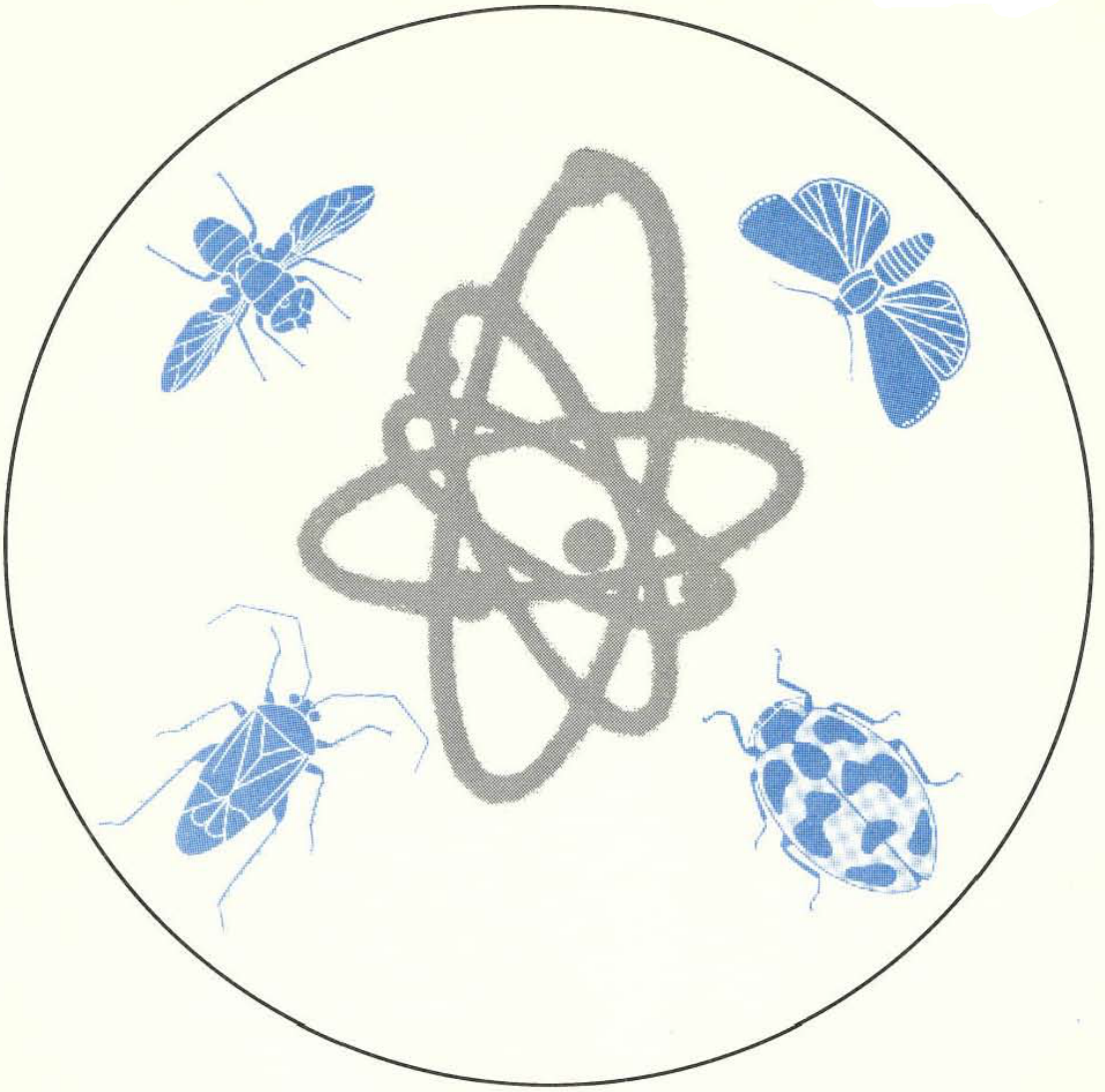

TECHNICAL REPORTS SERIES No. 61

Laboratory Training Manual on the Use of Isotopes and Radiation in Entomology

Second Edition

A JOINT UNDERTAKING BY FAO AND IAEA

INTERNATIONAL ATOMIC ENERGY AGENCY, VIENNA, 1977

LABORATORY TRAINING MANUAL ON THE USE OF ISOTOPES AND RADIATION IN ENTOMOLOGY

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A JOINT UNDERTAKING BY THE
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INTERNATIONAL ATOMIC ENERGY AGENCY

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FOREWORD

The use of isotopes and radiation in entomology has brought many advances in both basic knowledge and practical control of insect populations. The Interregional Training Courses on the Uses of Isotopes and Radiation in Entomology, sponsored by the Food and Agriculture Organization (FAO) and the International Atomic Energy Agency (IAEA) in cooperation with the United States Department of Agriculture, the United States Energy Research and Development Administration and the Department of Entomology of the University of Florida, have met the demand for specialist introduction to this applied use of nuclear techniques, and a total of seven such courses have now been held.

Eleven years have passed since the first edition of this manual made available in printed form much of the material taught at these courses. During that period, keeping pace with developments in the field, there have been changes in the theoretical part and, more especially, in the applied part. In addition, recent changes in radiation units and the philosophy underlying certain radiation measurements were felt to be of particular importance to students starting a training in the use of nuclear techniques. A revision was, therefore, undertaken.

The revised manual is based upon material drawn from the previous edition, the Tracer Manual on Crops and Soils (IAEA Technical Reports Series No.171, 1976) and numerous sources mentioned in the relevant reference lists. It is divided into seven parts, the main ones having a number of blank pages for adding new information and notes.

Professor H. Cromroy of the University of Florida provided the completely new Part II, Radiation Biology, Professor W. Kloft of Bonn University revised and extensively augmented Part V, Applied Part.

The Lecture Material (Part I), and Appendixes (Part VI) were revised by Professor H. Cromroy and Mr. E.R.A. Beck of the IAEA, the latter also being responsible for the introduction of the material on the new radiation units and for the revision of a Glossary (Part VII) based on that in TRS No.171. The co-ordination of the revised edition was undertaken by Dr. I. Moore of the Joint FAO/IAEA Division of Atomic Energy in Food and Agriculture.

It is hoped that this edition of the manual will assist the FAO and IAEA personnel responsible for providing training in the use of nuclear techniques in entomology, governments and institutes planning such didactic courses, as well as the individual scientists and students.

The FAO and IAEA wish to express their indebtedness to the contributors to this edition and to the authors of all other source material drawn upon by the present revisors.

The Joint FAO/IAEA Division of Atomic Energy in Food and Agriculture would welcome comments and suggestions on their training manuals, which should be addressed to:

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60	1972	Laboratory Training Manual on the Use of Isotopes and Radiation in Animal Research (3rd Edition) – see last entry.
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171	1976	Tracer Manual on Crops and Soils
—	1978(?)	Laboratory Training Manual on the Use of Nuclear Techniques in Animal Research (in preparation)

CONTENTS

PART I. LECTURE MATERIAL

SOME BASIC SYMBOLS AND UNITS FREQUENTLY USED IN THIS MANUAL

Symbol	Description	Dimensions and/or units
Z	atomic number, i.e. proton number	
A	mass number	
A_r	relative atomic mass	unified atomic mass units (u)
M, M_{Ca}, M_{CaSO_4}	gram-atomic or gram-molecular mass	grams (g)
N_A	Avogadro's constant (number)	$N_A = 6.022 \times 10^{23}$ mol^{-1}
$T_{\frac{1}{2}}$	radioactive half-life	time, e.g. years (a), days (d), hours (h), minutes (min), seconds (s)
λ	radioactive decay constant	inverse time, i.e. s^{-1}
t	time in general	time, i.e. s
T	counting time (duration of)	time, i.e. s
C	accumulated counts in time T	counts
R (= C/T)	count-rate including background or blank	counts per second (counts/s)
R_b	count-rate of background or blank	counts per second (counts/s)
R_s	count-rate of sample	counts per second (counts/s)
ϵ	counting efficiency = counting yield	
A^*	activity	becquerels (Bq), curies (Ci) (becquerels ≡ disintegrations per second)
A	specific activity of a radioisotope *or* atoms % excess of stable isotope	activity per gram or mole (e.g. kBq/g, TBq/mol, mCi/g, etc.)
S	amount of tracee (substance being traced)	mass (i.e. grams) or moles
$T_{\frac{1}{2},\,biol}$	biological half-life (physiological elimination)	time, e.g. s
$T_{\frac{1}{2},\,eff}$	effective half-life (including effects of physiological elimination and radioactive decay)	time, e.g. s

PART I. LECTURE MATERIAL

I–1. PROPERTIES OF RADIONUCLIDES AND RADIATIONS

I–1.1. Atomic model. Radioactivity

An atom is composed of a positively charged nucleus surrounded by shells of negatively charged (orbital) **electrons.**[1] The nucleus contains **protons** and **neutrons** as its major components of mass. A proton carries a positive (elementary) charge, and a neutron has no charge. The nucleus has a diameter of the order of 10^{-12} cm and contains almost the entire mass of the atom. The atom, including the orbital electrons, has a diameter of the order of 10^{-8} cm (or 1 ångström).

The number of protons, **Z**, in the nucleus, which is characteristic of a chemical element, is called the **atomic number** (proton number). The atomic nuclei of a particular element may, however, not all have the same **neutron number, N.** Atoms that have the same **Z**, but different numbers of neutrons, are called **isotopes** (of the chemical element corresponding to **Z**) because they occupy the same place in the periodic chart of the elements.

As the neutrons and protons represent the major part of the mass of the atom and each has an atomic 'weight', i.e. an atomic mass[2], close to unity, the **mass number, A,** which is equal to the sum of protons and neutrons, is the nearest whole number to the relative atomic mass, A_r. Thus:

$$Z + N = A \triangleq A_r \text{ in unified atomic mass units (see footnote 2)}$$

Nuclides (any species of nuclei) are described symbolically by the designation:

$${}^{A}_{Z}\text{El}$$ *or* $${}^{A}\text{El}$$ *or* element-A (for example $${}^{59}_{26}\text{Fe}$$ *or* $${}^{59}\text{Fe}$$ *or* iron-59)

where El represents the chemical symbol for the element.

The nuclei of some nuclides are not stable. One by one they disintegrate spontaneously, each nuclide at a characteristic rate, and they are called 'radioactive'. In nature a number of unstable nuclides are known, and nowadays radioactive isotopes of nearly every element are produced artificially (e.g. in atomic reactors and by particle accelerators). The disintegration of radioactive nuclei is accompanied by the emission of various kinds of ionizing

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[1] Many of the basic terms are included in a glossary for easy reference (Part VII).

[2] The unified atomic mass unit (abbreviation u) is defined as exactly 1/12 the mass of the nuclide ^{12}C: $1\ u = 1.66053 \times 10^{-27}$ kg approximately [1].

radiation. Radioactive nuclides are termed radionuclides, and other similar abbreviations are used (i.e. radioactive isotope → radioisotope, etc.).

Radioactive nuclei, upon disintegration, may emit **alpha** (α) or **beta** (β) particles as well as **gamma** (γ) rays. Alpha particles are fast-moving helium nuclei ($^4_2$He), i.e. a combination of two protons and two neutrons. Beta particles are fast-moving electrons of either negative (β^-) or positive (β^+) charge. Gamma rays are electromagnetic energy packets (photons) of very short wavelength compared with that of visible light, but travelling at the characteristic speed of light.

Natural isotopes of elements with low Z-numbers (except ordinary hydrogen) have approximately the same number of neutrons as protons in their nuclei ($N \triangleq Z$), and they are usually stable. As the atomic number of the element increases, the number of neutrons increasingly exceeds the number of protons with stability being maintained, but finally only unstable nuclei occur (above $Z = 83$, bismuth). Thus, the majority of radioisotopes in nature are found for elements of high Z-number with a neutron-to-proton ratio of the order of $1\frac{1}{2}:1$. The emission of alpha particles is characteristic of these heavy, unstable elements. The alpha particle is a very stable nuclear form which is ejected as a single particle from the nucleus of the heavy atom when it disintegrates.

There appears to be a more or less well-defined optimum N:Z ratio for the stability of each element. When the number of neutrons in the nucleus is excessive, the N-number tends to decrease by ejection of a negative beta particle and a neutrino[3] from the nucleus. This beta particle accompanies the transformation of a neutron into a proton:

$$n \rightarrow p^+ + \beta^- + \nu \quad \text{(see footnote 3)}$$

An excess of protons in a nucleus may be counteracted by the ejection of a positron, i.e. a positive beta particle (regarding the definition of the energy unit MeV, see §I–1.5):

$$1.02\ \text{MeV} + p^+ \rightarrow n + \beta^+ + \nu$$

Here, 1.02 MeV (1.63×10^{-13} J) is the minimum energy required for β^+-emission and is equivalent to the rest mass of a positron plus an electron. An excess of protons in the nucleus may alternatively be reduced by the nucleus capturing

---

[3] A neutrino (ν) possesses energy, but no charge and practically no mass, and will therefore not be detected by any of the instruments used in isotopic tracer techniques.

one of its own orbital electrons, a process known as **electron capture** (EC) or K-capture:

$$p^+ + e^- \rightarrow n + \nu$$

EC is accompanied by the emission of a characteristic X-ray, most frequently representing the energy difference between an L and a K-shell electron in the element formed (a 'hole' in the K-shell being filled by an L-electron).

After the ejection of an alpha or beta particle, or after EC, the energy level of the daughter nucleus may not be at its ground state. The excess energy of a nucleus thus excited is emitted in the form of one or more gamma photons.

The excited nucleus may, however, interact with an orbital electron in the decaying atom, whereby the electron is expelled from the atom at a given velocity, and the expected gamma photon is not emitted. This process results in the combined emission of a fast electron and a characteristic X-ray, and is known as **internal conversion** (IC). The X-ray photon may in turn undergo IC, producing a so-called **Auger electron**.

In some instances, two alternative modes of decay of the nucleus may occur. An example is seen for potassium-40 decay:

$$^{40}_{19}K \rightarrow {}^{0}_{-1}e + {}^{40}_{20}Ca \qquad (\beta^- \text{ decay, } 89\% \text{ of disintegrations})$$

$$^{40}_{19}K + {}^{0}_{-1}e \rightarrow {}^{40}_{18}Ar \qquad (\text{EC, } 11\% \text{ of disintegrations})$$

In the case of the EC mode a gamma photon is also emitted.

Another example is

$$^{65}_{30}Zn \rightarrow {}^{0}_{+1}e + {}^{65}_{29}Cu \qquad (\beta^+ \text{ decay, } 1.5\% \text{ of disintegrations})$$

$$^{65}_{30}Zn + {}^{0}_{-1}e \rightarrow {}^{65}_{29}Cu \qquad (\text{EC, } 98.5\% \text{ of disintegrations})$$

In this case, in 45.5% of disintegrations, EC is followed by the emission of a gamma photon.

When a large nucleus such as ^{235}U captures a neutron, the nucleus will divide into two parts of approximately equal masses. This process is called **fission**. The primary fission products are unstable (excessive N), and each forms a series of radioactive daughter nuclides terminating with a naturally occurring stable nuclide.

I–1.2. The equation of Einstein

In almost every atomic or nuclear reaction a small quantity of mass is transformed to energy or vice versa. An example of the energy, Q, involved in an alpha decay is given as follows:

$$^{226}_{88}\mathrm{Ra} \rightarrow {}^{4}_{2}\mathrm{He} + {}^{222}_{86}\mathrm{Rn} + \mathrm{Q}$$

The Q of a nuclear reaction is related to the decrease in atomic mass in accordance with the general equation of Einstein:

$$E = mc^2$$

where E = energy release (in the above case Q),
m = mass decrease,
c = speed of light.

Alternatively, Einstein's equation may be expressed either as:

$$E = 931\,m$$

where E = energy release in mega-electronvolts (MeV),
m = mass decrease in unified atomic mass units,

or as

$$E = 5810\,m$$

where E = energy release in attojoules ($aJ \equiv 10^{-18}$ J).

I–1.3. Radioactive decay law. Specific activity

The decay of radioactive atoms is comprised of individual random (unpredictable) events. However, if a sample contains a sufficiently large number of atoms of a radionuclide, their average statistical behaviour can be described by a precise law. The radioactive decay law is developed as follows:

Let N be the number of radioactive atoms of a given radionuclide present at any time t. The change in N per unit time at any moment, dN/dt, is proportional to the number of atoms present at that moment, or:

$$\frac{dN}{dt} = kN = -\lambda N \qquad (I–1)$$

where λ is (numerically) the proportionality constant, termed the **decay constant**. The negative sign is used because the number N decreases with time and λ is chosen to be positive.

Rearranging Eq.(I–1) to solve for λ:

$$\lambda = -\frac{1}{N}\frac{dN}{dt} \qquad \text{(I–2)}$$

Thus, the decay constant is the fraction of radioactive atoms decaying per unit time at any moment.

Equation (I–1) may be integrated[4] to give:

$$N = N_0 e^{-\lambda t} \qquad \text{(I–3)}$$

where N_0 is the number present at any starting time ($t = 0$), and N is the number remaining after a period of time t. (e is the base of natural logarithms and equal to 2.71828.. .)

It can be seen from Eq.(I–3) that the decay of radioactive atoms is exponential with time. Also, the time for N_0 to be reduced to half its initial value is a constant, as shown below, *independent of* N_0 *and* t.

Let N_0 be reduced to $\frac{1}{2} N_0$ in a period of time ($t = T_{\frac{1}{2}}$) termed the **half-life**. Then, from Eq.(I–3):

$$\tfrac{1}{2} N_0 = N_0 e^{-\lambda T_{\frac{1}{2}}} \qquad \text{(I–4)}$$

Hence:

$$\tfrac{1}{2} = e^{-\lambda T_{\frac{1}{2}}} \qquad \text{or} \qquad e^{\lambda T_{\frac{1}{2}}} = 2 \qquad \text{(I–5)}$$

Thus, taking the natural logarithm of both sides:

$$\lambda T_{\frac{1}{2}} = \ln 2 = 0.693 \qquad \text{(I–6)}$$

Therefore, since λ is a constant characteristic of a given radionuclide, the same is true of $T_{\frac{1}{2}}$. The dimension of $T_{\frac{1}{2}}$ is given in time units, whereas the decay constant is indicated in reciprocal time units.

---

[4] The steps in this procedure are given in Part VI, Appendix VI–5.

Since the rate of decay, -dN/dt, is termed the radioactivity or, simply, activity, A*, of the sample, then according to Eq.(I–1):

$$A^* = \lambda N \quad \text{(Rutherford's equation)} \tag{I–7}$$

From Eq.(I–3), one therefore obtains:

$$A^* = A_0^* e^{-\lambda t} \tag{I–8}$$

Substituting $\lambda = (\ln 2)/T_{\frac{1}{2}}$ from Eq.(I–6) into Eq.(I–8), the following alternative equation is obtained:

$$A^* = A_0^* \, (\tfrac{1}{2})^{t/T_{\frac{1}{2}}} \tag{I–8a}$$

The half-life of a radionuclide may be determined graphically by plotting the disintegration rate (or a constant fraction thereof, as determined by a suitable counting instrument) versus time on log-linear graph paper. Referring to Eq.(I–8a), if the common logarithm (logarithm to the base 10) is taken on both sides, the result is:

$$\log A^* = \log A_0^* - \frac{\log 2}{T_{\frac{1}{2}}} \, t \tag{I–8b}$$

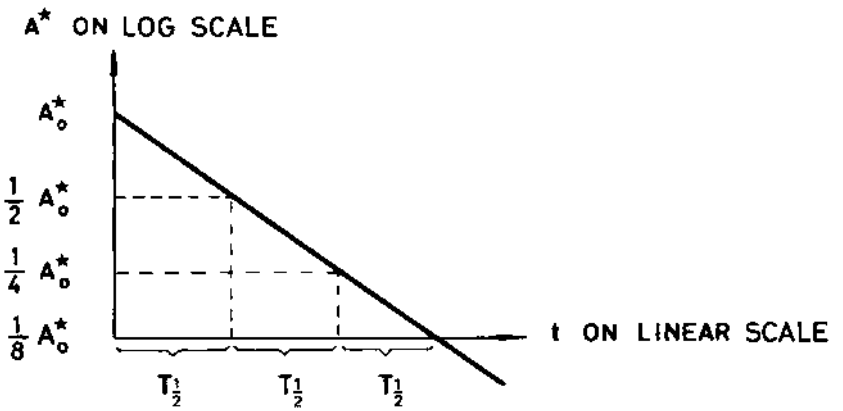

FIG.I–1. Decay curve of a single radionuclide (log-linear plot).

Therefore, a plot of A^* (or count-rate) on the log co-ordinate versus time on the linear co-ordinate will be a down-grade straight line with a numerical slope of $0.301/T_{\frac{1}{2}}$. This is graphically illustrated in Fig.I–1. $T_{\frac{1}{2}}$ can be calculated, for example, as one third of the time it takes for A_0^* to fall to $\frac{1}{8}$ A_0^*.

The special unit of activity (radioactivity) has for many years been the curie (abbreviated Ci). This was originally defined as the radioactivity associated with the quantity of radon in equilibrium with 1 g of radium (1910). The formal definition agreed in 1964 by the CGPM, when the curie was accepted for use with the International System (SI), was:

$$1\ \text{Ci} = 3.7 \times 10^{10} \text{ disintegrations per second}$$
$$= 3.7 \times 10^{10}\ \text{s}^{-1} \text{ (exactly)}$$

Since 1976, a new unit of activity, the becquerel (Bq), has been defined as a derived unit of the SI:

$$1\ \text{Bq} = 1 \text{ disintegration per second}$$
$$= 1\ \text{s}^{-1}$$

Hence

$$1\ \text{Ci} = 3.7 \times 10^{10}\ \text{Bq} = 37\ \text{GBq (exactly)}$$
$$1\ \text{Bq} = 27.\dot{0}\dot{2}\dot{7}\ \text{pCi} \cong 27.03\ \text{pCi}$$

The old special unit, the curie, is to be phased out in the next few years. The list below will assist in obtaining a *feel* for the interrelationship.

10^{6} Ci = 1 MCi = 37 PBq = 3.7×10^{16} Bq
10^{3} Ci = 1 kCi = 37 TBq = 3.7×10^{13} Bq
10^{0} Ci = 1 Ci = 37 GBq = 3.7×10^{10} Bq
10^{-3} Ci = 1 mCi = 37 MBq = 3.7×10^{7} Bq
10^{-6} Ci = 1 μCi = 37 kBq = 3.7×10^{4} Bq
$\sim 2.7 \times 10^{-11}$ Ci = $\sim$ 27 pCi = 1 Bq = 10^{0} Bq
10^{-12} Ci = 1 pCi = 37 mBq = 3.7×10^{-2} Bq

In practice, a radionuclide will often be accompanied by variable quantities of one or more stable isotopes of that element. The stable form is called the carrier. **Specific activity** is the term used to describe the ratio of radioactive atoms to carrier atoms. The specific activity is defined in general as the activity of a particular radionuclide per unit mass of its element or compound. Common

units of specific activity are microcuries per gram of substance (i.e. kilobecquerels per gram of substance).[5]

The absolute (carrier-free) specific activity of a (carrier-free) radionuclide, i.e. the special case in which all atoms of the element present are of the same radioisotope, can be obtained from Rutherford's equation (Eq.(I–7)) by substituting N_A, Avogadro's constant[6], for N, the number of atoms:

$$A_0 = \lambda N_A \qquad \text{(I–9)}$$

If λ, the decay constant, is in reciprocal seconds (s^{-1}), A_0 is obtained directly in becquerels (i.e. disintegrations per second) per mole, or in curies per mole after dividing by the curie-to-becquerel conversion factor:

$$A_0\ (\text{Bq/mol}) = \lambda N_A \qquad \text{or} \qquad A_0\ (\text{Ci/mol}) = \frac{\lambda N_A}{3.7 \times 10^{10}} \qquad \text{(I–10a)}$$

The carrier-free specific activity per gram is obtained by dividing the appropriate form by the gram atomic mass of the radioisotope, M:

$$A_0\ (\text{Bq/g}) = \frac{\lambda N_A}{M} \qquad \text{or} \qquad A_0\ (\text{Ci/g}) = \frac{\lambda N_A}{(3.7 \times 10^{10})M} \qquad \text{(I–10b)}$$

It is worth bearing in mind that, from Eq.(I–6), λ can be obtained from the half-life (expressed in seconds):

$$\lambda\ (s^{-1}) = \frac{\ln 2}{T_{\frac{1}{2}}} = \frac{0.693}{T_{\frac{1}{2}}} \qquad \text{(I–11)}$$

An example of the production of radioisotopes that are carrier-free will be given in § I–1.6.4.

I–1.4. Principles of radiocarbon dating

The method of radiocarbon dating, originally suggested by Libby, is based on two assumptions. The one is that, when living matter dies, the carbon atoms contained in the dead material do not exchange with carbon atoms (e.g. in CO_2)

---

[5] Counts per second per gram are also frequently used in experimental work.

[6] Avogadro's constant (or number), N_A, is the number of atoms or molecules per mole of substance: $N_A \cong 6.022 \times 10^{23}\ \text{mol}^{-1}$.

outside the material. The other is that the specific activity of ^{14}C in nature has been in a steady state and has thus remained at an essentially constant value for many thousands of years (up to the detonation of nuclear weapons).

Carbon-14 is continually produced in the upper atmosphere by the effect of cosmic rays on ^{14}N. Carbon-14 (frequently termed radiocarbon) is converted to CO_2 and enters the biosphere. On the other hand, ^{14}C is continually decaying radioactively with a half-life of about 5700 years. The steady-state specific activity, A_0, has been determined to be approximately 0.25 Bq per gram of carbon.

Hence, for example, a tree that died t years ago had at that time ($t = 0$) a specific activity of ^{14}C equal to A_0. If a piece of wood from that tree is found today (t years later) and the remaining specific activity, A, is determined by a special counter, then t can be calculated from the decay equation:

$$A = A_0 \left(\tfrac{1}{2}\right)^{t/T_{\frac{1}{2}}} \qquad \text{(I–12)}$$

For instance, if the value of a is found to be 0.063 Bq/g, i.e. disintegrations per second per gram, then the specimen is dated as being about 11 thousand years old (i.e. $2T_{\frac{1}{2}}$).

I–1.5. The energy of radiations

The energy unit most commonly used with regard to radiation is the electronvolt (eV). This is equivalent to the kinetic energy acquired by an electron (or any other singly charged particle) accelerated through a potential difference of one volt in a vacuum. This unit is used with SI and has been determined experimentally to be:

$$1 \text{ eV} = 1.602\ 19 \times 10^{-19} \text{ J (approximately)}$$

A commonly used multiple is mega-electronvolts (10^6 eV = 1 MeV).

$$1 \text{ MeV} \cong 1.6 \times 10^{-13} \text{ J} \cong 1.6 \times 10^{-6} \text{ erg}$$

The kinetic energies of the particles and photons emitted by radionuclides have characteristic values. The energies of alpha particles, characteristic gamma and X-ray photons are constant or discrete. The energies of beta particles ejected by a given radionuclide vary, however, from zero up to a certain maximum energy (E_{max}) that is available to the beta particles. This is because a variable part of E_{max} is carried away by a neutrino in every beta particle decay. Neutrinos cannot be detected by ordinary methods as they have no charge and essentially no mass. As a consequence, the beta particles

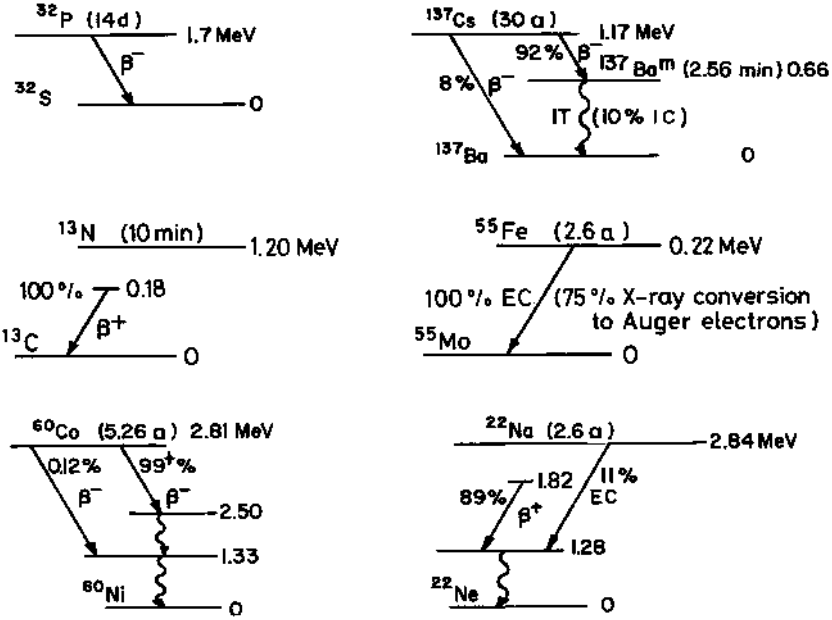

FIG.I–2. Decay schemes showing characteristic radiations and energies of six radionuclides.
IT = Isomeric transition. Different types of the same nucleus are called isomers.
IC = Internal conversion of gamma photon.
EC = Electron capture (K-capture).

show a *continuous spectrum* of energies from zero to E_{max}. The beta energies given in a table or chart of nuclides are E_{max}-values. The average beta-particle energy is usually about one-third of E_{max}. Internal conversion electrons, on the other hand, are monoenergetic.

The characteristic radiations and energies for a given radionuclide are often shown in the form of a decay scheme. Examples of the decay schemes of six radionuclides are shown in Fig.I–2.

I–1.6. Interaction of radiation with matter

I–1.6.1. Alpha particles

The alpha particles ejected from any particular radionuclide are mono-energetic. Their initial kinetic energies are of the order of several MeV and, since ionization potentials and bond energies are in the range 1 – 12 eV, the alpha particles are capable of causing many ionizations as well as electronic excitations of the atoms or molecules along their path. Ionization is complete

removal of the valence electron, and excitation is raising electrons to higher energy levels in their orbits. Since the valence electron participates in any chemical bond of the atom, ionization destroys the integrity of that bond. Alpha particles are doubly charged and of comparatively heavy mass and, therefore, form a dense track of ion pairs (i.e. ejected electrons and positively charged ions) along their path. Hence alpha particles lose energy in matter relatively rapidly by these processes. As the alpha particle dissipates its energy along its path, its velocity decreases, and at zero kinetic energy the particle acquires two electrons from its surroundings and becomes a helium atom. The **range**, i.e. the distance that an alpha particle can penetrate into any matter (absorber), depends on the initial energy of the particle and the density of the absorber. The range of an alpha particle is relatively small and amounts to several centimetres in air and several micrometres (1 μm = 10^{-3} mm) in tissue for energies of the order of 1 to 10 MeV.

Since all the energy of an alpha particle is lost in a relatively thin layer of matter, the LET (linear energy transfer) is high.

I–1.6.2. Beta particles

Beta particles lose energy in matter through ionization and excitation in the same way as alpha particles. The mass of the beta particle, however, is only 1/7300 of the mass of the alpha particle, and beta particles have only unit charge. They will, therefore, be scattered more, penetrate further into matter and produce a less dense track of ion pairs (i.e. electrons have a lower LET) than alpha particles. The range of beta particles in matter is also a function of the initial energy of the particle and of the density of the absorber, but this range is not well defined because of the tortuous path (due to scattering) of the electron. The range of beta particles of 1 MeV initial energy is approximately 3 m in air and 4 mm in tissue. (See also Part VI, Appendix VI–4.).

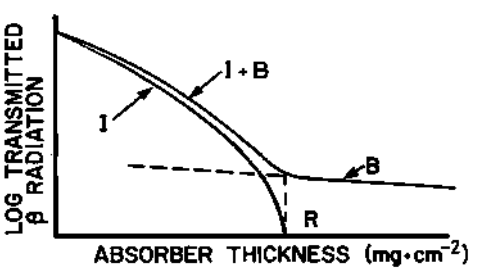

FIG.I–3. Curve demonstrating the beta radiation as a function of absorber thickness.
I = Intensity of transmitted beta radiation.
B = Bremsstrahlung component (and gamma-ray component).
R = Approximate maximum range of beta particles in absorber material.

Partly owing to the fact that beta particles have a continuous spectrum of energies up to E_{max}, their absorption in matter is by chance approximately exponential with absorber thickness. Thus, when the beta radiation transmitted by an absorber is plotted on log-linear graph paper as a function of the mass per unit area[7] of the absorbing material, a fairly straight line is obtained over a portion of the curve (Fig.I–3).

The total transmission curve becomes almost horizontal at R, which is the range of straight-pathed beta particles with an initial energy close to E_{max}. Although all the beta particles are stopped by this thickness of absorber, there is still some transmission of radiation, because the beta particles interact with the atoms of the absorber giving rise to non-characteristic X-rays, the **bremsstrahlung**. In addition, any gamma rays will contribute to this component. By subtracting this component (B) from the composite curve (I + B); the pure beta-transmission curve (I) is obtained.

Positive beta particles, termed positrons, lose their kinetic energy in matter in very much the same manner as negative beta particles. However, when the kinetic energy of the positron has been reduced to zero through ionization and excitation, the positron undergoes **annihilation** with a nearby negative electron, giving rise to two characteristic annihilation photons of 0.51 MeV (8.17×10^{-14} J) each. (In accordance with Einstein's equation, 0.51 MeV (8.17×10^{-14} J) is the equivalent energy of the rest mass of an electron.)

Absorption and scattering of beta particles are important in the measurement of the activity of beta samples. Absorption and scattering will occur in the sample cover, the detector window, the walls of the shield, the intervening air, and in the sample itself (self-absorption). These effects will all influence the count-rate, self-absorption being the most important.

I–1.6.3. Gamma and X-ray photons

Electromagnetic radiation is considerably more penetrating than particulate radiation of the same energy. This is because the photon must first undergo a special absorbing event, whereby either one or two 'secondary ionizing' electrons are produced, before any photon energy becomes dissipated. Gamma rays will be absorbed in matter as a function of the photon energy, as well as the Z and the density of the absorbing material (gamma rays, X-rays and annihilation rays differ only as to their origin, and they interact identically

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[7] Mass per unit area is a product of the density of the absorber multiplied by its 'thickness' parallel to the incident radiation. Its units are: g/cm^2; mg/cm^2; kg/m^2 (SI). Variously called thickness, area density, surface density, density thickness, etc.

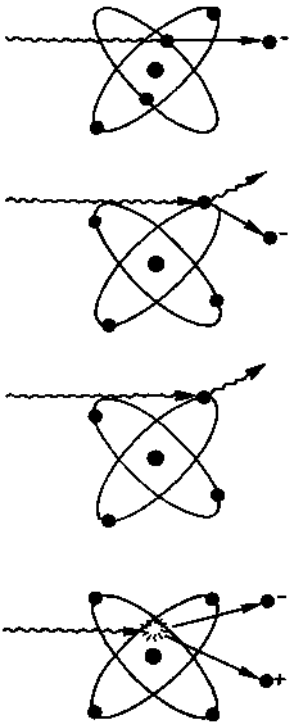

FIG.I–4a. Gamma-ray interactions.

in matter). The following, illustrated in Fig.I–4a and described below, are the four types of attenuating event considered in the ICRU Report 19 [2]:[8]

(a) Photoelectric absorption
(b) Compton effect (absorption and scattering)
(c) Coherent scattering
(d) Pair-production absorption

[8] This section has been revised to correspond with the concept of mass attenuation coefficient as defined in Radiation Quantities and Units, ICRU Report 19[2]. The attenuation coefficient is defined as that fraction of particles (including photons) that experiences interactions in traversing a given distance in a certain medium, where ". . . the term *interactions* refers to processes whereby the energy or direction of the indirectly ionizing particles is altered."

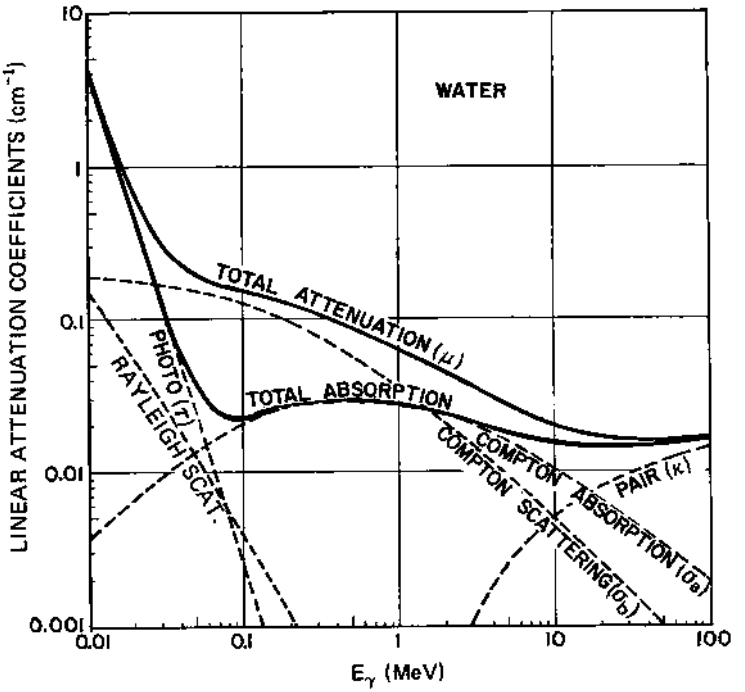

FIG.I–4b. Linear attenuation coefficients for gamma rays in water (the total Compton attenuation, $\sigma_c = \sigma_a + \sigma_b$).

(a) **Photoelectric absorption** is predominant for relatively low-energy gamma photons and for absorbing material of high-Z. The gamma ray interacts with a K or L electron of the absorber atom and expels the electron from the atom with a kinetic energy equal to the initial gamma-photon energy minus the binding energy of that K or L electron. Thus, an electron is ejected with kinetic energy, enabling it to produce ionizations and excitations along its path exactly in the manner of a beta particle.

In Fig.I–4b, the coefficient for photoelectric absorption (τ) is given for water as a function of gamma-photon energy, E_γ. The absorption coefficient is a measure of the probability of absorption (average number of events per cm).

(b) **Compton effect** is the interaction of the gamma photon with an outer electron of the absorber atom or with a free electron. Part of the initial kinetic energy is absorbed (transferred to the electron), and the photon is scattered off in a new direction at a lesser energy. The photon will eventually after multiple scattering be absorbed through the photoelectric effect. As can be

seen from Fig.I–4b, the coefficient for Compton absorption (σ_a) is at a maximum in water for gamma rays of about 0.5 MeV. The effect rises only slightly with increasing Z. The fast electron arising from a Compton event will produce ionizations and excitations again exactly in the manner of a beta particle.

(c) **Coherent scattering** is a process in which photons are scattered after interaction with orbital electrons. The electrons return to their original state, there is no change in photon energy, and there is a relation in phase of the scattering from different electrons of the atom. Rayleigh scattering is another name for this process. Such scattering is important only for low-energy photons (< 0.1 MeV) and high-Z materials.

(d) **Pair-production absorption** may occur when the gamma photon has an initial energy of at least 1.02 MeV (1.63×10^{-13} J). In this process, the gamma-ray photon interacts with the positive field of the nucleus of the absorber atom and is completely used up in producing a positron-electron pair.[9] Since it requires 1.02 MeV (1.63×10^{-13} J) for the formation of the two electron rest masses, this is the energy threshold for the pair-production event. Any gamma-photon energy above the required 1.02 MeV is imparted as kinetic energy to the positron-electron pair. Both the positron and the negative electron cause ionizations and excitations along their respective paths. The two 0.51 MeV photons produced upon annihilation of the positron are subsequently absorbed by a photoelectric event or a combination of Compton and photoelectric events. In Fig.I–4b, the absorption coefficient for pair production is labelled as κ.

Considering the above processes (single, random events), a beam of monoenergetic gamma rays is absorbed exponentially as a function of thickness, x, of the absorbing material. For a beam of intensity I, the change in intensity per unit absorber thickness, dI/dx, is proportional to the intensity of the beam at that point. Thus,

$$\frac{dI}{dx} = -\mu I \qquad \text{(I–13)}$$

Equation (I–13) is identical with the well-known Lambert-Beer law for attenuation of monochromatic light. The proportionality constant (μ) is termed the **total attenuation coefficient** (Fig.I–4b). Exactly analogous to the radioactive decay constant, λ, μ is the fraction of the original intensity removed from the beam per unit linear thickness of absorber. Equation (I–13) is

[9] When the term electron is used in this text, it can be taken to refer to the negative electron, unless otherwise stated.

mathematically identical with the radioactive decay law (Eq. (I–1)) and may be integrated to give:

$$I = I_0 e^{-\mu x} \tag{I–14}$$

Here, μ, the total attenuation coefficient for gamma and X-ray photons is given by:

$$\mu = \tau + \sigma_c + \sigma_{coh} + \kappa \tag{I–15}$$

where τ is the photoelectric attenuation coefficient;
σ_c is the total Compton attenuation coefficient ($= \sigma_a + \sigma_b$, see Fig.I–4b);
σ_{coh} is the attenuation coefficient for coherent scattering;
κ is the pair-production attenuation coefficient.

The numerical value of μ is dependent on the gamma-photon energy (E_γ) and the type of absorber material. Figure I–4b illustrates the attenuation probabilities for water as a function of E_γ.

Again analogous to radioactive decay, the thickness at which I is reduced to one-half its initial value is termed the **half-thickness** or **half-value layer** (HVL), $X_{\frac{1}{2}}$, and one finds:

$$X_{\frac{1}{2}} = \frac{\ln 2}{\mu} = \frac{0.693}{\mu} \tag{I–16}$$

an equation of a form similar to Eq.(I–11), i.e. Eq.(I–6).

Equation (I–14) may alternatively be expressed as follows:

$$I = I_0 \left(\tfrac{1}{2}\right)^{x/X_{\frac{1}{2}}} \tag{I–14a}$$

an equation of a form similar to Eq.(I–8a).

An understanding of the interactions of high-energy electromagnetic radiation with matter is necessary in considerations of shielding, dose calculations, and measurement of gamma, X and annihilation photons.

I–1.6.4. Neutron production and interaction processes

Neutrons have no charge and, therefore, cannot ionize directly. However, they can produce ionization indirectly and are generally considered in discussions of ionizing particles.

Neutrons are produced by fission processes, and the most common sources of neutrons are nuclear reactors that control fission chain reactions. Neutrons can also be produced by small laboratory sources, and these sources will be discussed in greater detail in § I–6.1.

Neutrons lose energy and interact with matter by the following processes:

(a) Elastic collisions. Neutrons of high initial energy, *fast neutrons*, interact with other nuclei in billiard-ball-like collisions, losing a fraction of their kinetic energy per collision. By this moderation process they eventually reach an energy that is the same as that of molecules in thermal equilibrium with their particular environment. They are then termed *thermal neutrons.* The light elements, especially hydrogen, are the most efficient for this moderating process.

A fast neutron undergoing an elastic collision with another atom or molecule will generally produce a fast-recoil ionized atom (e.g. a proton from hydrogen). This recoil ion will then cause ionizations and excitations along its path.

(b) Absorption reactions. Examples are given below of the four principal types of absorption reactions. These occur predominantly with slow or thermal neutrons.

(i) (n,γ) reaction

$$^{59}_{27}\mathrm{Co} + {}^{1}_{0}\mathrm{n} \rightarrow {}^{60}_{27}\mathrm{Co} + \gamma$$

This type of reaction is used to produce many of the artificial radionuclides. In the example, the ^{60}Co produced cannot be chemically separated from the stable ^{59}Co in the sample, and this is an example of production of a non-carrier-free radionuclide.

(ii) (n,p) reaction

$$^{14}_{7}\mathrm{N} + {}^{1}_{0}\mathrm{n} \rightarrow {}^{14}_{6}\mathrm{C} + {}^{1}_{1}\mathrm{H}$$

This is the reaction by which *cosmic-ray* neutrons produce ^{14}C activity in the biosphere. This reaction is also used to produce ^{14}C commercially and, since the ^{14}C can be chemically separated from the nitrogen compound in the sample, it is an example of the production of a carrier-free radionuclide.

(iii) (n, fission) reaction

$$^{235}_{92}\mathrm{U} + {}^{1}_{0}\mathrm{n} \rightarrow {}^{90}_{36}\mathrm{Kr} + {}^{144}_{56}\mathrm{Ba} + 2\ {}^{1}_{0}\mathrm{n}$$

This illustrates fission of ^{235}U into two fission fragments and two additional neutrons.

(iv) (n, α) reaction

$$^{10}_{5}B + {}^{1}_{0}n \rightarrow {}^{7}_{3}Li + {}^{4}_{2}He + \gamma$$

This is a reaction used to detect neutrons.

I–1.6.5. Neutron activation analysis

If a stable element is exposed to a flux of neutrons there is a finite probability that a stable nucleus can capture a neutron to produce an isotope of that element, with an increase of one in the mass number. As discussed above, this activation process is a primary method of producing artificial radionuclides. An example of such a capture reaction is:

$$^{23}_{11}Na + {}^{1}_{0}n \rightarrow {}^{24}_{11}Na + \gamma$$

often abbreviated

$$^{23}_{11}Na\ (n,\gamma)^{24}_{11}Na$$

In general, such activation reactions are most probable with thermal or slow neutrons. The activation reaction is utilized in an analytical technique termed *neutron activation analysis* described below.

Let n_T be the number of nuclei of a specified stable nuclide exposed to a flux, ϕ, of thermal neutrons. Let σ represent the cross-section per nucleus or the probability of a capture reaction occurring. Then the rate of production of radioactive atoms, N, will be:

$$\left(\frac{dN^*}{dt}\right)_{prod.} = \sigma\, n_T\, \phi \qquad \text{(I–17)}$$

where σ = cross-section (cm^2 per nucleus) (see footnote 10);
n_T = total number of specified stable nuclei exposed;
ϕ = thermal neutron flux density ($cm^{-2} \cdot s^{-1}$).

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[10] Cross-sections are often given in a unit called a barn; $1\ b = 10^{-24}\ cm^2 = 10^{-28}\ m^2$.

However, the radioactive atoms, N, produced by activation will immediately begin to decay at their own rate, characterized by the $T_{\frac{1}{2}}$ of the radionuclide produced. Therefore, the above equation must be modified to include this rate of decay. Thus:

net production rate = (rate of production) − (rate of radioactive decay)

$$\frac{dN^*}{dt} = \sigma n_T \phi - \lambda N^* \qquad (I-18)$$

This equation can be integrated to give the *neutron activation equation:*

$$A^* = \sigma n_T \phi (1 - e^{-\lambda t_{irr}}) \qquad (I-19)$$

where σ, n_T and ϕ have the same meaning as given above, and

A^* = activity produced at end of irradiation period (Bq, i.e. disintegrations per second);

λ = decay constant of radioisotope produced (s^{-1}) (see Eq.(I−11));

t_{irr} = duration of irradiation period (s).

Usually the technique is used to determine the number of atoms, n_T, of a certain stable nuclide in a sample. If the flux, ϕ, irradiation time, t_{irr}, and cross-section, σ, are known, and the activity produced, A^*, is determined, this theoretically allows calculation of n_T. Otherwise, a comparator technique can be used (i.e. simultaneous irradiation of a standard with a known amount of the sample element), as is frequently done in practice.

With a nuclear reactor as the source of neutrons, the technique is extremely sensitive for small amounts of certain substances (e.g. selenium) that cannot be analysed by conventional chemical methods.

The element for analysis should have a high abundance of the reacting stable isotope, and (as seen from the neutron activation equation) this isotope should have a high cross-section, σ. The half-life of the radioisotope produced should neither be so short as to preclude measurement or so long as to preclude using the correspondingly long irradiation period needed. The detection limit for certain elements can be as low as a picogram (10^{-12} g).

I–2. RADIATION DETECTION AND ASSAY OF RADIOACTIVITY

The radiations which come from radioisotopes interact with all matter (gaseous, liquid or solid), causing chemical changes, ionizations and excitations. These effects are utilized in the various methods of detection and measurement.

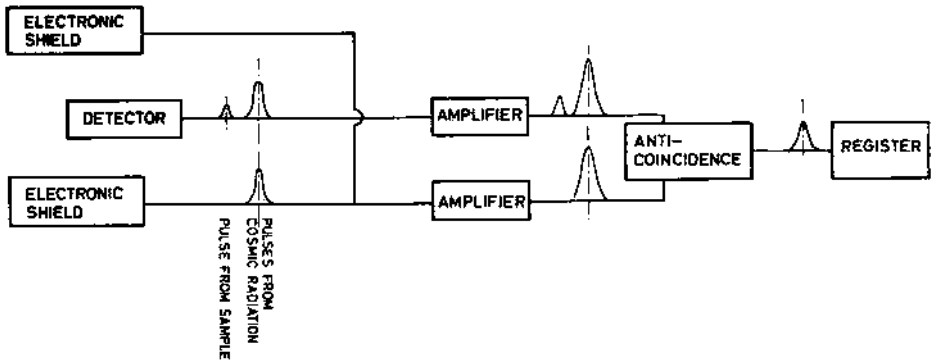

FIG.I–5. Block diagram illustrating an anti-coincidence unit used as an electronic shielding against cosmic radiation.

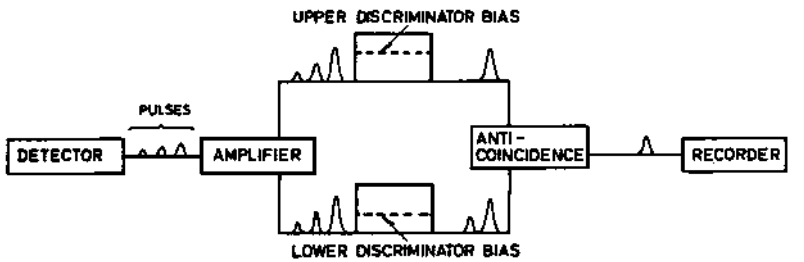

FIG.I–6. Block diagram illustrating a pulse-height analyser.

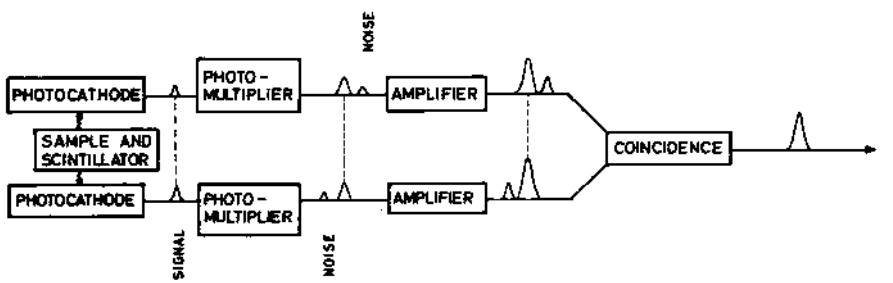

FIG.I–7. Block diagram illustrating a coincidence unit.

In radiography, for example, ionizing radiations are detected by their effect on photographic, X-ray or nuclear emulsion.

In the ionization chamber, the gas-flow detector, the Geiger-Müller tube and the neutron detector, ions produced directly or indirectly by the radiation are collected on charged electrodes.

In solid and liquid scintillation counting, emission photons (in the blue to ultraviolet region) form the basis of detection.

Solid-state detectors, more properly called semiconductor radiation detectors, are crystals whose electrical conduction is altered by the absorbed radiation. Their operation depends on their semiconductor properties, and this class of detector has become of great importance in dosimetry.

Besides a radiation detector, a monitoring or measuring set-up includes one or more of the following electronic units:

Power unit. The primary source of power is either a battery or the mains supply. The detector potential requirements range from a few hundred to a few thousand volts, and good stabilization is generally necessary.

Amplifier. The primary signal is often an electronic pulse or electric current that is too small for registering unless amplification is used. Furthermore, in proportional counting the amplification must be linear, i.e. the magnification factor must be independent of pulse size.

Timing unit. This ranges from a stop-watch to an automatic unit which stops the detector at the end of a predetermined time interval or registers the time necessary for accumulation of a pre-set number of counts.

Pulse input sensitivity. An electronic discriminator biased to reject all pulses below and/or above a certain size. This improves the signal-to-noise ratio.

Anti-coincidence unit. This electronic unit rejects pulses that arrive 'in coincidence', i.e. both arriving within a very short time interval (e.g. 1 μs). An anti-coincidence unit is used for so-called electronic shielding against cosmic radiation (see Fig. I–5) and for pulse-height analysis.

Pulse-height analyser. This consists essentially of two variable discriminators (a lower and an upper one), together with an anti-coincidence unit. With this auxiliary equipment, only pulses within a set pulse-height interval are registered (see Fig. I–6).

Coincidence unit. This unit rejects all single pulses but passes one pulse when two pulses arrive in coincidence (e.g. within 1 μs). A coincidence unit is generally used in conjunction with two scintillation detectors, in order practically to eliminate photomultiplier noise pulses (see Fig. I–7).

Registering unit. This may be a scaler, i.e. a set of decades displaying the sum count or a certain fraction thereof, a count-rate meter (visible or audible), a voltmeter reading out accumulated radiation dose, a sensitive electric-current meter displaying dose rate, or even a recording potentiometer.

Historically, it might be noted that de Hevesy used a simple metal-leaf electrometer for his pioneer work, and since then a great deal of useful work in biological research has been done, and is still being done, with a Geiger-Müller counter, a stop-watch and a pocket dose meter or film badge.

A number of detectors and some associated electronic equipment will now be described in more detail.

I–2.1. Autoradiography

This method is a photochemical process and the one used by Becquerel in 1896 in the discovery of radioactivity. In autoradiography, ionizing radiations interact with the silver halide in photographic emulsions. When radioactive material is placed near a photographic plate or film, a blackening will be produced on development of the emulsion. The blackened areas constitute a *self-portrait* of the activity in the material. The intensity of the blackening (as determined by eye or with a densitometer) at a given place will be a function of the exposure time and the amount of activity in the sample at that place. It also is a function of the LET (linear energy transfer) of the particular radiation. Gamma rays and X-rays have a low LET and are of little use in autoradiography, because the photons from a given place in the sample material will penetrate throughout a large area of emulsion, producing an almost uniform fogging on development. Conversely, alpha particles and low-energy beta particles (^{3}H, ^{14}C, ^{35}S, ^{45}Ca), which have a high LET, are very effective. High-energy beta particles produce diffuse radiograms owing to the relatively long path-lengths of these particles in the emulsion. The properties of the emulsion should be a compromise between fine grain to increase the resolution and high sensitivity to reduce the exposure time. Usually, exposure times are long because, to obtain a good image, an absorption of about 10^{7} soft beta particles is needed per square centimetre of emulsion. Thus, a thin histological section containing 1 to 10 Bq/cm^2 (1 to 10 dis$\cdot$s$^{-1}\cdot$cm^{-2} = 27 to 270 pCi/cm^2) will require several weeks exposure to show optimal blackening.

The method of autoradiography is particularly suitable when the *distribution* of a radioactive compound in biological material is to be studied. However, precautions should be taken that there is no chemical or pressure effect of the material on the emulsion as this may also produce an image.

Various techniques have been worked out, each with specific advantages and disadvantages. Apart from the chemical effect on emulsions, complications with regard to the drying or pretreatment of samples, the transport of radioactive compounds under moist conditions and the self-absorption of low-energy particles in the biological material may occur; film development conditions will also affect the image. Hence the interpretation of autoradiograms of biological material is not straightforward.

Autoradiography is frequently applied to the determination of the components of a paper chromatogram.

Micro-autoradiography is useful when the distribution of radioactive compounds in a section is to be studied. Either the sections on the slides may be coated with melted emulsion, or a stripping film may be used to cover the sections on the slides.

A more recent technique is the combined use of autoradiography and electron microscopy, for example in the study of sub-cellular organelles labelled with ^{3}H.

I–2.2. Ionization detectors

A number of detectors are based on the principle that, in an electric field, negative particles will move to a positive electrode and positive particles to a negative electrode. Charged particles which arrive at an electrode will give rise to an electronic pulse, which can be amplified and registered. Alternatively, the pulses may be merged to form an electric current, which again can be amplified and measured.

Alpha and beta particles and IC electrons have a high specific ionization, i.e. produce a great number of ion pairs per unit length of track. Gamma and X-rays have a much lower primary specific ionization; but at least one fast electron will be released by each photoelectric effect or Compton scattering (or pair production if the energy is very high), and these fast electrons will ionize just as do beta particles. Neutrons may also produce ions, directly (by collision) or indirectly (following nuclear absorption), as described in § I–1.6.4 above. Detection by ionization of these kinds of radiation is based on the fact that atoms of a gas (in the detector) will become ionized when they are hit by the radiation particles or photons. The number of ionizations in the gas is a direct measure of the quantity of ionizing particles or photons (α,β, e, γ, X or n) that reach the detector. When an electric field is created in the detector, the negative ions and/or electrons will start moving and, by hitting the positive electrode (anode), discharge. Likewise the positive ions will move toward the cathode.

Five different types of ionization instrument will now be described.

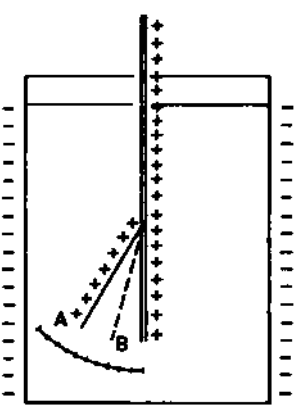

FIG.I–8. Electroscope.

I–2.2.1. Electroscope

In the electroscope or simple electrometer (see Fig. I–8) the positive electrode is a rod with a wing or a metal string, and the negative electrode is the wall of the detector.

When the electroscope is fully charged, the deflection of the wing or string will be maximum (A), the amount of deflection being a function of the charge accumulated. When a radioactive source is brought near the detector, the air in the detector will become ionized and electrons will move in the direction from wall to rod. As a consequence, the deflection will decrease (B).

This type of detector is commonly used as a 'pocket dose meter' and gives a measure of the accumulated dose of external radiation (gamma, X and hard beta radiation) to which a worker has been exposed during a certain period.

I–2.2.2. Gas-filled detectors with collector/cathode voltage bias

Not all the ions will discharge on the electrodes of an electroscope, since a certain number will have recombined before they reach the electrodes.

If a bias voltage is applied between the cathode and collector (anode), the losses due to recombination in the volume of the detector decrease until, eventually, all the ions will be discharged on the electrodes. If the bias voltage is further increased up to a certain limit, the number of ion pairs that discharge will remain constant, i.e. each ionizing particle or photon that interacts will give rise to an electric pulse on the electrodes. Detectors operating in this mode are termed ionization chambers.

Figure I–9 shows a plot of pulse size against bias voltage. Region I is the ionization chamber region. Curves are drawn for an alpha and a beta particle traversing the sensitive volume of the detector.

As the bias voltage is increased, the ions produced will move towards their respective electrodes with greater velocities, and at some voltage they will gain sufficient kinetic energy to cause further ionization in the gas, called secondary ionization. This process is known as gas amplification, and the flood of ions produced is termed the Townsend avalanche. As a result of gas amplification, each incident ionizing particle will lead to the formation of a relatively large electronic pulse. The pulse size produced increases rapidly with applied voltage.

When the bias voltage results in gas amplification (region II), the size of the pulse produced by a particle (at a given voltage) is proportional to the number of primary ion pairs formed by the particle in the initial event. This is termed the proportional region.

In region III, the bias voltage is so great that the charge collected on the anode attains a maximum size independent of the number of primary ions formed.

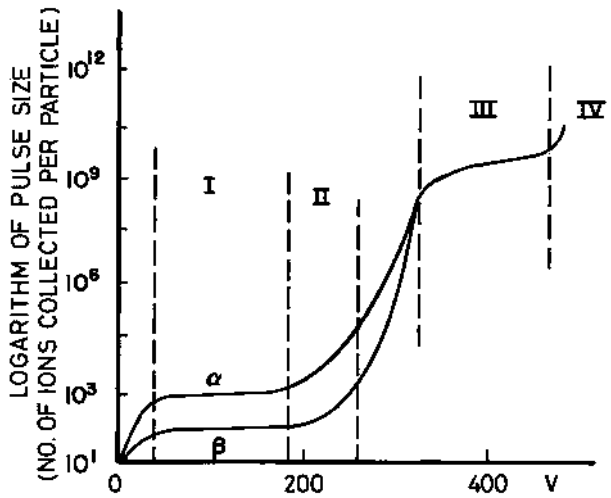

FIG.I–9. Plot of logarithm of pulse size versus electrode voltage; I – ionization-chamber region; II – proportional region; III – Geiger-Müller region; IV – region of continuous discharge. (The voltage corresponding to a given region varies greatly from one make of instrument to another.)

In this region, at a given voltage, all pulses are of the same size, irrespective of the number of primary ions, and this is termed the Geiger-Müller (GM) region.

In region IV (and to some extent in region III) the discharge in a GM tube would continue indefinitely if it were not stopped or quenched. For this purpose certain quenching-gas molecules are added to GM tubes to stop the discharge, for example, gaseous halogens such as chlorine. When they collide with positive ions, quenching gas molecules dissociate rather than become ionized themselves and, in this fashion, the discharge is stopped. The halogen gas atoms subsequently recombine.

I–2.2.3. Ionization chamber

When a detector is operating in region I (Fig. I–9) each ionizing particle or photon gives rise to an electric pulse on the electrodes. A constant stream of particles or photons gives rise to a continuous series of pulses, forming a weak electric current, which may be amplified and registered by an electronic circuit. Such detectors are termed *ionization chambers*, and they are often filled with air. A typical measuring circuit is shown in Fig. I–10. The final scale reading will then be a measure of the energy dissipated in the ionization chamber per unit of time by the ionizing particles or photons. This kind of detection instrument is thus a *dose-rate meter*. (A well-known version of this type of meter

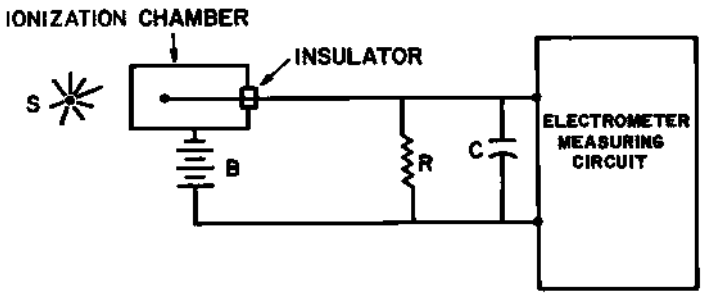

FIG.I–10. Schematic diagram of ionization-chamber circuit; B = voltage source (battery); R = resistor; C = capacitor; S = radioactive source.

is the Cutie Pie.) When the walls of the ionization chamber are constructed of air-equivalent material, the instrument can be used to measure absorbed dose of gamma or X-rays in air. There are also chambers having biological tissue equivalence.

A small, electrically-charged ionization chamber, held in place for instance by a finger ring, may be used to measure accumulated exposure dose. An electronic vacuum-tube voltmeter is often necessary to measure the charge reduction, which is proportional to dose.

I–2.2.4. Proportional counter

Proportional counters operate in region II (Fig. I–9), when secondary ionization has become important. The electrons that have arisen from primary ionization will produce secondary ion pairs of the gas atoms in the counter tube as they are accelerated towards the anode. This process of secondary ionization becomes increasingly important as the voltage difference between the electrodes is further increased. The final pulse size will be proportional to the energy of the initial ionizing particle (as long as all this energy is dissipated in the detector), provided the applied voltage remains constant during the measurement. Usually the radioactive sample will be placed inside the detector, which will be transfused by a gas at atmospheric pressure (gas-flow counters). In this way particles of low energy, such as the β^- from ^{14}C, may be counted effectively ('windowless' counting), provided suitable amplification precedes the register.

I–2.2.5. Geiger-Müller (GM) counter

When the voltage difference between the electrodes of the detector is still further increased, secondary ionization becomes predominant and each primary

ionizing event results in a discharge of a great number of electrons (avalanche). At this stage the large output pulse is independent of the energy of the initial particle or photon, and a further increase of the high voltage does not appreciably alter pulse size or count-rate. Geiger-Müller counter detectors (GM tubes) operate at this high voltage *plateau*. The discharges of secondary electrons initiated by one ionizing particle or photon would continue if the detector were of an open design, as in the gas-flow counter (atmospheric pressure). GM tubes operate at a reduced gas pressure (about one-tenth atmosphere), and contain a certain amount of *quenching gas*. Usually the closure of a GM tube is a very thin mica window (1–3 mg/cm^2), and the filling gas is often a noble gas like argon with, for example, alcohol or halogen as the quenching gas. A certain number of molecules is dissociated during the quenching of a discharge with alcohol. Therefore, the quantity of quenching gas in the GM tube decreases steadily, and consequently the life of the tube is limited by this effect. This disadvantage does not exist when a halogen gas, e.g. chlorine, is used for quenching, because the atoms of the dissociated chlorine molecule recombine; the life of the tube is therefore determined by other effects, such as corrosion and leakage.

Energetic beta particles, electrons and gamma or X-photons emitted by radioactive liquids may be counted with a thin glass wall 'dip-counter' GM tube, which is immersed in the liquid, or with a specially designed liquid detector that consists of a cylindrical glass container around the GM tube. The radioactive liquid thus surrounds the GM tube in both cases. Particles of low energy can obviously not be counted in this way because of absorption in the wall of the GM tube.

Detectors operated in the Geiger-Müller region are very sensitive to beta particles, and very little additional amplification of the pulse is necessary to drive a counting circuit. In addition, they are almost insensitive to normal voltage fluctuations. Furthermore, they are relatively inexpensive. A simplified GM counter circuit is shown in Fig. I–11.

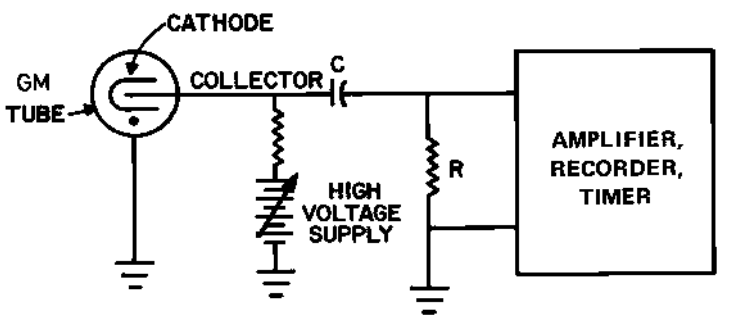

FIG.I–11. Counter input circuit with a GM tube detector; C = capacitor; R = resistor.

The fact that some time is required to collect the flood of positive ions from each discharge, as well as for the quenching process to take place, implies that during this period the detector tube will be insensitive to other ionizing particles entering its sensitive volume. This period is 100 to 300 microseconds for GM tubes and is termed the dead time, t_{dead}. The time needed for a complete recovery of pulse size *after* the dead time is termed the recovery time, t_{rec}. The time required before a subsequent pulse will again be recorded in the counting system, termed the resolving time, τ, lies between t_{dead} and $t_{dead}+t_{rec}$; as the voltage amplification system is made more sensitive, so τ approaches t_{dead}. Since the dead time, and hence the resolving time, vary in a given tube, even from pulse to pulse, it is common practice to fix τ at a value somewhat greater than $t_{dead,max}$ *electronically* in the counting system. At high count-rates, counts will be lost due to the inoperative period of the counting system, and hence a resolving-time correction must be made to obtain the true counts from the figure registered.

Let R be the observed counts of a GM counter collected in one second and τ (fraction of a second) the resolving time of the counter. During one second, the counter will have been ineffective for $R\tau$ (fractions of a second). Therefore, R counts have really been registered (to a first approximation) in only $(1 - R\tau)$ seconds. The true count-rate, R_{true}, is therefore (approximately):

$$R_{true} = \frac{R}{1 - R\tau} \qquad \text{counts/s} \qquad \text{(I–20)}$$

A method for determining the resolving time of a counter is given in Part IV, §IV–1.2. Correction is normally not necessary unless the count-rate exceeds about 50 counts/s. Equation (I–20) is only approximate, therefore it should not be used to make corrections that would be more than about 10% of R, otherwise it would be better to dilute the sample or to count it at a greater distance from the detector.

GM counters are used most widely for the detection and measurement of beta particles. For gamma rays they are not very effective (1–3% efficiency), because most of the photons will penetrate the gas without any interaction. For the detection of beta particles on glass-ware, benches or trays, monitors are used. A monitor consists of a GM tube connected to a power unit and a count-rate meter. Often a small loud-speaker is connected to the rate meter, so that a noise will warn the operator when the tube is in the vicinity of a contaminated spot.

The necessary associated equipment for a GM counting system includes (besides the GM tube) a high-voltage supply, an amplifier (possibly with a discriminator), a recorder and a timer. This is illustrated in Fig. I–11.

I–2.3. Solid scintillation counting

A scintillator emits a small flash of light when struck by a fast, charged particle. An example is (Ag) ZnS hit by an alpha ray.

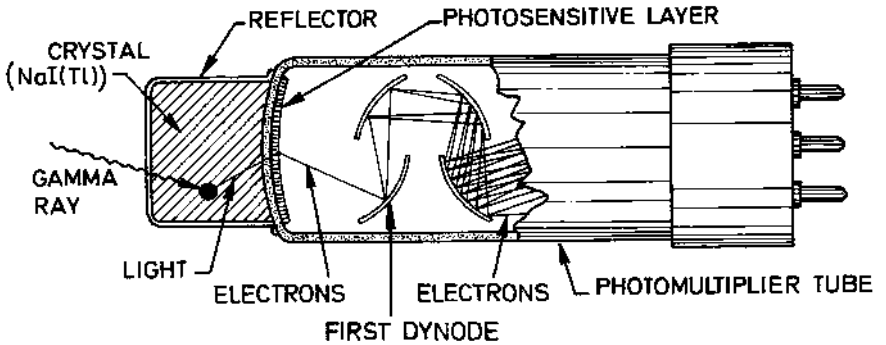

FIG.I–12a. Scintillation detector.
Total light to tube is nearly proportional to gamma-ray energy. If 1 electron ejects 5 from a dynode, 11 dynodes result in 5^{11} electrons, i.e. about 50 million electrons output.

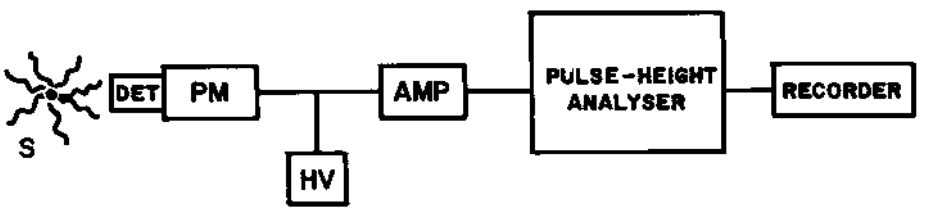

FIG.I–12b. Block diagram of NaI(Tl) scintillation counter system.
S = Gamma-ray source.
DET = NaI(Tl) scintillation detector.
PM = Photomultiplier tube.
HV = High-voltage power supply.
AMP = Linear amplifier.

Solid scintillators (fluors) are particularly suited for the detection of gamma rays (besides X-rays and annihilation radiation) because of the high density and high-Z of certain solid crystals. The alkali halides, in particular NaI (activated with Tl), have been the most useful. A typical scintillation crystal detector is shown in Fig. I–12a, and a diagram of a counter system in Fig. I–12b.

When a gamma-ray photon is partially or totally absorbed in the scintillation crystal, at least one fast electron is liberated (it will be either a photoelectron, a Compton electron or pair-production electrons, depending upon the absorbing event). These fast electrons cause excitation and ionization along their paths in the crystal. When the atoms, thus excited, return to their ground-state they emit light photons with an intensity maximum in the violet or near-ultraviolet

spectral region. The total number of light photons emitted will be proportional to the amount of the gamma-photon energy that is lost in, and absorbed by, the crystal.

The photocathode of a photomultiplier tube is optically coupled to one face of the scintillation crystal, and the light photons produced in the crystal are internally reflected until they reach the photocathode. Here, by the photoelectric effect, they release photoelectrons. The number of these photoelectrons again is proportional to the gamma-photon energy originally absorbed in the crystal.

In the photomultiplier tube the photocathode is connected to a series of electrode stages or dynodes, each at a potential more positive than that of the preceding stage. Thus, photoelectrons released from the photocathode surface will be attracted to the first dynode and will gain sufficient kinetic energy to release two or more secondary electrons from the surface of this dynode. This multiplication process occurs at each stage and, at the end of ten or more stages in a typical photomultiplier tube, a large number of electrons will arrive at the anode. The size of this pulse of electrons will be proportional to the original gamma-ray energy lost in the crystal. The pulse is then amplified linearly and directed to a scaler or to a pulse-height analyser.[11]

In a pulse-height analyser, the pulses are sorted according to their size by pulse height and stored in the appropriate portion of an electronic memory. After counts have been collected for a period of time, the read-out of the memory will be a gamma-ray spectrum of the radiation absorbed by the scintillation detector.

The energy lost in the scintillation crystal by an incident gamma photon will range from zero to E_γ depending upon the absorption event. For instance, the gamma ray can be absorbed by the photoelectric effect, or by a Compton interaction followed by photoelectric absorption of the scattered photon, or by any combination of processes that lead to total absorption of the gamma-photon energy within the crystal. If this occurs, then the output pulse will be stored in a location corresponding to the full E_γ-value. A typical monoenergetic gamma-ray spectrum is shown in Fig. I—13 and the resulting peak is labelled as the *total-absorption peak.* If, however, the primary interaction in the crystal is of the Compton type and the scattered photon escapes from the detector, then the energy absorbed within the crystal will be less than E_γ. The range of possible Compton interactions results in a distribution of pulse sizes ('Compton smear'). This distribution is labelled as the Compton region in Fig. I—13.

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[11] The pulse height (voltage level) is used to determine the lower and upper levels of discrimination for pulse analysis. The term pulse size is sometimes used synonymously, and sometimes for the area under a pulse, which is obtained using a computer program linked to the pulse-height analyser output. In this manual, the common (commercial) term, pulse-height, is used wherever *voltage-level discrimination* would be used in the measurement.

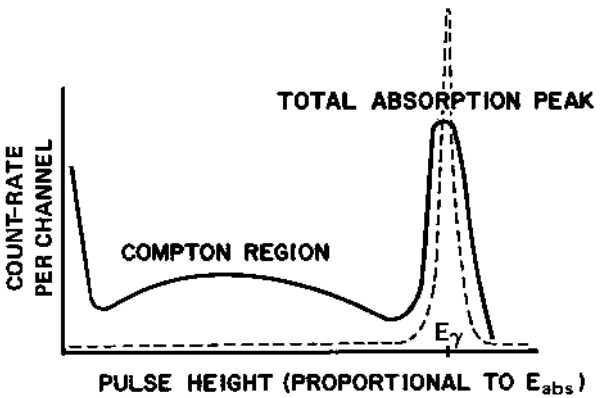

FIG.I–13. Observed gamma-ray spectrum of a radionuclide emitting mono-energetic photons.
E_{ab} = *Gamma-photon energy absorbed by crystal.*
---- *Incident gamma-ray energy distribution.*

The location of the total-absorption peak is characteristic of E_γ and is useful in identifying the corresponding gamma-ray emitter in any sample. The area under the total-absorption peak is proportional to the activity of that radionuclide in the sample. The peak is actually broadened into a distribution due to (a) instrumental broadening and (b) statistical broadening as a result of the several conversion steps from gamma-photon absorption to final pulse.

Multi-channel pulse-height analysis is only required when it is necessary to measure the activity of one gamma-ray emitter in the presence of one or more others, e.g. in neutron-activation analysis or multiple-tracer experiments. Generally, a less expensive single-channel pulse-height analyser is sufficient.

Another distinct advantage of solid scintillation counting is the very short resolving time of such systems. This enables high count-rates to be determined (up to about 1000 counts/s) without the necessity of resolving-time corrections.

I–2.4. Liquid scintillation counting

Liquid scintillation counting techniques have promoted the application of radionuclides in the biological and agricultural sciences because they have allowed much wider use of low-energy beta-particle emitters such as ^3H and ^{14}C to be made.

In this technique, the sample to be counted is placed in solution, together with an organic scintillator (the detector material), in an organic solvent. Since each radioactive atom or molecule is closely surrounded by molecules of the scintillator, self-absorption preventing detection (see §I–2.6) is greatly reduced,

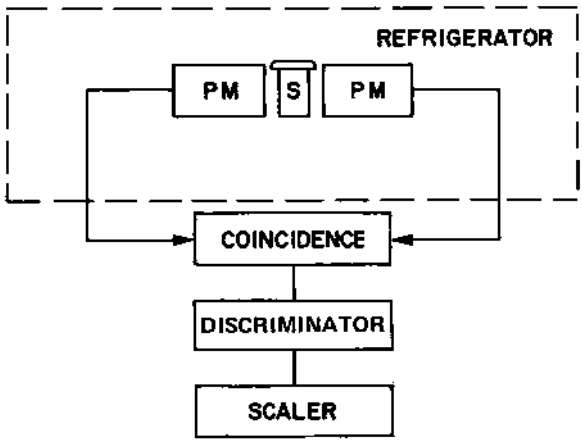

FIG.I–14. Block diagram of a typical liquid scintillation counter.
S = Counting vial containing liquid scintillation solution and sample.
PM = Photomultiplier tubes.
Refrigerator = Refrigerating unit (optional).
Coincidence = Prompt coincidence and sum circuit.
Discriminator = Upper- and lower-level discriminator.

and the counting efficiency greatly increased. If the sample is insoluble in the organic solvent, it may often suffice to put it into uniform suspension.

There are now many solute/solvent liquid scintillator systems in use. A very common one is PPO (2–5 diphenyloxazole) with toluene or dioxane as the solvent.

The ionizing particles from the radioactive material cause excitation and ionization of the solvent molecules. These transfer their excitation energy to the PPO molecules which in turn fluoresce or scintillate, i.e. give rise to light photons on returning to their ground-state. The number of light photons emitted from the counting vial due to any one ionizing particle is proportional to the energy lost by that particle in the solution.

The counting vial is optically coupled to a photomultiplier tube system to collect the emitted light. A block diagram of a simple liquid scintillation system is shown in Fig. I–14.

Normally, two photomultiplier tubes are used to collect the light emitted from the scintillation vial. This is done to increase the sample-to-background counting ratio as follows: After each single ionizing event, light photons will normally be registered at both photocathodes simultaneously. The coincidence circuit (Fig. I–14) is designed to produce one output pulse if it receives an input pulse from each of the two photomultipliers simultaneously (within about 1 μs), i.e. in coincidence. Background or electronic noise pulses from

either photomultiplier tube will seldom be in coincidence with those in the other, and will therefore be rejected. Thus, the ratio of true count-rate to background, and thereby the sensitivity, is increased. Since the size of the output pulse is proportional to the energy lost in the liquid scintillator, limited pulse-height analysis is possible. It is limited (a) because of the shape of beta spectra and (b) because the pulse-height resolution is poor. However, it is generally possible to count, for instance, ^{3}H and ^{14}C simultaneously.

The detector part of the system is often refrigerated to reduce thermally produced electron noise in the photomultiplier tubes.

For certain high-energy beta emitters ($E_{max} > 0.26$ MeV), it is often possible to employ so-called Cerenkov counting techniques using the liquid scintillation counter. In such cases, the radioactive sample need only be dissolved or suspended in water. An ionizing particle travelling through a medium (here water) at a velocity greater than the velocity of light in that medium produces a flash of Cerenkov light. The rate of production of the light flashes is proportional to the activity of the sample. Cerenkov counting techniques have proved useful with ^{42}K, ^{24}Na and other high-energy beta emitters. In particular, with this technique ^{32}P can be counted in the presence of ^{33}P without any interference from the latter ($E_{max} < 0.26$ MeV).

One of the main sources of error in liquid scintillation counting is the 'quenching' of light, often caused by compounds in the solution to be measured. This can be due to light absorption by coloured compounds or by certain chemicals. Since quenching commonly occurs, and its degree can be variable, it must always be considered. The three most important methods of correction are listed below:

(i) Removal of coloured material. The solution may be filtered through activated charcoal or treated by an ion-exchange technique to remove the quenching agent.

(ii) Channel-ratio method. In general, the net loss due to quenching is greatest for the most intense light flashes produced by the highest-energy particles. Therefore, when quenching occurs, the output pulse spectrum is shifted towards lower energy. If, by discriminator settings, the ratio of a lower energy part (channel) to a higher energy part (channel) of the spectrum can be obtained, then it is possible to observe the relation between counting efficiency, ϵ, and channel ratio. This is done using a set of standards with a known constant amount of activity and measuring with increasing amounts of chemical quencher (i.e. increasing the channel ratio). A standard curve of ϵ versus channel ratio can then be prepared and subsequently used to correct sample measurements for any decrease in count-rate due to quenching. However, this curve will not account for quenching due to coloured material.

(iii) External standard technique.[12] In some instruments a standard source may be moved into position near the vial counting position. Thus, the relative decrease in the *standard count* for each sample counting vial will be proportional to the amount of quenching material and will provide the quench correction to be used for that vial count.

I–2.5. Semiconductor radiation detectors

The art of particle and gamma-ray spectroscopy has more recently been significantly advanced by the development of semiconductor radiation detectors. In particular, lithium-drifted germanium detectors have been very useful in gamma-ray spectroscopy. The energy resolution of such systems is improved by at least a factor of 10 over NaI(Tl) for gamma rays from e.g. $^{137}Cs/^{137}Ba^{m}$. Semiconductor detectors have the disadvantage that they cannot be made as large as NaI(Tl) crystals, and therefore the counting yield is decreased. Also, they must be continually maintained at very low temperature (normally that of liquid air, ~80 K). In neutron-activation analysis, for instance, where high gamma-ray energy resolution is necessary, use of such detectors is warranted. Advances are being made in these techniques, and these detectors will become cheaper and simpler to use in the coming years.

I–2.6. Inverse-square-law effect

A relationship met with in various branches of physics is that known as the inverse-square law. As applied to radiation, it states that the intensity of radiation emanating uniformly over the full solid angle (4π) from a point source in a vacuum decreases proportionally and monotonically with the square of the distance from the source. If I is the intensity of the radiation and d the distance from the point source, this can be expressed as:

$$I \propto \frac{1}{d^2} \tag{I–21}$$

or

$$I = \frac{k}{d^2} \tag{I–22}$$

where k is the constant of proportionality.

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[12] An internal standard technique may also be used; however, it makes it necessary to handle each sample twice.

If distances d_1 and d_2 correspond to intensities of I_1 and I_2, from Eq. (I–22):

$$\frac{I_1}{I_2} = \frac{d_2^2}{d_1^2} \qquad \text{or} \qquad I_1 d_1^2 = I_2 d_2^2 \tag{I–23}$$

Hence it can be seen that if $d_2 = 10\, d_1$, the intensity will have decreased 100 fold at d_2, i.e. a detector placed at 10 cm from a *point source* will see only 1% of the radiation seen by the detector placed at 1 cm.

This indicates one method of reducing the count-rate from a source that may have too high an activity for the detection system.

Since a point source is a theoretical concept, it is useful to note some practical requirements in making use of this relationship:

(a) The radiation must not be focussed or collimated in any way.

(b) A source can be considered as a point source if the detector is placed at a distance which is at least ten times larger than the largest dimension of the source, i.e. with a needle source of 1 mm dia and 1.0 cm long the detector would have to be placed at least 10 cm from the source ($<1\%$ error).

(c) Since radioactive decay is a random phenomenon, counting statistics have to be considered. Simply summarized, the counting time must be sufficiently long in relation to source activity to make random fluctuations in decay negligible (see §1–2.8).

(d) Measurements are rarely made in a vacuum. The important consideration is that matter (gas, liquid or solid) that is between the source and the detector should not attenuate the beam due to any kind of interaction (absorption, scattering, etc.) by more than $\frac{1}{2}\%$ in practical counting. This will vary with type of radiation and with the medium (see §I–1.6). For example, air at atmospheric pressure will not affect gamma counting at typical experimental distances.

I–2.7. Counting efficiency (counting yield)

Practically every tracer experiment involves a number of samples containing radioactivity, and the assay of the activity of these samples is an integral part of the complete experiment. When a radioactive atom decays, often more than one particle or photon is emitted. For example, a ^{60}Co nucleus emits either one beta particle and two gamma photons or occasionally one of each (see Fig. I–2). However, metastable states excepted, a disintegration including the emission of particle(s) and/or photon(s) requires only 10^{-10} s or less, whereas the resolving times of even the fast counters are of the order of 10^{-7} s. Thus, no practical counter will have a counting efficiency, ϵ (counts per disintegration), of greater than unity. The efficiency of a given counter in assaying a given sample is

defined as follows:

$$\epsilon = \frac{\text{count-rate of sample}}{\text{disintegration rate in sample}} = \frac{R - R_b}{A^*} \qquad (I-24)$$

in counts per second per becquerel or curie.

In most counters the counting efficiency is considerably less than unity, that is to say, only a fraction of the total disintegrations in the sample are detected and registered by the counting system. With the exception of liquid scintillation counting, the reduction in ϵ is caused by the following:

(a) Geometry factor. Events in the source are not 'seen' by the detector. This is a function of the geometry factor, i.e. the solid angle of the source/detector arrangement divided by 4π. For a small source close to the detector window the solid angle is about 2π and the geometry factor about 0.5.

(b) Air and window absorption. Particles, particularly alpha and low-energy beta, and to a lesser degree photons, may be absorbed in the air or in the window or walls of the detector, never reaching the sensitive volume of the detector.

(c) Self-absorption in the sample. Alpha and beta particles, and to a much lesser extent gamma photons, can be absorbed by the sample material in which the radionuclide is contained, and a significant fraction of the activity radiation will not be counted. This is a very important consideration for low-energy beta particles. In consequence, the count-rate from a given sample will not increase in proportion to its thickness. For a sample of a given area, as the sample thickness of constant activity-concentration material increases, the count-rate will tend towards a maximum (Fig. I–15). At thickness X (measured in units of mass per unit area, see footnote 7) the sample is considered to be of **infinite thickness.** A common method for assay of low-energy beta emitters using GM counting is to count all samples at *infinite thickness.* The count-rate, R_X, is then proportional to the activity concentration in the sample. The value for infinite thickness of beta emitters is approximately equal to the range of beta particles in units of mass per unit area.

(d) Scattering. Particles or photons may be scattered towards or away from the sensitive volume of the detector. This scattering occurs in the backing material of the sample holder, the walls of the shield, and the air between the source and the window.

When it is necessary to know the value of ϵ, it need seldom be determined by investigating each of the above effects individually. Instead, a calibrated standard, i.e. a source of known activity, prepared in identical fashion to the samples, is counted under the same geometry to determine ϵ.

Calibrated standards may be purchased from radionuclide suppliers. A local standard may be prepared from the radioactive material to be used in the experiment.

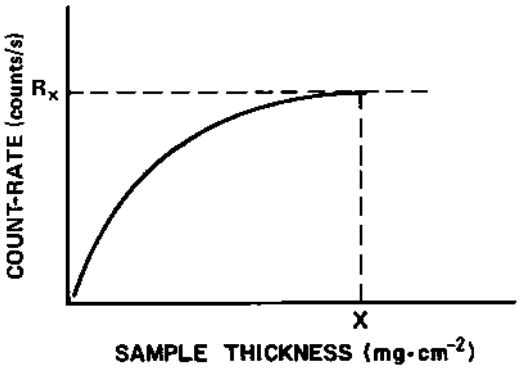

FIG.I–15. Count-rate as a function of mass-thickness for samples of constant activity concentration.

In the latter case, the count-rate of all experimental samples can be compared, for instance, as a percentage of the experimental amount of tracer activity administered (% of dose). In purely comparative investigations it is sufficient if ϵ can be kept constant from sample to sample, and its actual value need not be known.

In 1972, the ICRU published a report on the measurement of low-level radioactivity [3]. It is of interest for users of this manual, and particular reference is made to the definition and discussion of a *figure of merit* (Ref.[3], §1–3) which should be studied in connection with this and the following section (§I–2.8).

I–2.8. Counting statistics (natural uncertainty)

If a single radioactive sample is counted several times under identical conditions using a *perfect* counter, and the count is corrected for radioactive decay (or the decay correction is negligible) then the individual number of counts will be observed to fall in the neighbourhood of a mean value. These deviations are due to the random nature of the radioactive decay (§ I–1.3). This phenomenon may be termed **natural uncertainty**, as opposed to normal **technical uncertainty** due to the operator or the apparatus. The understanding of these statistical effects is necessary in the consideration of experimental design and in the interpretation of counting results.

Disintegration statistics follow closely the Poisson probability distribution law. As a special consequence of the Poisson distribution, the natural standard deviation ($\sigma_{nat,C}$) of a registered number of counts (C), irrespective of the time it takes to accumulate them, is closely equal to the square root of that number, C, under the assumption that the duration of the counting is much less than the half-life of the radionuclide being counted. So, for C accumulated counts, to a close approximation:

$$\sigma_{nat,C} = \sqrt{C} \qquad \text{(I–25)}$$

TABLE I–1. NATURAL STANDARD DEVIATION OF ACCUMULATED COUNTS

Accumulated counts (C)	Natural standard deviation $\sigma_{nat,C} = \sqrt{C}$	Natural standard deviation[a] as % of C
100	10	10
1 000	31.6	3.2
10 000	100	1.0
100 000	316	0.3
1 000 000	1 000	0.1

[a] $\%\,\sigma_{nat,C} = \left(\frac{\sqrt{C}}{C} \times 100\right)\%$.

Table I–1 gives the calculated natural standard deviations for some given numbers of accumulated counts. As can be seen from the table, although $\sigma_{nat,C}$ increases as the square root of C, the natural uncertainty expressed as a percentage of the counts decreases as C increases.

Referring to Eq. (I–25), if both sides are divided by the counting time, T, the result is the natural standard deviation of the count-rate, R, since R = C/T and T in this respect is constant. Thus:

$$\sigma_{nat,R} = \frac{\sigma_{nat,C}}{T} = \frac{\sqrt{C}}{T} \qquad \text{(I–26)}$$

However, since C = RT:

$$\sigma_{nat,R} = \sqrt{\frac{R}{T}} \qquad \text{(I–27)}$$

When C becomes large (> about 10), the Poisson distribution is closely approximated by the normal distribution. From the normal distribution, one standard deviation on either side of the mean value accounts for 68% or about 2/3 of the total area under the probability curve.

A useful rule of counting is to try to accumulate so many counts that the percentage natural standard deviation is 2 to 3 times less than the percentage technical standard deviation. If 10 000 counts, for instance, are accumulated, then from this single assay it can be stated that there is a 68% probability that the true mean C-value is within 10 000 ± 100, or in the range of 9 900 to 10 100.

Two standard deviations (2σ) account for approximately 95% of the area under a normal distribution curve, and in this case it can be stated that there is a 95% probability that the true mean C-value is within $10\,000 \pm 200$.

The accumulated counts (C) collected in any counting interval are due to true counts of the sample (C_s), plus those from background (C_b). There is a significant radiation background in almost any location. This background comes from cosmic rays and cosmic-ray induced activity, such as ^{14}C, and from naturally occurring radioactive materials in the earth's crust and elsewhere, e.g. ^{226}Ra, ^{232}Th and ^{40}K. The latter all have associated gamma rays. The cosmic-ray contribution varies with altitude, and the composition of the earth's crust, etc., varies with location. All radiation detector/counter systems have an associated background from the above sources and from electronic noise. The background count-rate is commonly reduced by shielding or by special electronic circuitry.

Obviously, every sample count is made in the presence of a background count-rate for that particular system. The background will be a function of the type of detector, as well as shielding, location, discriminator settings, etc.

The deviation of a background count is independent of that of a sample plus background count, so the appropriate uncertainty terms add as the sum of the squares. Therefore, the variance of the net sample count (C_s) is, since $C_s = C - C_b$:

$$\sigma^2_{C_s} = \sigma^2_C + \sigma^2_{C_b} \tag{I–28}$$

where $\sigma^2_{C_s}$ = variance of net sample count;

σ^2_C = variance of sample plus background count;

$\sigma^2_{C_b}$ = variance of background count.

The natural uncertainty of the net sample count then follows from Eqs (I–25) and (I–28):

$$\sigma_{\text{nat},C_s} = \sqrt{C + C_b} \tag{I–29}$$

where σ_{nat,C_s} = natural standard deviation of accumulated net sample counts;
C = the total of accumulated counts due to sample plus background;
C_b = the part of accumulated counts due to background.

Similarly, it follows from Eq. (I–24) that the natural standard deviation (σ_{nat,R_s}) of the *net count-rate* ($R_s = R - R_b$) of the sample is given by

$$\sigma_{nat,R_s} = \sqrt{\frac{R}{T} + \frac{R_b}{T_b}} \qquad (I-30)$$

where R = count-rate of sample plus background;
R_b = count-rate of background;
T = time used for counting sample plus background;
T_b = time used for counting background.

In tracer experiments, the net count-rate of samples very commonly approaches, or is even less than, background count-rates. In order to divide a given total period of counting time between T and T_b in such a way as to statistically minimize σ_{nat,R_s}, the following formula may be used (by inserting preliminary values for R_b and R, obtained during short periods of counting):[13]

$$\frac{T_b}{T} = \sqrt{\frac{R_b}{R}} \qquad (I-31)$$

where the symbols have the meanings given below Eq. (I–30). However, strict adherence to this criterion for optimum statistical partition of counting time is not critical in practice.

I–3. RADIATION PROTECTION

It is imperative that a knowledge of the safe use of radionuclides and radiation be gained before they are applied as tools in research. Ionizing radiation is hazardous to all biological systems, but with proper considerations for health protection measures, the hazard to personnel or experimental systems can be reduced to a tolerably low level.

The health physics involved in the safe use of radionuclides and radiation is discussed in some detail under three headings:

§I–3.2. Protection of personnel
§I–3.3. Control of contamination
§I–3.4. Waste disposal

However, before considering these categories, an insight must be gained both into basic considerations involved in radiation protection and into units.

[13] Derivation of Eq. (I–31) is given in Part VI, Appendix VI–6.

I–3.1. Basic considerations and units

The liberation of ion pairs by energetic photons is termed the **exposure, X**, and is defined as:

$$X = \frac{dQ}{dm}$$

where dQ is the absolute value of the total charge of ions of one sign produced when all the electrons (e^+ and e^-) liberated by photons in a volume of air of mass dm are stopped in air. In SI units, the exposure is measured in coulombs per kilogram, while the present special unit of exposure, the roentgen (R), is defined as:

$$1\ R = 2.58 \times 10^{-4}\ C/kg$$

To obtain some impression of the magnitude of such interaction, the following approximate figures are of assistance. The charge on an electron is about 1.6×10^{-19} C, and 1 cm^3 of air at STP has a mass of around 1.3 mg; hence, one roentgen will produce about 2.1×10^9 ion pairs per cubic centimetre of air and, since release of an ion pair requires some 33 eV, about 1.1 J of radiation energy are absorbed per cubic centimetre of air. Thus, one roentgen will dissipate 8.5×10^{-3} J/kg air (i.e. 85 erg/g).

As discussed in §I–1.6, the energy of gamma rays absorbed per gram of various materials is a function of properties such as Z and density. Thus, the energy absorption per gram from exposure to 1 R will be slightly different for soft tissue, for water and for air, and it will be a function of photon energy. Further, for example, the energy absorption per gram of bone tissue from exposure to 1 R of X or gamma photons of energy below 0.01 MeV will be 2 to 5 times that of soft tissue exposed to 1 R of the same photons. **In general, there is no simple relationship between the energy absorbed per gram and the exposure.**

The biological effect from a *given* type of irradiation is, however, proportional to the energy absorbed per gram, and a unit of absorbed dose was, therefore, introduced.

The unit of absorbed dose in SI is the gray (Gy), representing the absorption of 1 joule per kilogram of the irradiated material. The special unit of absorbed dose at present is the rad, use of which is being phased out during the period to 1986. The interrelationships of interest are:

$$1\ Gy = 1\ J/kg = 100\ rad\ (= 10^4\ erg/g)$$

The dose absorbed by a material subject to a given exposure will vary with the nature of the material, depending to a large extent on the scattering power

(electron density) of the constituent atoms. The units gray and rad may be used irrespective of the type of ionizing radiation being considered.

It is clear, however, that radiation dissipating 1 Gy (100 rad) with a high specific ionization, i.e. a high linear energy transfer (LET), will have a greater biological effect on an organism than a different quality of radiation dissipating 1 Gy with a low specific ionization. For example, alpha particles have a much higher LET than beta particles, and hence the biologically damaging effect of alpha particles will be greater than that of beta particles. This observation resulted in the use of a factor termed the Relative Biological Effectiveness (RBE), which was defined using the biological effect of irradiation with 200 keV X-rays as the basis for comparison:

$$\text{RBE} = \frac{\text{Absorbed dose due to 200 keV X-rays causing a specific effect}}{\text{Absorbed dose due to other radiation causing the same effect}} \qquad \text{(I 32)}$$

As a practical indication of the use of the term, Van der Vloedt has shown that the RBE of neutrons is 4 to 5 times greater than that of gamma rays in achieving sexual sterilization in tsetse flies.[14]

Originally, the product of the absorbed dose in rads and the RBE were taken to give a value in rems (originally derived from the idea roentgen equivalent man) such that a figure of, say, 100 rems would represent the same biological effect of an irradiation, irrespective of the type of radiation used, i.e. the effect 100 rem beta rays = effect 100 rem gamma rays. This was to make it possible to sum such 'effective absorbed doses' or 'weighted absorbed doses' resulting from different exposures to different radiations at different times, a matter of practical importance. **The use of the term rem for such values is no longer applicable,** and the product absorbed dose and RBE is termed 'effective dose' or 'weighted dose', still measured in rads, grays or J/kg. For example:

$$D_{\text{eff},1}\,(\text{rad}) = D_1 \times \text{RBE}_1;\quad D_{\text{eff},2}\,(\text{rad}) = D_2 \times \text{RBE}_2$$

$$D_{\text{eff,tot}}\,(\text{rad}) = D_{\text{eff},1} + D_{\text{eff},2} \qquad \text{(I 33)}$$

although the applicability of this idea *must* be proved for the biological system under investigation.

The modern concept of dose equivalent, H, has been defined with regard to the human and his organs by the ICRP and ICRU [4] as follows:

$$H = QND \qquad \text{(I 34)}$$

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[14] Van der Vloedt, A., et al., Laboratory studies on the sexual sterilization of the tsetse fly *Glossina palpalis palpalis* Rob. Desv. by ionizing radiation (submitted for publication, 1977).

TABLE I–2. VALUES OF QUALITY FACTOR Q[a] USED IN DEFINING DOSE EQUIVALENT [7]

Radiation	Q
X-rays, γ-rays, electrons and β-rays	1.0
Fast neutrons and protons up to 10 MeV[b]	10
α-particles from radioactive decay (for internal exposure)	10
Heavy recoil nuclei	20

[a] These values of Q are those chosen specifically for use in defining maximum permissible doses.
[b] In the case of irradiation of the lens of the eye with particulate radiation of high LET, an additional modifying factor, N, must be used. N should be 3 when $Q \geqslant 10$.

where D is the absorbed dose, Q is the quality factor of the radiation and N is the *product* of all other modifying factors. Q and N are dimensionless, and hence the dimensions of H are J/kg, as for absorbed dose. The use of dimensionally similar units for these two different concepts could have serious consequences in radiation protection, etc., and, hence, either rem or J/kg (dose equivalent) should be used.[15] Hence 1 J/kg (dose equivalent), i.e. 100 rem, of one kind of ionizing radiation is, for radiation protection purposes, defined as having the same biological effect in man as 1 J/kg (dose equivalent) or 100 rem of another kind of ionizing radiation. In considering irradiation of the whole or part of the human body, the dose equivalent would be computed from the known or estimated absorbed dose, making use of the factors published by the ICRP [5] (see also Ref. [6]). Dose equivalents are additive for a given person or organ. Some data concerning quality factor, Q, are appended in Table I–2. Apart from an exception noted in Table I–2, N is assigned the value unity for all radiations from external sources [2, 4, 7].

I–3.2. Protection of personnel

The International Commission on Radiological Protection (ICRP) has recommended that the yearly dose to radiation workers must not exceed 5 rem per year. This is equivalent to an average rate of 0.1 rem per week; however,

[15] A proposal to replace the special unit rem by a new SI unit, the sievert (Sv), is at present under consideration; the relationship would be 1 Sv = 1 J/kg *when* Q times N in the expression above are both unity.

TABLE I -3. APPROXIMATE VALUE[a] OF THE HALF-THICKNESS OF LEAD AS A FUNCTION OF GAMMA-RAY ENERGY (>0.2 MeV)

E_γ (MeV)	Approximate half-thickness of lead shielding[a] (cm)
0.25	0.25
0.5	0.5
1.0	1
1.5	1.5
2 to 4	2

[a] The value will depend on the geometrical relationship between source and absorber. With a point source *very* close to the absorber, the radiation meets the absorber at a wide range of angles of incidence (broad geometry). The half-thicknesses in such a case will be different to those if the absorber is sufficiently distant for the incident radiation to be nearly parallel (in effect a collimated beam; narrow geometry). The half-thickness values could vary by 20 to 30% down or up from the values given in the table above, which are for intermediate geometries more normally met with in measuring and experimental practice.

the ICRP recommendation does not stipulate any weekly rate [5, 6]. The yearly maximum of 5 rem applies to the dose (from both internal and external radiation) to the whole body, the gonads alone or the red bone marrow alone.

I–3.2.1. External exposure

Radiation dose to personnel must always be kept as low as practicable and any unnecessary exposure must be avoided. In the case of external exposure, this can be accomplished by an optimum combination of (i) shielding, (ii) increasing working distance from the source and (iii) minimizing exposure time. Shielding of alpha emitters for external radiation is not required because the wall of the container or a few centimetres of air will absorb all particles. The same considerations generally apply to low-energy beta emitters such as ^{3}H, ^{14}C or ^{45}Ca. High-energy beta emitters require only 1 to 2 cm of low-Z material, such as polymethyl methacrylate (Lucite, Perspex, etc.) for shields.[16] In the case of gamma rays, a high-Z material, such as lead, provides the best shielding. Table I–3 gives approximate values of half-thicknesses (half-value layers) of lead for shielding against gamma rays.

[16] For strong beta-emitting sources the production of bremsstrahlung must be considered.

To obtain the approximate half-thicknesses of water, the corresponding half-thickness of lead may be multiplied by 10. (The density of water is about one tenth that of lead.) To obtain the approximate half-thickness of any other material, the necessary half-thickness of water is divided by the density of that other material.

The photon-intensity attenuation factor, F, and the number, n, of half-thicknesses ($X_{\frac{1}{2}}$, see Eq. (I–14a)) are related as follows:

$$F = 2^n, \text{ i.e. } n = \frac{\log_{10} F}{0.3} \qquad \text{(I–35)}$$

Work with radioactive sources must always be performed with sufficient shielding for personnel. The calculated dose rate after shielding must always be checked with a dose-rate meter, preferably an ionization chamber type. Sources not in use should always be stored behind shielding and access to the sources strictly controlled. Warning signs such as the following should be used:

Radioactive materials; external dose rates less than 2.5 $mR \cdot h^{-1}$ ($<6.5 \times 10^{-7}$ $C \cdot kg^{-1} \cdot h^{-1}$)

CAUTION RADIOACTIVE MATERIALS

External dose rates 2.5 to 100 $mR \cdot h^{-1}$ (6.5×10^{-7} to 2.5×10^{-5} $C \cdot kg^{-1} \cdot h^{-1}$)

CAUTION RADIATION AREA

External dose rates exceeding 100 $mR \cdot h^{-1}$ ($>2.5 \times 10^{-5}$ $C \cdot kg^{-1} \cdot h^{-1}$)

CAUTION HIGH RADIATION AREA

It is important that before beginning any work with gamma-ray emitters the researcher should know how great the radiation dose from the source will be. The gamma-ray dose constant Γ (in various units for 1 m from a *point source*) is given in Table I–4 for various radionuclides.

For point sources of activity, gamma-ray intensity is inversely proportional to the square of the distance (see §I–2.6). Thus, once the exposure dose is known at any one distance, it may be calculated at any other distance by the inverse-square law.

It was seen in §I–2.6 that distance is a very important factor in minimizing dose. Consider a *point* source from which the gamma-ray exposure dose was 1 $mR \cdot h^{-1}$ at 10 cm. Any manipulations with the source by means of long forceps or tweezers would produce a negligible finger or whole-body dose. However, if the source were handled without tweezers, for instance with rubber gloves as the only protection, the radiation exposure dose at 1 mm distance would be 10 000 $mR \cdot h^{-1} = 10$ $R \cdot h^{-1}$ to the skin of the finger tips.

TABLE I–4. GAMMA-RAY EXPOSURE DOSE LEVEL AT 1 m FROM A POINT SOURCE (Γ) FOR SOME SELECTED RADIONUCLIDES

Radionuclide	Γ (at 1 metre)			Predominant gamma-photon energy (MeV)
	($R \cdot h^{-1}$ per Ci)	($R \cdot h^{-1}$ per Bq)	($C \cdot kg^{-1} \cdot h^{-1}$ per Bq)	
Na-22	1.2	3.2×10^{-11}	8.4×10^{-15}	1.3 and 0.5[a]
Na-24	1.8	4.9×10^{-11}	1.3×10^{-14}	2.7 and 1.4
Mg-28 (+ equil. Al-28)	1.6	4.3×10^{-11}	1.1×10^{-14}	1.8 and 1.4
K-42	0.14	3.8×10^{-12}	9.8×10^{-16}	1.5
Cr-51	0.02	5.4×10^{-13}	1.4×10^{-16}	0.3
Mn-54	0.47	1.3×10^{-11}	3.3×10^{-15}	0.8
Co-58	0.55	1.5×10^{-11}	3.8×10^{-15}	0.8 and 0.5[a]
Fe-59	0.67	1.8×10^{-11}	4.7×10^{-15}	1.3 and 1.1
Co-60	1.3	3.5×10^{-11}	9.1×10^{-15}	1.3 and 1.2
Cu-64	0.12	3.2×10^{-12}	8.4×10^{-16}	0.5[a]
Zn-65	0.27	7.3×10^{-12}	1.9×10^{-15}	1.1
Se-15	0.20	5.4×10^{-12}	1.4×10^{-15}	0.4, 0.3 and 0.1
Rb-86	0.05	1.4×10^{-12}	3.5×10^{-16}	1.1
Zr-95	0.41	1.1×10^{-11}	2.9×10^{-15}	0.8 and 0.7
I-131	0.22	5.9×10^{-12}	1.5×10^{-15}	0.4
Cs-137 (+ equil. Ba-137m)	0.31	8.4×10^{-12}	2.2×10^{-15}	0.7
Ta-182	0.68	1.8×10^{-11}	4.7×10^{-15}	1.2 and 0.2
Au-198	0.25	6.8×10^{-12}	1.7×10^{-15}	0.4
Ra-226 (+ equil. decay chain) with 0.5 mm Pt cover for calibration	0.825	2.3×10^{-11}	5.9×10^{-15}	many different

[a] Annihilation photons following β^+.

Reduction of exposure time is also important in minimizing dose. Manipulations with sources should be performed rapidly *but carefully*.

Monitoring of external dose can be accomplished by the use of personal dose meters [8]. These can be worn on the body, or attached to the hands or wrists if necessary. They provide an integrated dose reading, i.e. a dose value summed over the total working period. Pocket dose meters (electrometers), film badges or solid-state thermoluminescent dose meters [9] are the most common systems currently in use.

I–3.2.2. Internal exposure

The internal hazards of radionuclides involve some distinctly different considerations. Beta emitters and particularly alpha emitters become extremely hazardous on entry into the body. The protection against internal contamination largely involves prevention of accidental ingestion, of inhalation or of skin absorption of radionuclides. The International Commission on Radiological Protection (ICRP) has calculated (i) maximum permissible body burdens of all the radionuclides and (ii) the maximum permissible annual intakes in water and air that would produce such body burdens if chronic exposure conditions existed [5, 6]. The factors that determine the maximum permissible body burden of any radionuclide are:

(a) Particle radiation energy, LET and radioactive half-life;
(b) Absorption from the gastro-intestinal (GI) tract or lung tissue into body fluids;
(c) Distribution into body organs;
(d) *Biological half-life*, i.e. the time required for a given body burden to decrease physiologically by one-half. The combined effect of radioactive decay and physiological excretion is given by the relation:

$$\frac{1}{T_{\frac{1}{2},\,\mathrm{eff}}} = \frac{1}{T_{\frac{1}{2}}} + \frac{1}{T_{\frac{1}{2},\,\mathrm{biol}}} \qquad \text{(I–36)}$$

where $T_{\frac{1}{2}}$ = radioactive half-life
$T_{\frac{1}{2},\,\mathrm{eff}}$ = effective half-life
$T_{\frac{1}{2},\,\mathrm{biol}}$ = biological half-life

Factors (b) and (c) also depend on the chemical and physical form of the radionuclide. Solubility in body fluids will largely determine the absorption and transport of the radionuclide.

The relative radiotoxicities of all known radionuclides are given in Part VI, Appendix VI–3, while some typical examples are shown in Table I–5.

I–3.3. Control of contamination

Contamination of laboratory, benches, glass-ware and operators by radionuclides must be avoided for two reasons:

(i) Laboratory contamination can result in internal exposure of the laboratory personnel, and it may even be spread to areas where other personnel may be exposed.
(ii) Experimental results are likely to become uncertain.

TABLE I–5. LIMITATION ON RADIOACTIVITIES IN VARIOUS TYPES OF WORKING PLACE OR LABORATORY[a] [7]

Radiotoxicity of radionuclides (and examples of each)	Minimum significant quantity (μCi)	(kBq)	Working place or laboratory required		
			Type C	Type B	Type A
1. Very high (Sr-90, Po-210, etc.)	0.1	3.7	10 μCi or less 0.37 MBq or less	10 μCi to 10 mCi 0.37 MBq to 0.37 GBq	10 mCi or more 0.37 GBq or more
2. High (Na-22, Ca-45, Co-60, Sr-89, I-131, etc.)	1.0	37	100 μCi or less 3.7 MBq or less	100 μCi to 100 mCi 3.7 MBq to 3.7 GBq	100 mCi or more 3.7 GBq or more
3. Moderate (C-14, P-32, S-35, K-42, Zn-65, Br-82, etc.)	10	370	1 mCi or less 37 MBq or less	1 mCi to 1 Ci 37 MBq to 37 GBq	1 Ci or more 37 GBq or more
4. Slight (H-3, Rb-87, etc.)	100	3700	10 mCi or less 0.37 GBq or less	10 mCi to 10 Ci 0.37 GBq to 0.37 TBq	10 Ci or more 0.37 TBq or more

[a] Type C, Type B and Type A have the meanings normally used in the classification of laboratories for handling radioactive materials. Type C is a good quality chemical laboratory. Type B is a specially designed radioisotope laboratory. Type A is a specially designed laboratory for handling large activities of highly radioactive materials. In the case of a conventional modern chemical laboratory with adequate ventilation and fume hoods, as well as polished, easily cleaned, non-absorbing surfaces, etc., it would be possible to increase the upper limits of activity for Type-C laboratories towards the limits for Type-B laboratories for toxicity groups 3 and 4.

A number of laboratory rules must, therefore, be strictly adhered to:

(a) Eating, drinking, smoking and application of cosmetics in the laboratory are strictly prohibited, as is combing of hair (due to the electrostatic charge induced).

(b) Each person should wear a laboratory coat. This coat should be worn in the laboratory space where the experiments with radionuclides are done, but not in separate counting rooms or outside the area of radioactivity.

(c) When there is a significant risk that the hands may become contaminated, thin surgical gloves or disposable plastic gloves should be worn. The surgical gloves have to be put on and taken off in such a way that the inside never touches the outside in order to prevent direct contamination of the skin. A detailed description of the procedure for putting on or removing gloves is given in Part VI, Appendix VI–1. As soon as the risk for contamination of the hands is no longer present, the gloves should be removed, as they constitute a source of contamination of glass-ware, equipment, faucet handles, etc.

(d) Pipetting or the performance of any similar mouth action is strictly prohibited. Syringes or propipettes must be used.

(e) Protective eye glasses or shields are advantageous and should always be worn in high radiation areas of a radiochemistry laboratory. This will shield the lens of the eye from beta particles and will minimize eye injury in the event of a chemical accident.

(f) To prevent contamination of gloves, hands or equipment, paper tissues should be at hand and should always be used as a preliminary means of decontamination (see Part IV, §IV–3). After use, these tissues should be disposed of in foot-operated waste bins or large drums.
(g) All operations involving volatile materials, heating or digestion must be done under a fume hood. The air velocity (suction) at the hood face should be approximately 1 m/s.
(h) Any operation in which radioactive dust may arise should be carried out in a glove-box in which slightly negative pressure is maintained. In the exhaust system a dust filter must be present to collect radioactive particles. These precautions are imperative in the case of alpha activity.
(i) All operations should be carried out over shallow trays. The bottoms of the trays should be covered with absorbent paper.
(j) Storage bottles should be available for dumping of liquid waste (see Part VI, Appendix VI–2). These bottles should contain a small amount of *ion-exchange resin* to concentrate the activity.
(k) Avoid cross-contamination by using glass-ware, tin openers, tweezers, etc., for *one particular radionuclide only*.
(l) A thin-window GM survey meter should be available for contamination detection. In addition, it would be preferable to have an ionization-chamber survey meter for exposure dose measurements.
(m) Frequent surveys of laboratory work areas, equipment and personnel should be performed with the GM survey meter to detect contamination. In the case of alpha emitters, ^{3}H or other low-energy beta emitters, filter paper should be used to swab the suspected areas. The swabs should be counted with an appropriate detector.
(n) Before leaving the laboratory the hands, clothing and shoe soles should be checked with a suitable survey instrument or swabbed, the swabs being counted.

I–3.3.1. Decontamination

Decontamination of the skin should first be attempted with soft soap and water, possibly with a soft brush. Care should be taken to avoid damaging the skin by excessive washing. Often washing with a carrier solution will aid in removal through exchange with the radioactive isotope. Obviously, the carrier solution must be non-toxic to the skin.

Generally, the contamination of glass-ware, metal surfaces or painted surfaces which have been contaminated with radioactive material of high specific activity is greatly reduced by repeated washings with carrier solution. Stocks of carrier solution should therefore be present where contamination is likely to occur. A spreading agent may be very effective. Otherwise, materials may be decontaminated as follows:

MATERIAL	DECONTAMINATION SOLUTION
Glass	Either 10% nitric acid, or 2% ammonium bifluoride, or chromic acid, or carrier in 10% hydrochloric acid.
Aluminium	10% nitric acid, sodium metasilicate or sodium metaphosphate.
Steel	Phosphoric acid plus a spreading agent.
Lead	4N hydrochloric acid until a reaction starts, then a dilute alkaline solution, followed by water.
Linoleum	Xylol or trichlorethylene to remove wax surface.
Painted surfaces	Spreading agent and ammonium citrate or ammonium bifluoride.
Wood and concrete	Difficult to decontaminate. Partial or complete removal of the contaminated material will usually be the only effective method.

I–3.3.2. Special laboratory design features

A laboratory in which work with radioactive materials is done should have facilities that:

(a) minimize the incidence and spread of contamination
(b) make possible rapid decontamination.

These facilities are further determined by the nature of the work that is to be carried out. Three types of laboratory may accordingly be described (see Table I–5 and Ref. [7]). Usually, a Type A laboratory will be associated with reactor operations or waste processing plants. For biological research, Type B or C laboratories will generally be adequate.

A Type C laboratory may be any ordinary laboratory that has a good ventilation system and an exhaust hood. Floors and benches should have a surface that can be cleaned easily.

If larger quantities of radionuclides are to be used, for example for the dilution of stock solutions or the preparation of labelled compounds, then a Type B laboratory will be required.

The characteristics of a Type B laboratory may be listed as follows:

(a) The laboratory room should preferably be separate from the counting room(s).
(b) Ventilation of the laboratory should be sufficient to exchange the total room volume 12 times hourly. The air flow should be from least active to most active areas. The fan for each hood should be at the top of the vent

duct so there is negative pressure throughout the vent duct. Multiple hoods should automatically be vented simultaneously at the same air velocity. The ventilation to the room should be separate from that to other rooms, *particularly counting rooms*. There should be a particle filter in each exhaust duct.

(c) Shielded, lockable, separate storage areas should be available for highly radioactive sources.

(d) To facilitate decontamination, benches should be covered with melamine laminate and floors with vinyl or linoleum, preferably without seams. Under no circumstances should uncovered wooden or concrete floors and bench tops be allowed. Furniture should be of non-porous material.

(e) The GM survey meter, the hand and foot monitoring station and laboratory coat hooks should be located just inside the entrance to the laboratory.

(f) Water faucets should be of a foot or elbow-operated design to prevent contamination.

(g) If possible, a shower for personnel decontamination should be located close to the laboratory.

(h) Drains should be located in the floor.

(i) There should be no ridges and corners in which dust may accumulate and which are difficult to clean.

I–3.4. Waste disposal

Radioactive waste should be controlled and disposed of according to the recommendations of the ICRP and IAEA (see Part VI, Appendix VI–2). Generally, liquid waste should be stored in polyethylene containers and not disposed of into the sanitary sewer system through sinks. High-volume, low-activity liquid waste may be treated by ion-exchangers to reduce the volume. Solid waste should be placed in foot-operated bins. All waste containers must have the appropriate label as well as a label stating the date and quantity of each radionuclide added.

If possible, it is advisable to store all liquid and solid waste until the activities present have been reduced by radioactive decay such that it might be disposed of by usual methods. If this is not possible, as in the case of long-lived emitters, land burial may be necessary. In some countries a central organization is in charge of collection, storage and/or burial of radioactive materials.

Waste disposal can be a serious problem, and if work with appreciable activity of long-lived radionuclides is expected, expert advice should be sought.

I–4. TRACER METHODOLOGY

The tracer method is a technique used to investigate certain characteristics of a population of specific objects such as molecules, organisms or other entities

by observing the behaviour of the **tracer**. The substance to be traced is generally termed the **tracee**. The criteria for an ideal tracer are that it must be chemically and physically indistinguishable from the tracee, at the tracer concentration used, and that the introduction of the tracer must not disturb the system. Both these criteria are almost perfectly met by radioactive or stable isotopic tracers. Isotopes have identical chemical properties (only slight mass differences), and they can be obtained in high specific activity or tracer abundance. Therefore, the introduction of an isotopic tracer generally adds negligible mass to the system and does not disturb its kinetics.

In principle a stable isotope can be used as a tracer just as well as a radioisotope. In practice, however, one radioactive atom is detectable, whereas 10^{12} or more atoms of a stable isotope constitute the smallest detectable amount. Conversely, the stable isotope is better than the radioisotope in two respects, namely (a) there is no radiation hazard and (b) the life of the tracer is infinite. Generally, these advantages do not outweigh the supreme sensitivity of radiotracer detection.

I–4.1. Pathway identification

A most common use of an isotopic tracer is to follow the pathway of an entity in a chemical, physical or biological system.

When the entity is, for example, an intact organism or an inorganic object, the radioactive label used may belong to any element. The choice of label will then be governed (a) by the ease of incorporation and attachment of the label and its stability of attachment, (b) by the ease of detection of its radiation, and (c) by the half-life (in order to be able to follow the label over the desired period of experimentation, yet not allow unduly long-termed contamination of the environment after the experiment is finished).

When the entity to be studied (tracee) is an organic material or compound, the radionuclide must belong to one of the elements in the tracee. This often reduces the choice to ^{14}C, ^{3}H or ^{15}N plus perhaps ^{32}P, ^{35}S, ^{36}Cl or ^{131}I. The label may be incorporated into the tracee through biological growth, chemical synthesis or exchange processes.

If the tracee is a mineral nutrient, the label should be an isotope of that element. In general, elements in the same chemical group, e.g. alkali metals, have similar chemical properties, but not sufficient for an isotope of one element to serve in general as the tracer for another element in the same group.

If the tracer is introduced into the system, its identification in other parts of the system infers information about the possible pathways of the tracee in the system. A well-known example is the use of ^{14}C-labelled glucose to observe the pathways and intermediates in the glycolytic cycle. Another example might be the migration of insects and identification of their predators in an ecological study.

I–4.2. Tracer dilution

The tracer dilution technique has been very useful in determining the isotopically exchangeable mass of a substance in a system. The **isotope dilution** principle is: for a given amount of isotopic tracer, the specific activity (or enrichment) at any time is inversely proportional to the total exchangeable mass of tracee mixed uniformly with the tracer at that time.

This technique, introduced by de Hevesy, is particularly useful when quantitative separations are not possible or are too tedious for the systems under study. In addition, it is the principal technique used to measure the exchangeable mass in vivo.

I–4.2.1. Derivation of equations

Consider a system that contains an unknown amount, S grams (or moles), of a test substance. To this system is added a known amount of a radioactive (or stable) tracer of initial specific activity (or initial atom per cent excess), A_{init}. Then, in the case of a radiotracer:

$$A_{init} = \frac{A^*}{s} \tag{I–37}$$

where A* = activity of the tracer (usually μCi or kBq, or corrected counts/s)
s = known amount of test substance associated with the tracer added, i.e. the carrier.

If the tracer is allowed to mix completely in the system, a final specific activity (or final at.% excess), A_{fin}, is reached. In the case of a radiotracer:

$$A_{fin} = \frac{A^*}{S + s} \tag{I–38}$$

Then in general, according to the isotope dilution principle:

$$\frac{A_{init}}{A_{fin}} = \frac{S + s}{s} \tag{I–39}$$

In the case of a radiotracer, Eq. (I–39) may also be obtained by dividing Eq. (I–38) into Eq. (I–37).

Solving for S in Eq. (I–39), we find:

$$S = s\left(\frac{A_{init}}{A_{fin}} - 1\right) \tag{I–40}$$

where S and s are both in either grams or moles of substance.

(Equation (I–40) is valid for a stable isotopic tracer, if A stands for at.% excess, and s is the amount of test substance added as tracer.)

Therefore, to determine S, only A_{fin} need be determined, as A_{init} and s are known. Quantitative separation of the tracee from the sample that has been isolated from the system is not necessary because specific activity (or enrichment) is independent of sample size, recovery, handling losses, etc. However, it is a necessary condition that the tracer be homogeneously mixed with the tracee in the system. The fulfilment of this condition becomes very important in tracer dilution studies in vivo.

Very commonly, s is negligible compared with S. This is the case with carrier-free or high specific activity radiotracers. By inspection of Eq. (I–38), if s is negligible with respect to S, then:

$$S = \frac{A^*}{A_{fin}} \qquad (I–41)$$

and only the total tracer activity and the final specific activity need be known.

A variation of the tracer dilution technique, called **inverse tracer dilution,** enables determination of an unknown amount, S, of an isotopically labelled test substance or tracee in a system, by the addition and mixing in of a known amount, s, of unlabelled test substance as tracer. Let A_{init} and A_{fin} be the initial and final specific activities (initial and final enrichments, i.e. at.% excess) respectively, in the tracee system. In the case of a radiotracer:

$$A_{init} = \frac{A^*}{S} \qquad (I–42)$$

where A* is unknown and S is sought, and (cf. Eq. (I–38)):

$$A_{fin} = \frac{A^*}{S + s} \qquad (I–43)$$

Then, according to the isotope dilution principle, or by division of Eq. (I–43) into Eq. (I–42):

$$\frac{A_{init}}{A_{fin}} = \frac{S + s}{S} \qquad (I–44)$$

Solving for S in Eq. (I–44), we find:

$$S = s\left(\frac{1}{\frac{A_{init}}{A_{fin}} - 1}\right) = s\left(\frac{A_{init}}{A_{fin}} - 1\right)^{-1} \qquad (I–45)$$

where S and s are both in either grams or moles of substance.

Therefore, the determination of the specific activities (enrichments) before and after the complete mixing in of s grams (or moles) of unlabelled tracer enables calculation of the amount of labelled tracee in the system. (Equation (I–45) is valid for a stable tracer, in the same way as Eq. (I–40).)

In general, if both the tracee and the tracer are labelled, it can be shown that:

$$\frac{S}{s} = \frac{A_s - A_{S+s}}{A_{S+s} - A_S} \qquad \text{(I–46)}$$

where A_S and A_s = initial specific activities (abundances) of tracee and tracer respectively

A_{S+s} = final specific activity (abundance) of tracee and tracer mixture

Equations (I–40) and (I–45) are easily deduced from Eq. (I–46).

I–4.2.2. Example of a closed system

Consider the problem of estimating the volume of water in a vessel. This would be an example of a **closed system**, since no water can enter or leave, that is there is no communication with the external environment. If s (ml) of tritiated water, ^{3}HOH, of specific activity A_{init} (μCi·ml^{-1} or kBq·ml^{-1}), is pipetted into the vessel and allowed to mix, then the unknown volume of water in the vessel, S (ml), can be calculated directly by Eq. (I–40)[17] when the specific activity of the mixture, A_{fin}(μCi·ml^{-1} or kBq·ml^{-1}, respectively), has been determined.

I–4.2.3. Example of an open system

Now consider the estimation of the volume of water in an animal. This would be an example of an **open system**, presumably in the steady state with respect to water exchange with the external environment. In the steady state, the intake rate is equal to the outgoing rate and the exchangeable mass is constant. If A* (μCi or kBq) of very high specific activity tritiated-water tracer is injected into the animal and allowed to mix with the body water, then the water volume (total body water), S, can be calculated using Eq. (I–41). The final specific activity, A_{fin}(μCi·ml^{-1} or kBq·ml^{-1}), is determined by sampling plasma or urine after mixing is complete. However, the animal will have lost some fraction of the initial activity, A*, via excretion during this mixing period. Therefore, the

[17] Although specific activity is expressed in this and the next example as μCi or kBq per ml of water, it is actually the mass of exchangeable hydrogen that is being determined.

total activity excreted during the mixing should be collected and subtracted from A*. Equation (I–41) is then modified to:

$$S = \frac{A^* - A^*_{exc}}{A_{fin}} \qquad (I–47)$$

where S is in ml, A* is usually in net μCi or kBq, or in net (corrected) counts/s, and A^*_{exc} is the activity (in corresponding units) of tritiated water excreted via all routes up to the time the sample containing A_{fin} is taken.

I–4.3. Tracer kinetics

The principal difficulty in the tracer dilution technique is to ensure the uniform mixing of tracer and tracee. To determine the degree of mixing as a function of time it is necessary to take repetitive samples from the system. Such data contain valuable information on the kinetics of the mixing processes. It is such analysis as well as the response of the system at *tracer equilibrium* that is treated by the field of tracer kinetics.

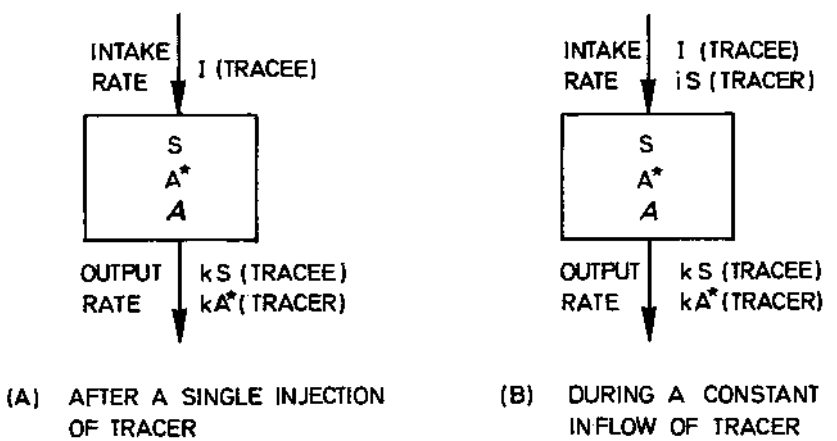

FIG.I–16. Schematic models of an open compartment (in tracee steady state).

S = Tracer in grams (constant)
A* = Radioactivity of tracee, e.g. in μCi, kBq, or net (corrected) counts/s
A = Specific activity = A*/S, e.g. in μCi/g, kBq/g or counts·s^{-1}·g^{-1}
I = Intake in grams of tracee per unit time
i = Input of tracer in μCi, kBq or counts/s, as appropriate, per unit time per gram of tracee in compartment
k = Output rate constant

Most biological systems are open, that is, there is exchange with their environment. Consider an **open compartment** as shown in Fig. I–16. A compartment is a subdivision of a system in which the tracer specific activity, at any given time, has the same value everywhere within the boundaries of the subdivision. Thus, the tracer specific activity defines the boundaries of a compartment and the boundaries may or may not coincide with any chemical, physical or physiological boundaries. Mixing within a compartment is assumed to be rapid compared with the rate that tracer leaves the compartment.

When the compartment is in the steady state with respect to the tracee, the intake rate is equal to the output rate, i.e.:

$$I = kS \tag{I–48}$$

Therefore, the amount of tracee, S, is constant, but the tracer activity, A*, and hence the specific activity, A, may vary with time. (See caption to Fig. I–16 for the symbol definitions.)

I–4.3.1. Single injection of tracer into open compartment

Consider now a steady-state, open compartment in which a single dose of tracer, A_0^*, has been injected and allowed to mix rapidly at zero time. Many compartments in nature are observed to follow **first-order kinetics.** That is (see Fig. I–16A):

$$\frac{dA^*}{dt} = -kA^* \tag{I–49}$$

or (dividing both sides by S):

$$\frac{dA}{dt} = -kA \tag{I–49′}$$

and on integration

$$A = A_0 e^{-kt} \tag{I–50}$$

where k is the **first-order rate constant** (and A_0 is A_0^*/S). Thus, the specific activity of the tracer is observed to decline exponentially.

A log-linear plot of specific activity (after mixing) versus time would appear as in Fig. I–17.

The slope of the line allows calculation of k. Assuming that the tracer behaves exactly as the tracee, it is thus possible to calculate the output rate, kS, since S can be determined by tracer dilution (i.e. $S = A_0^*/A_0$).

Exactly analogous to radioactive decay, the biological half-life $T_{\frac{1}{2},\,biol}$ may be determined graphically or from Eq. (I–51):

$$T_{\frac{1}{2},\,biol} = \frac{0.693}{k} \tag{I–51}$$

As can be inferred from Eqs (I–48) and (I–51), the biological half-life is not a true *biological constant* but inversely related to the intake. If the intake rate increases by a factor of two (and S remains constant), the biological half-life decreases by a factor of two, etc.

If the tracer undergoes significant radioactive decay during the experiment, the observations as plotted in Fig. I–17 must be corrected for this radioactive decay. If this is not done, an **effective half-life** will be observed, accounting for both radioactive decay and biological loss. Since these processes are independent, we can define an effective decrease rate constant:

$$\lambda_{eff} = \lambda + k \tag{I–52}$$

where λ = radioactive decay constant of tracer
k = biological rate constant
both in similar units. For interest, Eq. (I–52) should be compared with Eq. (I–36).

The effective half-life then is

$$T_{\frac{1}{2},\,eff} = \frac{0.693}{\lambda_{eff}} \tag{I–53}$$

I–4.3.2. Constant flow of tracer into open compartment

Consider now the case when tracer, from zero time and onwards, is supplied to the steady-state compartment at a constant inflow rate (see Fig. I–16B, including the caption). Since a constant fraction of the tracer present in the compartment will simultaneously be lost per unit time, the radioactivity in the compartment will increase from zero and approach a maximum value ($kA^*_{max} = iS$). The differential equation describing this rate of change is given by

$$\frac{dA^*}{dt} = \text{(intake rate)} - \text{(output rate)} = iS - kA \tag{I–54}$$

or, dividing by S:

$$\frac{dA}{dt} = i - kA \tag{I–54′}$$

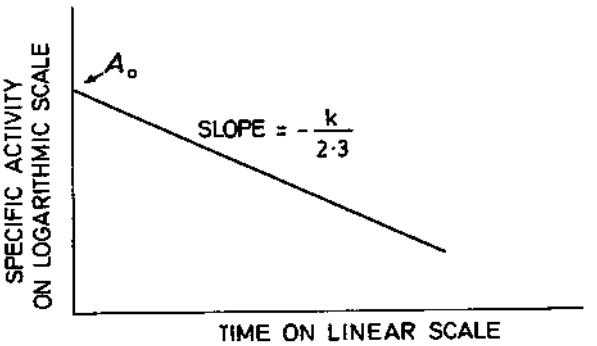

FIG.I–17. Logarithm of specific activity versus time in an open, steady-state compartment after rapid mixing of tracee and a single dose of tracer injected at zero time.

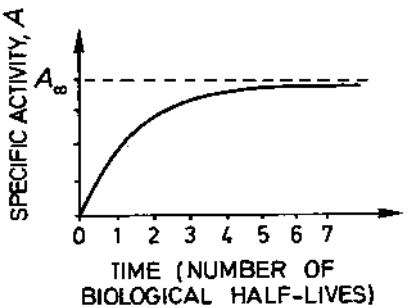

FIG.I–18. Specific activity in an open, steady-state compartment following initiation of constant inflow of radioactive tracer.

Since the specific activity, A, increases with time, the output rate will increase until it essentially equals the intake rate and $dA/dt = 0$. At that time the tracer as well as the tracee within the compartment will be in steady state.

Equation (I–54′) can be integrated to give:

$$A = \frac{i}{k}(1 - e^{-kt}) \qquad \text{(I–55)}$$

i.e.

$$kA = i(1 - e^{-kt}) \qquad \text{(I–55′)}$$

In this case, a plot of specific activity, A, versus time will be as shown in Fig. I–18. The specific activity will reach half the steady-state value ($A_\infty = i/k$) in a time equal to $T_{\frac{1}{2},\,biol}$, three quarters of this value in $2T_{\frac{1}{2},\,biol}$, seven eighths in $3T_{\frac{1}{2},\,biol}$, and so on.

The behaviour of the tracee in such steady-state systems is commonly termed *turnover*. The turnover time or average lifetime, $\bar{t}$, i.e. the average time a tracee atom or molecule spends in the compartment, is given by:

$$\bar{t} = \frac{1}{k} \tag{I–56}$$

or

$$\bar{t} = 1.44\,(T_{\frac{1}{2},\,biol}) \tag{I–57}$$

The derivation of Eqs (I–56) and (I–57) is given in Part VI, Appendix VI–7.

It should be apparent that, once a tracer has mixed completely in a whole system, though the system probably will be composed of many compartments, the behaviour of the tracer will be as in a single compartment.

I–4.3.3. Closed two-compartment system (exchange)

The general equations for a closed two-compartment model are not unduly rigorous and will be presented below. Consider the model as shown in Fig. I–19.

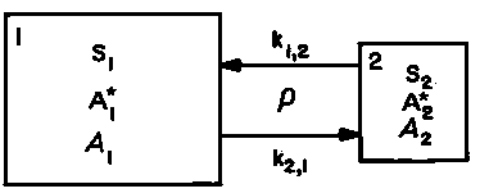

FIG.I–19. Closed two-compartment model (in tracee steady state) with S, A and A defined as in caption to Fig.I–16.*

$k_{1,2}$ = first-order rate constant describing transfer of tracee or tracer from compartment 2 to compartment 1

$k_{2,1}$ = first-order rate constant describing transfer of tracee or tracer from compartment 1 to compartment 2

ρ = rate at which tracee is exchanged, i.e. grams per unit time

If compartment 1 is initially labelled, the following differential equations may be written:

$$\frac{dA_1^*}{dt} = \frac{S_1 dA_1}{dt} = \rho(A_2 - A_1) \tag{I–58}$$

$$\frac{dA_2^*}{dt} = \frac{S_2 dA_2}{dt} = \rho(A_1 - A_2) \tag{I–59}$$

Let $\Delta_{1,2}$ equal the difference in specific activities at any time, that is

$$\Delta_{1,2} = A_1 - A_2 \tag{I–60}$$

From Eqs (I–58) and (I–59), it is then apparent that

$$dA_1 - dA_2 = d\Delta_{1,2} = -\rho\left(\frac{1}{S_1} + \frac{1}{S_2}\right)\Delta_{1,2}\, dt \tag{I–61}$$

Equation (I–61) is observed to be a first-order differential equation, identical in form to Eq. (I–1), and may be integrated directly to give

$$\Delta_{1,2} = A_1(0)\, e^{-\rho\left(\frac{1}{S_1} + \frac{1}{S_2}\right)t} = A_1(0)\, e^{-\rho \frac{S}{S_1 S_2} t} \tag{I–62}$$

where $S = S_1 + S_2$ and $A_1(0)$ has been substituted for $\Delta_{1,2}(0)$, in accordance with the condition that initially (t = 0) all the activity is in compartment 1.

Now, since the system is closed, the total activity is constant. This can be expressed as

$$S_1 A_1 + S_2 A_2 = S_1 A_1(0) \tag{I–63}$$

If Eqs (I–62) and (I–63) are solved simultaneously, the following solutions are obtained:

$$A_1 = \frac{A_1(0)}{S}\left(S_1 + S_2\, e^{-\rho \frac{S}{S_1 S_2} t}\right) \tag{I–64}$$

$$A_2 = \frac{A_1(0) S_1}{S}\left(1 - e^{-\rho \frac{S}{S_1 S_2} t}\right) \tag{I–65}$$

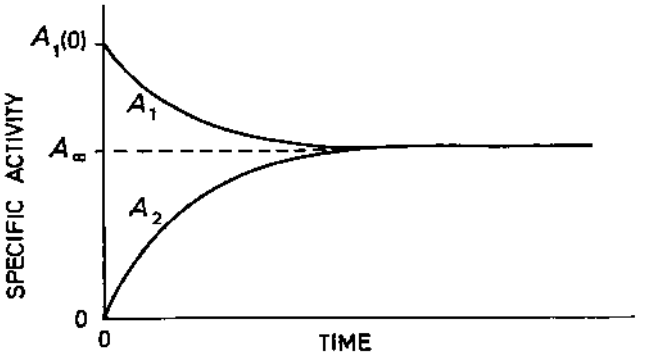

FIG.I–20. Specific activities in a closed, steady-state two-compartment system with compartment 1 initially labelled.

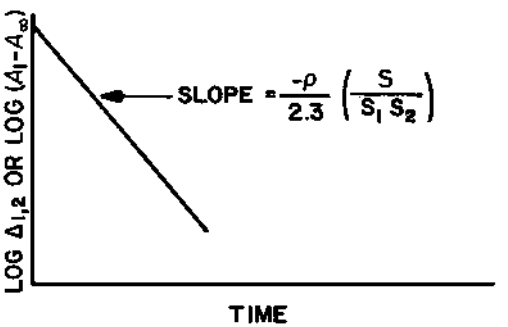

FIG.I–21. Log-linear plot of specific-activity excess versus time for closed two-compartment model in tracee steady state (exchange).

Plots of A_1 and A_2 versus time are shown in Fig. I–20.

Total tracee, S, may be determined by the dilution technique. Now, by inspection of Eqs (I–64) and (I–65) for long times, i.e. when the tracer is completely mixed, A_1 and A_2 equal the equilibrium value:

$$A_\infty = \frac{A_1(0)\,S_1}{S} \qquad \text{(I–66)}$$

Therefore, one can solve for S_1 from Eq. (I–66), and then for S_2 by difference from S.

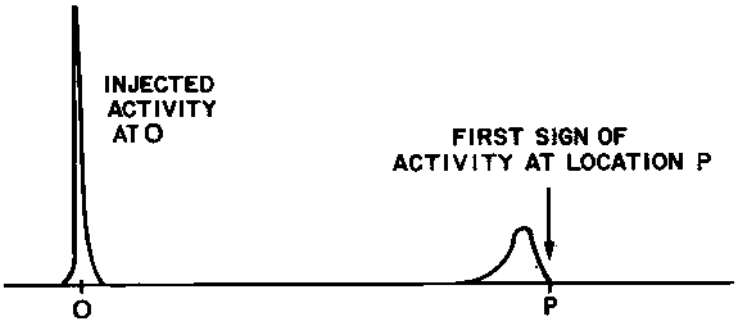

FIG.I–22. Diffuse appearance of tracer at location O following pulse injection at location P.

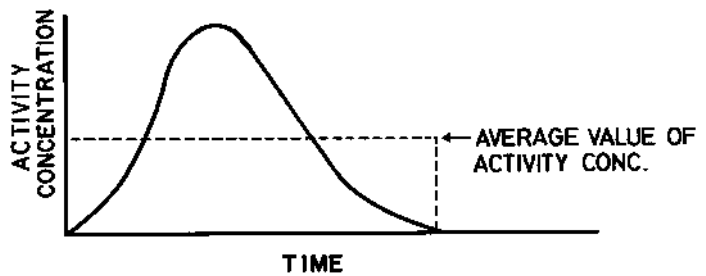

FIG.I–23. Schematic curve of tracer concentration at location P following single pulse injection at location O (see Fig.I–22).

From a log-linear plot of Eq. (I–62) or $(A_1 - A_\infty)$ versus time (see Eqs (I–64) and (I–66)), the numerical value of the slope, equal to $-\rho S/2.3 S_1 S_2$ (see Fig. I–21), may be used to calculate the transfer rate of exchange, ρ. To obtain transfer rates between compartments is the object of most tracer kinetic experimentation.

Finally, it should be noted that:

$$\rho = k_{1,2} \cdot S_2 = k_{2,1} \cdot S_1 \qquad \text{(I–67)}$$

which expresses the steady-state condition of the tracee.

I–4.3.4. Rate of flow determination

A final example of the use of tracers can be termed translocation or rate flow studies. If a radioactive tracer is injected at one location, O, in a system and its appearance observed at another location, P, the shape of the injection pulse will have become diffuse because of statistics and the number of possible pathways between locations O and P (see Fig. I–22).

If a known amount, A*, of label is injected at location O and the concentration of label appearing at P is measured as a function of time, then a typical curve as shown in Fig. I–23 may result.

If no tracer is lost between O and P (only a labyrinth in between), and if the area under the curve in Fig. I–23 can be determined (either graphically or by integration), then the flow rate, Q, in the system can be calculated, according to the so-called Stewart-Hamilton principle, as follows:

$$Q = \frac{A^*}{\text{area under curve}}$$

where Q is in millilitres per time interval (s), A* is, typically, in μCi, kBq or counts/s. The area under the curve has dimensions of activity concentration × time, the units being chosen appropriately. Calculation of the flow rate by this method has been very useful in physiological circulation studies and can be adapted easily to natural systems.

The application of tracer dilution techniques in entomology occurs, for example, in studies in which radioactive insects are released into the environment and then trapped. If the efficiency of the traps, the total number of radioactive insects released, the number trapped, and the number of unlabelled insects trapped are known, then an estimate can be made of the total insect population in a given area.

I–5. NITROGEN-15 DETERMINATION

In the case of nitrogen, the longest-lived radioisotope, ^{13}N, has a half-life of 10 minutes only, and the use of a stable isotope is consequently a necessity in most studies in which nitrogen is to be traced.

Natural nitrogen consists of two stable isotopes, ^{14}N and ^{15}N, and the *abundance*, Ab, of ^{15}N is approximately four ^{15}N atoms in every 1000 nitrogen atoms, or, more precisely, $Ab_{N\text{-}15} = 0.365$ atom per cent (at.%). Nitrogen enriched in ^{15}N is commercially available and is now widely used as a tracer. The *enrichment* (i.e. the ^{15}N at.% in excess of 0.365) is analogous to 'specific activity' in the case of a radioisotope.

In the following, ^{15}N is used as an example. Similar considerations apply to other stable isotopes such as ^{18}O and ^{13}C.

In entomology, the IAEA's laboratory at Seibersdorf has used ^{15}N as a tracer to determine the relative amounts of body nitrogen obtained by mediterranean fruit fly larvae from the two main ingredients of a larval diet, bran and yeast (unpublished data, 1976).

I–5.1. Measurement of isotopic abundance

For many years very accurate determinations of isotopic abundances have been carried out by mass spectrometry. More recently, a less expensive method employing photospectrometry has been developed. For ^{15}N analysis in research, the method chosen will depend upon the experimental circumstances.

I–5.1.1. Nitrogen-15 analysis by mass spectrometry

When ^{14}N atoms and ^{15}N atoms combine at random to produce nitrogen molecules, three types of molecules are formed. Let these be designated 28-N_2, 29-N_2 and 30-N_2, according to their masses.

The first step in ^{15}N analysis by mass spectrometry is ionization by electron bombardment of a slow stream of gas molecules from the nitrogen sample. The N_2^+ ions thereby produced enter a vacuum, where they are accelerated through a high voltage and passed on through a magnetic field perpendicular to the trajectories of the ions. In the magnetic field each ion travels along a circular path, the radius of which is proportional to $\sqrt{M}$ (where M is the mass of the ion). In this way, ions differing in mass are separated spatially. Finally, the various ion currents at appropriately positioned collectors are separately collected, amplified, and traced out by a recorder to give a mass spectrum in which nitrogen peaks are found at masses 28, 29 and 30.

The calculation of ^{15}N abundance from the relative peak heights observed is described in §I–5.1.3.

I–5.1.2. Nitrogen-15 analysis by photospectrometry

This method is based on the isotopic shifts found in the optical emission spectrum of molecular nitrogen.

Sample nitrogen gas in a closed tube of glass or quartz is discharged by the use of a microwave generator. The light emitted is dispersed by a prism or a grating, and a suitable section of the molecular nitrogen spectrum is recorded. For a given bandhead peak, separate peaks corresponding to 28-N_2, 29-N_2 and 30-N_2 are obtained.

I–5.1.3. Calculation of nitrogen-15 abundance

If it is assumed that peak-height, H, in a mass spectrum or photospectrum is proportional to the number of corresponding nitrogen molecules, then by definition ^{15}N abundance is given as follows:

$$Ab_{N\text{-}15}\ (at.\%) = \frac{H_{29\text{-}N_2} + 2H_{30\text{-}N_2}}{2(H_{28\text{-}N_2} + H_{29\text{-}N_2} + H_{30\text{-}N_2})} \times 100 \qquad (I\text{–}68)$$

where $H_{M\text{-}N_2}$ is the relative height of the peak corresponding to N_2 molecules of mass M. Assuming further that all ^{14}N and ^{15}N atoms pair randomly to form N_2 molecules, it can be shown (see Part VI, Appendix VI–8) that:

$$Ab_{N\text{-}15}\,(\text{at.}\%) = \frac{100}{2K + 1} \qquad \text{(I–69)}$$

where K is the $H_{28\text{-}N_2}/H_{29\text{-}N_2}$ ratio.

Equation (I–69) is applicable, for example, to the calculation of the relatively low ^{15}N abundances usually dealt with in agricultural research.

In mass spectrometry the values obtained by means of Eqs (I–68) or (I–69) are considered to be final, whereas in photospectrometry they must be taken as observed values, which need correcting by the use of a calibration curve before *true* values are obtained.

I–5.2. Nitrogen liberation for ^{15}N-analysis

In most tracer samples the ^{15}N-labelled nitrogen will be in some chemically bound form. Nitrogen in the gaseous form may be liberated from the sample by one of three procedures, namely:

(a) Kjeldahl-Rittenberg;
(b) Direct Dumas;
(c) Kjeldahl-Dumas.

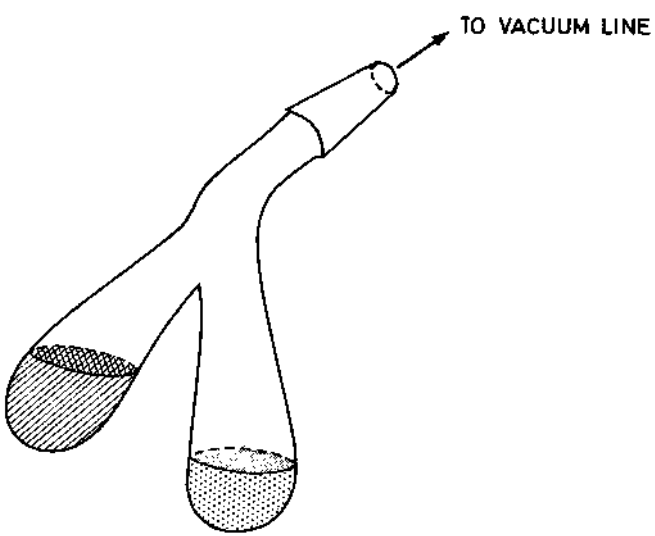

FIG.I–24. Rittenberg flask.

I–5.2.1. Kjeldahl-Rittenberg procedure

Following a traditional Kjeldahl wet combustion and distillation, the back-titrated solution containing the NH_4 salt is acidified and evaporated down to a few millilitres. An aliquot containing about 1 mg total N for mass spectrometry, or about 10 μg total N for photospectrometry, is placed in one side of a Rittenberg flask (Fig. I–24) containing an alkaline solution of NaBrO in the other side. After evacuation of air from the flask, the contents of the two sides are mixed and N_2 is liberated according to the reaction

$$2NH_4^+ + 3NaBrO + 2OH^- \rightarrow N_2 + 5H_2O + 3NaBr$$

Before letting the N_2 into the mass spectrometer or the optical discharge tube, the Rittenberg flask is cooled in liquid air (or nitrogen), whereby H_2O and other condensable impurities are frozen out. Unfortunately, CO is not frozen out, and its spectrum causes interference in both the mass spectrum and the optical spectrum of nitrogen.

I–5.2.2. Direct Dumas procedure

In this procedure, oxides of copper are used to perform a dry combustion of the sample at 500–1000°C for a period of time ranging (inversely to the temperature) from several hours to one hour. Above 600°C a quartz container is necessary.

The combustion products (CO_2, H_2O, etc.) other than N_2 are absorbed by oxides of calcium and aluminium or they are frozen out by liquid air (or nitrogen). Again, CO is not frozen out and causes interference.

In mass spectrometry, the N_2 is released from the combustion container by breaking it inside the evacuated inlet system of the mass spectrometer.

In photospectrometry, the discharge tube itself is used as the combustion container, so that no transfer of the chemically liberated N_2 is necessary. However, the following difficulties can be encountered:

(a) Samples consisting of plant or biological material are often difficult to combust completely in the discharge tube.

(b) A representative sample that is small enough for photospectrometry can be difficult to obtain; especially, large items, e.g. whole plants, have to be sampled directly.

(c) Natural nitrogen from air adsorbed onto or in the sample will become mixed with the sample nitrogen and decrease its enrichment.

I–5.2.3. Kjeldahl-Dumas procedure

This is a combination of the procedures described above in which an aliquot of NH_4 salt taken from a Kjeldahl combustion is treated by the Dumas procedure.

I–6. NEUTRON MODERATION AND GAMMA-RAY ATTENUATION TECHNIQUES

I–6.1. The neutron moisture meter

I–6.1.1. Principle involved

In 1932, Sir James Chadwick discovered that when alpha particles were allowed to fall upon a piece of beryllium in contact with a paraffin block, protons could be detected at the surface of the paraffin block. They were not detected when the paraffin was removed. Chadwick deduced that neutral particles were formed by the alpha reaction on beryllium, and these were able to eject protons from the paraffin block by collision. These natural particles he named neutrons. Thus the interaction and moderation of neutrons by hydrogen atoms was inherent in the discovery of the neutron itself. However, neutron moisture meters, which are based on this principle, were developed only about 30 years ago as an aid in speeding up soil moisture measurements during the construction of airfields, roads and buildings. Extensively tested in this work, the use of this technique was rapidly extended to soil moisture studies.

Neutrons are usually classified according to energy or velocity. Although the boundaries between the various divisions are ill-defined, a common classification of the energy, E, of neutrons is:

Thermal	$E = 0.025$ eV
Epithermal	$E \doteq 1$ eV
Slow	$0.03 \text{ eV} < E < 100 \text{ eV}$
Intermediate	$100 \text{ eV} < E < 10 \text{ keV}$
Fast	$10 \text{ keV} < E < 10 \text{ MeV}$
High-energy	$10 \text{ MeV} < E$

As neutrons traverse matter (see §I–1.6.4) they lose energy in a series of collisions and, like gas molecules, eventually come into thermal equilibrium with the surroundings. The term **thermal neutron** refers to a neutron in equilibrium at ambient room temperature. When in equilibrium, the neutron energies will have a Maxwellian distribution about the mean energy.

TABLE I–6. EFFECTIVENESS OF ELEMENTS IN SLOWING DOWN FAST NEUTRONS

Elements	Average number of collisions required for thermalization of 2 MeV neutrons	Elements	Average number of collisions required for thermalization of 2 MeV neutrons
Hydrogen	18.2	Phosphorus	288
Lithium	69.3	Sulphur	298
Beryllium	88.1	Chlorine	329
Boron	104.5	Potassium	362
Carbon	115.4	Calcium	371
Nitrogen	133.5	Titanium	442
Oxygen	152	Manganese	504
Sodium	215	Iron	514
Magnesium	227	Cadmium	1028
Aluminium	251	Uranium	2169
Silicon	262		

Moisture measurements are based on physical laws governing the scattering and moderation of neutrons. When a source of fast neutrons is placed in a soil, the neutrons ejected from the source collide with nuclei of the surrounding atoms and are scattered randomly in all directions. Each collision a neutron undergoes causes a loss of part of its kinetic energy. The scattering and energy reduction process continues until the kinetic energy of the neutron approaches the average kinetic energy of atoms in the scattering medium. At this lower energy level, the neutron is designated a slow or thermal neutron. The average energy loss by a fast neutron is much greater in collisions with atoms of low atomic weight than in collisions involving heavier atoms. The average numbers of collisions necessary to thermalize 2 MeV neutrons are given in Table I–6 for certain atoms. The loss of energy in a number of collisions, depending inversely upon the atomic weight, is a measure of the moderating properties of the nuclei in a given medium.

Table I–6 shows clearly that hydrogen is the most effective neutron moderator in the soil as, on an average, only about 18 collisions are needed to thermalize a fast neutron; for other elements commonly present in soils, a much greater number of collisions is required to achieve the same effect. If a source of neutrons and a slow-neutron detector are placed side by side in the soil, a portion of the neutrons moderated, mainly through collisions with hydrogen atoms, will be scattered back and detected. A relationship can thus be established between the soil moisture content per unit volume and the number of slow neutrons arriving per unit time at the detector.

I–6.1.2. Neutron source

It is mostly the (α,n) reaction that is used to provide a neutron flux, for example:

$$^{4}_{2}\mathrm{He} + ^{9}_{4}\mathrm{Be} \rightarrow ^{1}_{0}\mathrm{n} + ^{12}_{6}\mathrm{C} + 5.6\ \mathrm{MeV}$$

To use the short-range alpha particles to best advantage, a fine powder of beryllium is intimately mixed with a somewhat smaller amount of radioactive material, and the mixture is compressed to approximate a point source. Ra-Be is a very commonly used neutron source but has the disadvantage of a high γ/n emission ratio.

When a scintillation crystal is used as the slow-neutron detector, the radiation source must either emit very low-energy gamma rays or none at all, otherwise adequate shielding is not possible (americium-beryllium or actinium-beryllium sources are then used).

I–6.1.3. Slow-neutron detectors

Boron trifluoride detectors. The most widely used detectors are BF_3 tubes based on the following reaction:

$$^{10}_{5}\mathrm{B} + ^{1}_{0}\mathrm{n} \rightarrow ^{7}_{3}\mathrm{Li} + ^{4}_{2}\mathrm{He} + 2.78\ \mathrm{MeV}$$

The following are important considerations if a high measurement efficiency is to be obtained:

(a) The detection tube is made relatively large in volume (50–100 cm^3).
(b) To benefit from the large cross-section of the isotope ^{10}B (18% of natural boron), about 96% ^{10}B-enriched BF_3 is used as the filling gas.
(c) Detection tubes are operated in the proportional region (see Fig. I–9) under sub-atmospheric pressure to benefit from (i) the small dead-time of proportional counters (allowing for the measurement of very high count-rates), (ii) a moderate gas amplification factor of about 10^3, and (iii) the high alpha energy in discriminating between pulses resulting from neutron capture and those arising from relatively strong gamma-ray intensities.

Helium-3 detectors. Helium-3 detectors, based on the following reaction:

$$^{3}_{2}\mathrm{He} + ^{1}_{0}\mathrm{n} \rightarrow ^{1}_{1}\mathrm{p} + ^{3}_{1}\mathrm{H} + 764\ \mathrm{keV}$$

were recently developed. They are operated in the proportional region. They have filling pressures up to 10 atm, and give much higher efficiency in measuring

thermal and especially *epicadmium* neutrons (2 to 10 times greater than a BF_3 tube). BF_3 detectors are somewhat better with respect to gamma-ray rejection. (Helium tubes are used in the neutron moisture meters produced by some manufacturers).

Scintillation detectors. A scintillation crystal of europium-activated ^6LiI is also commonly used to detect slow neutrons, using the following reaction:

$$^6_3Li + ^1_0n \rightarrow ^4_2He + ^3_1H + 4.8 \text{ MeV}$$

The relatively small surface of a scintillation crystal through which the slow neutrons must pass for detection is partially compensated for by the higher efficiency of detection. Because of the high gamma sensitivity of a scintillation crystal, americium- or actinium-beryllium sources are usually used in this case.

I–6.2. The gamma density probe

Density probes use a gamma source, mainly of ^{226}Ra or ^{137}Cs, and either GM tubes or a scintillation crystal for the detection of the gamma photons, which are either backscattered from, or transmitted through, the medium involved in the measurement. The most commonly used instruments (depth and surface probes) are backscattering devices, as these are simpler to handle and cheaper in cost.

The measurement of density is based on the known interaction of gamma rays and the orbital electrons of atoms (see §I–1.6.3). Gamma photons with an energy less than 1 MeV and emitted by a radioactive source that is placed in or on the soil will interact with the surrounding electrons by the Compton effect and/or photoelectric absorption.

As the number of electrons per unit volume of soil (density) is increased, the Compton scattering power of the medium increases proportionately. With each scattering process, however, the gamma photon loses some of its energy. Thus, although an increase in electron density of the scattering medium increases the probability of multiple scattering of the gamma photon, the probability that the gamma photon will suffer photoelectric absorption before it can reach the detector is also increased. The combined effect of these two probabilities is that a smaller number of gamma photons will reach the probe as the surrounding material becomes more dense.

Since the number of electrons present per unit volume of material is approximately proportional to the density of the material, the number of gamma rays scattered back and detected per unit of time is an inversely correlated measure of density.

Since absorption of gamma photons by hydrogen is about twice as high per nucleon as for nucleons of other common elements in soil, this would lead to an error in bulk density caused by variation in water content. However, this error is usually reduced in magnitude, either by a circuitry technique that discriminates against low-amplitude pulses coming from the detector, or by a judicious use of lead screening to attenuate low-energy radiation reaching the detector.

The density probe measures the wet density of the soil. Therefore, the weight of water should be subtracted if dry-density data are desired.

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WORKING NOTES TO PART I

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PART II. RADIATION BIOLOGY

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II-1. INTRODUCTION

This part of the manual provides the reader with some of the fundamental concepts of radiation biology. It is impossible in a short part to discuss this rather complex field and for this reason only basic concepts will be covered as well as some of the few generalities that are known about the subject. A suggested bibliography is presented at the end of this part for those readers who are interested in fully developing their knowledge of the field of radiation biology.

Radiation biology could be defined as the study of the effects of radiation on living organisms. The term encompasses all types of radiation in the electromagnetic spectrum as well as particulate radiation. However, for the purposes of this manual the term will be restricted to the effects of *ionizing* radiation or those types of radiation which produce ionization within living organisms.

The interaction between ionizing radiation and the cell begins with a purely physical transfer of energy to atoms and molecules, irrespective of their organization, and into living structure. Then follows a sequence of events, the nature of which depends on the absorbed dose and the chemical and physico-chemical composition of the irradiated material. The interactions that arise may be either excitations or ionizations or both. In the excitation process, electrons of atoms and molecules are raised to a higher energy level without being expelled. These excited atoms are more reactive chemically than atoms in the unexcited state, and the chemical bonds may be disrupted leading to a break-up of complex molecules. Generally speaking, the energy absorbed in the excitation process is not considered to be as important as that absorbed in the ionization process. In the ionization process one (or more) of the external electrons from an atom is (are) removed, leaving the atom as an ion with a net positive charge. The electron that has been removed from the atom can recombine with the original system, but more frequently the electrons move at random until they become attached to a neutral atom to form a negative ion,or are captured by a positive ion thereby releasing ionization energy.

The major difficulty in understanding the mechanisms of cell death and destruction by irradiation is the lack of knowledge of the sequential events which occur in complex systems. A multitude of terms has been used by different investigators. Here we shall define two terms as follows:

1. Direct effects — those effects which are caused by ionizations contained within the biological macromolecule of interest or structure specified as being affected.

2. Indirect effects — those effects apparently caused in part by ionizations not contained or occurring within the biological macromolecule of interest or structure specified as being affected.

In conjunction with these two terms others are utilized which tend to confuse the novice reader in the field. These are terms associated with the models proposed to explain dose effect relations. The simplest of these models is the hit theory which is based on the assumption that the observed effect is caused only by ionizations occurring inside the 'target'. According to this theory the effect is directly and quantitatively determined by the transfer of a certain amount of energy from ionizing particles to a critically important volume whose geometrical configuration is considered to be delimited from the rest of the cell, this being in effect the target. Another model is the theory of indirect hit, which postulates that the effect of radiation has an intermediary agent which produces the biological effect; an example is the radiolysis of water which produces free radicals, which in turn interact with the critical target.

The oxygen effect is due to the efficacy of oxygen which enhances the action of X and gamma radiation on most cells. The oxygen increases the free radical formation in water radiolysis and also makes available more oxidizing radicals. Oxygen must be present during the irradiation process and it can increase the lethal effectiveness of X and gamma radiation by a factor of 2–3. This particular effect has been utilized in the field of radiotherapy where tumours with a low oxygen tension or being partially anoxic are oxygenated to increase their sensitivity to ionizing radiation and thus to increase the probability of cure.

II-2. RADIATION SENSITIVITY

Radiosensitivity or radiation sensitivity can mean many things to many people unless they use the same basic criteria for comparison. The parameters most commonly used to determine radiosensitivity are cell death, mitotic inhibition, and impairment or loss of biochemical and physiological functions. When dealing with the radiation sensitivity of higher animals and plants, survival after radiation is usually chosen as the end point and the measurement most commonly used is that of $LD_{50/30}$, which means the lethal dose necessary to kill 50% of a given population in 30 days. Cromroy [1, 2] used the criterion of $LD_{50/24h}$ for measuring the radiosensitivity of insects.

There is one law of radiosensitivity which was described within ten years after the discovery of the X-ray. In 1906, Bergonie and Tribondeau proposed a law of radiosensitivity based on work they had done with the germinal epithelia of rats. The law, most simply stated, specified that the most radiosensitive cells were those with the following characteristics:

1. Highest metabolic rate
2. Ability to retain the dividing capacity for the longest time
3. Least differentiation

Although many exceptions to the law are known, the basic formulation is valid. Radiation sensitivity of cells is now known to depend also on various other factors such as temperature, oxygen tension, hydration, ploidy, phase in the mitotic cycle, age, metabolic state, and particular animal strain or species.

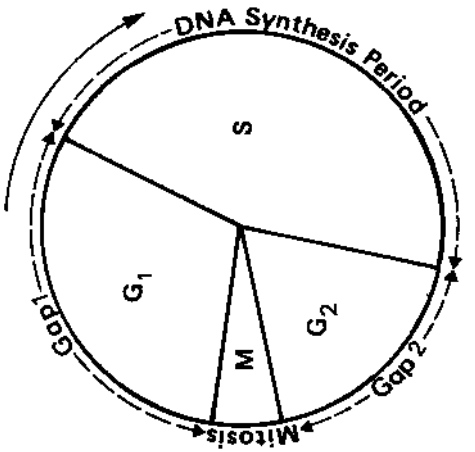

FIG.II-1. Schematic diagram of cell generation cycle.

Figure II-1 illustrates a schematic diagram of a cell generation cycle. It should be noted that the interphase period, which includes G_1, S, and G_2, lasts much longer than the mitotic period where division occurs. The radiosensitivity varies considerably throughout the cycle, depending on the cell phase. A general pattern has emerged of maximum radiosensitivity of cells in mitosis (M) and maximum radioresistance of cells in late S. It has also been shown that the G_2 period and early pro-phase are the most radiosensitive stages in the generation cycle of many cells. Somatic mammalian cells, however, may show no dependence of radiosensitivity on the division stage. There is still much which is not known about the G_2 period, and consequently the effects of radiation in this unique phase of the cell cycle cannot be defined at a molecular level at present.

In considering radiosensitivity at the cellular and tissue levels a distinction is made between radiation-induced lesions leading to reproductive death and those leading to interphase death. In reproductive death, the cells survive for a relatively

TABLE II-1. COMPARISON OF RELATIVE RADIOSENSITIVITIES OF VERTEBRATES, PLANTS AND INSECTS AS MEASURED BY LD_{50} [1, 2, 6, 9, 10]

A. VERTEBRATES

Species	$LD_{50/30\ d}$ (kR)	(mC/kg)
Dog	0.319	82.3
Goat	0.375	96.8
Swine	0.390	100.6
Man	0.450	116.
Mouse	0.940	243.
Rat	0.936	241.
Rabbit	0.890	230.

B. PLANTS

Species	Common name	$LD_{50/6\ months}$ (kR)	(mC/kg)
Pinus eliotti	Slash pine	0.375	96.8
Podacarpus macrophylla		0.600	155.
Zamia floridana	Coontie	0.610	157.
Juniperus conferta	Shore Juniper	0.640	165.
Araucaria excelsa		0.730	188.

C. INSECTS

Species	Common name	$LD_{50/24\ h}$ (kR)	(C/kg)
Periplaneta americana	American cockroach	53	13.7
Blattella germanica	German cockroach	91	23.5
Musca domestica	House-fly	93	24.0
Stomosys calcitrans	Stable-fly	115	30.
Culex quinquefasciatus	Mosquito	140	36.
Naupheta cinerea	A cockroach species	143	37.
Cimex lectularis	Bed bug	155	40.
Pediculus humanus humanus	Body louse	175	45.
Achaeta domestica	Cricket	125	32.
Silverfish		98	25.3
	Rice weevil	203	52.
	Saw-toothed grain beetle	210	54.
	Red flour beetle	310	80.
	Cigarette beetle	195	50.
Trogoderma inclusens	Beetle	245	63.
	Black carpet beetle	345	89.

long time and may undergo several divisions or form giant cells. The synthesis of nucleic acids and protein continues at a fairly normal rate over several generations. In contrast, interphase death may occur rapidly (possibly within a few hours) and is not associated with disturbances in cell division. Some of the cells for which interphase death has been reported are small lymphocytes, mammalian and insect primary oocytes, certain classes of primitive cells in the embryo and infant mammal, and insect ganglion cells.

Many studies have attempted to derive predictions of radiation sensitivity and to establish a biological indicator cell which would be of value in making such a prediction. The rationale for such research was based on cellular radiation biology which indicated that if radiation-induced genetic damage is the primary lethal event in animal and plant cells, then there should be some relationship between the amount of genetic material and some parameter of cell inactivation. Sparrow and Evans [3] were able to show in plants of the diploid species that the larger the nuclear volume the more radiosensitive the organism. This was later modified by Sparrow et al. [4] to the concept of interphase chromosome volume (ICV), which is the nuclear volume divided by the diploid chromosome number. Sparrow et al. [5] have shown that when ICV values and deoxyribonucleic acid content per chromosome were compared with mutation rates per roentgen an increase in mutation rate per roentgen was highly correlated with an increase in both ICV and DNA per chromosome. Cromroy utilized the columnar epithelial cells of the duodenal intestinal mucosa in mammals and the endothelial cells lining the midgut in insects for his biological cell indicators. Sparrow used the non-dividing, interphase nuclei of the tunica and outer corpus layer of the terminal meristem in plants. Cromroy selected as his parameters for measuring radiosensitivity either the $LD_{50/30\,d}$ for mammals or $LD_{50/24\,h}$ or $LD_{50/28\,d}$ for insects. Cromroy's research indicated that interphase chromosome volume did not serve as the best indicator for animals as it had for plants in Sparrow's work. Finally he used the mineral content of insect species for his predictor formulas, which are summarized in several papers [6, 7, 8]. Table II-1 is taken from the work of Cromroy [1, 2, 6, 9] and Conger and Cromroy [10] and compares the radiosensitivity of mammals, plants and insects, based on the parameter of species death at either 30 days (for vertebrates), 6 months (for plants) or 24 hours (for insects).

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WORKING NOTES TO PART II

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PART III. MENTAL EXERCISES

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(1) When the Z-number of all nuclides is plotted against the N-number, isotopes of a particular element will be found on a horizontal line. This kind of representation is usually given on nuclear charts. How can the decay products of a particular nuclide be found after the emission of one:

(a) alpha particle?
(b) β^--particle (electron)?
(c) β^+-particle (positron)?
(d) gamma ray?
(e) X-ray after electron capture?
(f) electron after internal conversion?
(g) neutron?

(2) With the aid of a nuclear chart, find the decay products of ^{14}C, ^{22}Na, ^{40}K, ^{90}Sr and ^{238}U.

(3) Calculate the weight of 100 μCi (3.7 MBq) of carrier-free ^{14}C and 100 μCi (3.7 MBq) of carrier-free ^{22}Na ($T_{\frac{1}{2}}$ of ^{14}C and ^{22}Na are 5730 and 2.62 years, respectively).

(4) If a solution has a concentration of 100 μCi (3.7 MBq) of carrier-free ^{14}C per ml, calculate its molarity ($T_{\frac{1}{2}}$ of ^{14}C = 5730 years).

(5) A sample of ^{60}Co has an activity of 1 Ci (37 GBq); calculate its activity 2 years later ($T_{\frac{1}{2}}$ of ^{60}Co = 5.3 years).

(6) A radionuclide has lost 15/16 of its original activity in 32 min; calculate the half-life of the nuclide.

(7) ^{137}Ba is formed from ^{137}Cs. How many curies and becquerels of $^{137}Ba^m$ will be formed from 100 mCi (3.7 GBq) of ^{137}Cs in exactly 1, 2 and 20min? ($T_{\frac{1}{2}}$ of $^{137}Ba^m$ = 2.55 min).

Answer: 23.8 mCi (881 MBq), 41.9 mCi (1.55 GBq) and 99.6 mCi (3.69 GBq).

(8) Determine the daily decrement, in percentage of the activity, of any ^{32}P preparation ($T_{\frac{1}{2}}$ of ^{32}P = 14.3 d).

(9) A ^{24}Na sample ($T_{\frac{1}{2}}$ = 14.8 h) had a count-rate of 400 counts/s. One hundred hours later it had a net count-rate of 4.40 counts/s. Roughly estimate the dead-time of the GM counter.

(10) The activity of ^{14}C in 8 g of natural carbon sample including background was found to be 10.2 counts/min. The background of the counter was 4.5 counts/min and the counting yield was 5%. Neglecting the statistical deviation, calculate the ^{14}C abundance in atom per cent ($T_{\frac{1}{2}}$ = 5700 years).

(11) The background count-rate of a GM counter system is 0.5 counts/s. A sample is counted giving a total of 450 counts in 100 seconds. The background is counted for 30 seconds. Calculate the net count-rate of the sample and the natural standard deviation as a percentage of the net count-rate.

(12) A 0.1 mg sample of pure ^{239}Pu underwent a decay of 2.3×10^6 Bq (disintegrations per second). Calculate the half-life of this radioisotope.

(13) Calculate the thickness of lead shielding necessary to reduce the exposure dose in air to 2.5 $mR \cdot h^{-1}$ (0.645 $\mu C \cdot kg^{-1} \cdot h^{-1}$) at 1 m from a 100 mCi (3.7 GBq) ^{60}Co source.
Hint: Use Eq.(I-35) and assume the half-thickness of lead for ^{60}Co gamma rays to be 1.3 cm.
Answer: 7.5 cm.

(14) Indicate the increase or decrease in the number of neutrons (N) and protons (Z) and in the mass number (A) after the following nuclear reactions:

(n,p)
(n,γ)
(n,α)

(15) Scandium (^{45}Sc) is to be determined by the activation method. Assuming the lower limit of the determination to be 1 count/s at 10% GM counting yield, compute the smallest measurable amount of scandium when the sample is subjected to a neutron flux of 10^{12} $cm^{-2} \cdot s^{-1}$ for 2 h. Assume that the (n,γ) reaction is the most probable and that the shielding effect is negligible. The cross-section of scandium is 23 b (23×10^{-28} m^2).

(16) What would be the specific activity of phosphorus (^{31}P), having a cross-section of 0.2 b (0.2×10^{-28} m^2), after irradiation by a neutron flux of 10^{12} $cm^{-2} \cdot s^{-1}$ for 1 h, 1 d and 10 d respectively? ($T_{\frac{1}{2}}$ of ^{32}P = 14 d).

(17) Calculate the energy absorbed by a 70 kg man who has received a whole-body dose of 700 rad (7 Gy), an amount almost certain to be fatal. Assume the body to have the specific heat of water (1.0 $cal \cdot g^{-1} \cdot degC^{-1}$ or 4.18 $kJ \cdot kg^{-1} \cdot K^{-1}$) and calculate the resulting temperature rise (1 joule = 0.239 calories) using both the special and SI-derived units.
Answer: 490 joules, 1.7×10^{-3} °C.

WORKING NOTES TO PART III

NOTES (cont.)

NOTES (cont.)

PART IV. LABORATORY EXERCISES

PART IV. LABORATORY EXERCISES

IV–1. EXPERIMENTS WITH A GM COUNTER

IV–1.1. The plateau of a GM tube

Geiger-Müller counter assemblies in normal operation often show an appreciable variation in performance from one time of measurement to another. It is thus useful to have a reference source by which day-to-day counting may be standardized. The half-life of such a standard should be so long that no correction for decay need be made. A suitable reference source may be made from black uranium oxide (U_3O_8). This combines the required chemical stability and long half-life (4.5×10^9 a). The oxide should not have been treated chemically for at least a year, during which time any significant daughter products removed by previous treatments will have again come to radioactive equilibrium.

The disintegration scheme of the mixture of isotopes which forms natural uranium is complex, and it is advisable to filter out all particles except the beta particles of 2.3 and 1.5 MeV. This may be done by covering the source with aluminium foil (35 mg/cm^2, i.e. 0.35 kg/m^2).

With this or a similar standard source the following properties of a GM tube may be determined:

(i) The threshold or starting potential;
(ii) The length and shape of the plateau.

It is then possible to deduce the optimum operating potential and the slope of the plateau.

PROCEDURE

(1) Obtain a source counting about 130 counts/s ($\cong$ 8000 counts/min).
(2) Put the source into the holder (in the lead castle), and increase the electrode bias voltage (high voltage) slowly until the first counts are obtained. This voltage level is called the *threshold voltage* (V_T).
(3) Determine the count-rates with increasing voltage. A total of 10 000 counts registered for each voltage step is adequate. (Voltage increments may be 20 to 50 volts.)
(4) When the count-rate does not change appreciably as the high voltage is increased, the GM tube is operating in the *plateau region*. No further high-voltage steps should be attempted once it is noticed that the count-rate is again beginning to increase, since at voltages above this (V_R in Fig. IV–1) the counter will start to race, and damage to the GM tube is likely to occur.

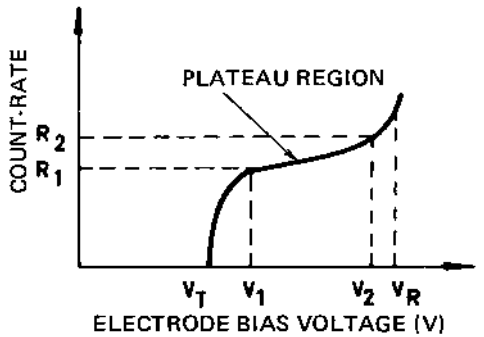

FIG.IV–1. Characteristic of a GM tube showing the relationship between count-rate and voltage.

(5) Calculate the slope as percentage increase in count-rate per 100 volts and the plateau length (see Fig. IV–1), $V_2 - V_1$.
The slope is:

$$\frac{(R_2 - R_1)/R_W}{(V_2 - V_1)/100} \times 100 \qquad (\% \text{ per } 100 \text{ V})$$

in which R_W is the count-rate at the working voltage (see below).

(6) As the counter ages, the threshold voltage (V_T) tends to increase and the racing voltage V_R to decrease. To allow for this, choose the working voltage at $V_T + 75$ volts or $\frac{1}{2}(V_T + V_R)$ if the plateau length ($V_2 - V_1$) is less than 150 volts. Occasional checks on the plateau characteristics of a tube are necessary with age (number of counts).

IV–1.2. The resolving time of a GM counter

The resolving time, i.e. the time after each pulse that the GM tube (and counting system) do not register pulses, can be determined in various ways. The method by which a series of samples of increasing strength is counted is straightforward. From the difference between the expected count-rate as extrapolated from low count-rates and the observed count-rate, the dead time can be estimated.

Let the true count-rate be R_{true} counts/s and the observed count-rate R counts/s. If the resolving time is τ seconds, the counter has been inoperative during $R\tau$ seconds per second. R counts have therefore been registered in $1 - R\tau$ effective seconds. The corrected count-rate R_{true} is thus as follows:

$$R_{true} = \frac{R}{1 - R\tau} \qquad (IV–1)$$

If the R of a radioisotope of *known* half-life is plotted against time on log-linear graph paper, R_{true} for the highest count-rates can be extrapolated from the R of the lowest count-rates, and τ can then be estimated approximately with the aid of Eq. (IV–1). (At very high count-rates, it may turn out that τ is no longer constant but equal to some function of R.)

Another approximation of the dead time τ may be obtained by the method of *twin samples*, i.e. from a comparison of the count-rate of two samples counted together with the sum of the count-rates of each sample counted separately. Let $R_{true,1}$, $R_{true,2}$, $R_{true,1+2}$ and $R_{true,b}$ be the correct count-rates (background included) of sample 1, sample 2, samples 1 plus 2, and a blank sample, respectively. Also let R_1, R_2, R_{1+2} and R_b be the corresponding observed count-rates. Then by definition:

$$R_{true,1} + R_{true,2} = R_{true,1+2} + R_{true,b}$$

and thus from Eq. (IV–1):

$$\frac{R_1}{1-R_1\tau} + \frac{R_2}{1-R_2\tau} = \frac{R_{1+2}}{1-R_{1+2}\tau} + \frac{R_b}{1-R_b\tau} \qquad \text{(IV–2)}$$

Since $R_i\tau \ll 1$ and $r\tau \ll R_i\tau$, the following approximations can be made:

$$\frac{R_i}{1-R_i\tau} \triangleq R_i + R_i^2\tau \quad \text{and} \quad \frac{R_b}{1-R_b\tau} \triangleq r \qquad \text{(IV–3)}$$

Therefore, after substituting, one obtains:

$$\tau \triangleq \frac{R_1 + R_2 - R_{1+2} - R_b}{R_{1+2}^2 - R_1^2 - R_2^2} \qquad \text{(IV–4)}$$

or

$$\tau \triangleq \frac{R_1 + R_2 - R_{1+2} - R_b}{2R_1R_2} \qquad \text{(IV–5)}$$

since

$$R_{1+2}^2 \triangleq (R_1 + R_2)^2$$

PROCEDURE

(1) Tap about 5 ml (approx. 420 counts/s ≙ 25 000 counts/min) from the $^{137}Ba^{m}$ column used in the experiment in §IV--2.2. Cover the counting dish with a plastic cap of 100–150 mg/cm^2, and immediately start making 6 to 8 one-minute countings separated by one-minute intervals.
(2) After half an hour determine the residual count-rate (background), and then plot the count-rates corrected for background of $^{137}Ba^{m}$ against time on log-linear graph paper.
(3) A straight line with its slope corresponding to the *tenth-life*[1] of $^{137}Ba^{m}$ (8.5 min) is parallel-displaced until it becomes a tangent to the last two or three points. From the small deviations of the first two or three points from this straight line, estimate the mean dead time of the GM counter, using Eq. (IV–1).
(4) Select two beta samples (e.g. ^{204}Tl or ^{36}Cl) of approximately 200 counts/s.
(5) Count the first sample in a sample holder with two holes. In the second hole insert an empty counting cup.
(6) After counting the first sample, remove the blank cup without touching the active sample. Put the second sample in the holder and count both samples together.
(7) Remove the first sample and replace by a blank cup without touching sample 2. Count sample 2.
(8) Count the background with the two blank cups in place.
(9) Calculate τ with the aid of Eqs (IV--4 or 5).

IV–1.3. Natural and technical uncertainty (statistics)

In scientific experimentation, the standard deviation (calculated from replicates) should always be given together with the results to permit assessment of the uncertainty.

When the standard deviation σ is calculated from replicates, it automatically includes all sources of uncertainty.

When a series of *identical* counts is made on a sample which is not moved between individual counts, assuming the counter functions correctly, the standard deviation of the sum-count will be found to be $\sigma_{nat} = C^{\frac{1}{2}}$, where C is the sum-count. This is a measure of the *natural* uncertainty inherent in radioactive decay. Note that this type of uncertainty can be calculated after a single counting. However, when the sample is moved between countings or a number of *identical* samples are counted in succession, a larger figure is likely to be obtained than can be explained by natural uncertainty alone. This is because of

[1] The time required for the radioactivity to decrease to one tenth of its initial value.

random irregularities in geometry and sample preparation. This form of added deviations (including erratic counter performance) we will call *technical* uncertainty. An experimental evaluation of these two types of uncertainty will be made.

MATERIALS

(1) ^{32}P solution of specific activity of about 0.05 μCi/ml (1.85 kBq/ml).
(2) 1 ml pipette, and a propipette (e.g. rubber bulb).
(3) 25 counting cups.

PROCEDURE

(1) Obtain a bottle of ^{32}P solution giving a count-rate of 170 counts/s ($\doteq$10 000 counts/min) per millilitre.
(2) Using a rubber bulb, pipette 25 samples containing 1 ml each; and as soon as each sample has been pipetted, start drying it under the infra-red lamp. Keep the lamp as close as possible to the samples *without* allowing the solution to boil.
(3) Place one of the dry samples in the sample holder, and make 25 countings of 2 min each, without moving the sample. Record the result of each counting.
(4) Calculate the natural standard deviation according to $C^{\frac{1}{2}}$ and the total standard deviation according to the following formula: Let $C_1, C_2, \ldots, C_n$ be the sum-counts registered in n countings. Calculate $\bar{C} = (\sum_n C_i)/n$, which is the mean sum-count. Calculate $C_1 - \bar{C}$, $C_2 - \bar{C}$, $\ldots$, $C_n - \bar{C}$. The total standard deviation of the sum-count is then

$$\sigma_{tot} = \left(\frac{\sum_n (C_i - \bar{C})^2}{n - 1} \right)^{\frac{1}{2}} \qquad \text{(IV–6)}$$

(5) Compare the values for σ_{tot} and σ_{nat}, and if they are found to be significantly different, explain. Calculate σ_{nat} in per cent.
(6) Now count each of the 25 samples separately for 2 min, and record the results.
(7) Repeat your calculation according to Eq. (IV–6), and compare with $C^{\frac{1}{2}}$. Calculate σ_{tech} in per cent, using the equation

$$\sigma_{tot}^2 = \sigma_{nat}^2 - \sigma_{tech}^2 \qquad \text{(IV–7)}$$

(8) Calculate the count-rate (R) and its total standard deviation in per cent and in counts/s (see Lecture Material, §I–2.7).

IV–1.4. External absorption of beta particles

The absorption of beta particles in matter is almost independent of the atomic number of the absorbing material, provided the thickness is expressed as mass per unit area. Beta particles, for example those ejected from ^{32}P, have a spectrum of energies running from zero to a maximum value. The average beta energy for ^{32}P is 0.6 MeV and the maximum energy 1.7 MeV. The thickness of matter which is able to stop all incident beta particles is called the *range*, and this is entirely determined by maximum energy particles; however, practically none of the beta particles have this maximum energy, and the range is therefore not sharply defined. For ^{32}P the range is approximately 800 mg/cm^2 (8 kg/m^2).

A transmission curve of ^{32}P beta particles through aluminium is to be obtained in the present experiment.

PROCEDURE

(1) Pipette 100 μl of a solution containing approximately 0.1 μCi ^{32}P/ml (3.7 kBq ^{32}P/ml) into a counting cup, and dry under an infra-red lamp.
(2) Prepare a second sample by pipetting 100 μl of a solution containing approximately 50 μCi ^{32}P/ml (1.85 MBq ^{32}P/ml) into a counting cup and drying.
(3) Count the weak source until 10^4 counts are registered. Place an aluminium filter of about 20 mg/cm^2 between the counter window and the source, and count again.
(4) Continue counting at increasing absorber thickness until a count-rate of about 200 counts/min (3.33 counts/s) is obtained.
(5) Repeat the two previous counts with the strong source and calculate the average ratio between strong and weak source activity. (Note: dead time is important for high count-rates, and background for low count-rates.) This factor serves to transform weak-source net count-rates into strong-source net count-rates. *Avoid direct exposure of the GM window to the strong source.*
(6) Continue counting with the strong source until an almost constant count-rate is obtained (*bremsstrahlung*).
(7) Plot the net count-rates on a log scale against the absorber thickness on a linear scale. Take the window thickness of the GM tube and the air thickness from GM tube window to sample into consideration when assessing the zero point of the linear scale. (This is particularly important in the case of soft beta emitters.)
(8) Deduct the extrapolated bremsstrahlung from the net count-rate and plot the corrected curve.

(9) Judge by inspection the point at which the uncorrected transmission curve appears to run into the almost horizontal bremsstrahlung line. This point corresponds to the *range* and should be of the order of 800 mg/cm^2 for ^{32}P.
(10) Repeat the exercise, using ^{14}C instead of ^{32}P. The range should then be about 30 mg/cm^2.
(11) The corrected curve should in both cases asymptotically approach a vertical line through the range point, as the beta-particle transmission becomes zero, i.e. as the log tends to $-\infty$ (see Lecture Material, §I–1.6.2).
(12) How well can the top part of the ^{14}C transmission curve, corresponding to the first decade or two on the log scale, be approximated by a straight line?

IV–1.5. Self-absorption and self-scattering of beta particles

It is often necessary to measure the radioactivity of sources which contain appreciable amounts of solid material. When a thick source is counted, errors from self-absorption and from source scattering are introduced. Absorption tends to decrease the count-rate below the expected value and is most important with soft beta emitters whose maximum energy is less than about 0.5 MeV. Scattering tends to increase the count-rate and is most noticeable with high-energy beta emitters. (The effect of self-absorption and self-scattering also exists with gamma-emitting sources, but is usually unimportant since this radiation has a greater power of penetration and the sources are relatively small.) A third source of error when voluminous samples of varying thickness are involved may be called *self-geometry*, i.e. the top of the sample is relatively closer to the counter as the thickness increases. The combined effect of self-absorption, self-scattering and self-geometry, which normally results in a diminution of count-rate, will be termed *self-weakening*.

Very often corrections for self-weakening in soft-beta samples of varying thickness may be circumvented, because only relative values are needed for the experiment. For instance, many experiments are based on formulae in which activity measurements are only entered in ratios such as A_1/A_2 where A_1 and A_2 are the specific activities of test substance in two samples. If in such a case the tracer is a low-energy beta emitter and the samples are unequal in thickness, their count-rates cannot in general be entered directly; however, correction to *true* count-rates need not be attempted, because correct *relative* specific activities will also suffice, since they are to be used as a ratio only. Correct specific activities relative to any arbitrary laboratory reference may easily be obtained (assuming that the cross-sectional area and the gross material of the experimental samples and the reference samples are the same). From a reference material containing any unknown but uniformly distributed specific activity of the radioisotope used in the experiment, a series of samples covering the thickness range

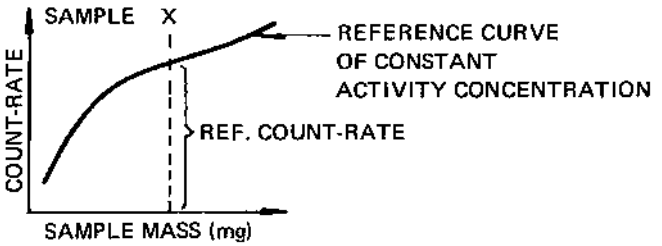

FIG.IV-2. Reference curve showing a relationship between count-rate and mass of samples of constant activity concentration.

of the experimental samples is prepared. From these reference samples a reference curve is constructed having count-rate plotted against mass of sample. This is now (whatever shape the curve may have) a reference of *constant* specific activity throughout the length of the curve, so the relative specific activity A_{rel} of any experimental sample is obtained by the weighing and counting of the sample (under the same conditions as used for counting the reference samples) and simple division of the net count-rate of the experimental sample by the net count-rate of the same mass (thickness) of reference material (see Fig.IV–2):

$$A_{rel} = \frac{\text{net count-rate of exp. sample of mass m}}{\text{net count-rate of ref. sample of mass m}} \qquad \text{(IV–8)}$$

When corrections for self-weakening to yield the *true* count-rates of beta samples are necessary, two different methods are possible. On the one hand, using the material in question, one may prepare a set of increasingly thick samples of constant specific activity (or constant total activity) and from these construct a self-transmission curve (count-rate plotted against mass per unit area (mg/cm^2)) or, on the other hand, one may make mathematical assumptions regarding the nature of the self-weakening effect.

In the first method one extends the self-transmission curve to samples which are as thin as possible, and this part of the curve is then extrapolated to yield the zero self-weakening value, or the so-called *true* value, of the activity concentration in count-rate per mg sample (or the total activity in counts/s or counts/min). This method can be difficult, because very thin samples often exhibit negative self-weakening; i.e. self-scattering into the detector slightly exceeds self-absorption.

The second method assumes that there is no significant self-scattering or self-geometry effect and that self-absorption proceeds in a simple exponential

manner. (This last assumption corresponds to approximating the curve obtained in the previous exercise (§IV–1.4) by a straight line, at least through the first decade or two.) Hence, accepting these assumptions:

$$a^* = a_0^* e^{-\mu x} \tag{IV–9}$$

where a_0^* = number of beta particles per cubic centimetre of the sample ejected in the direction of the GM tube;
μ = linear absorption coefficient (characteristic of $E_{\beta max}$ and density of sample material);
x = sample layer thickness (cm);
a^* = transmitted activity (per cm^3 sample) from depth x below the surface of the sample.

Differentiating and substituting Eq. (IV–9):

$$\frac{da^*}{dx} = -\mu a_0^* e^{-\mu x} = -\mu a^* \tag{IV–10}$$

i.e. the self-absorption loss per unit thickness is:

$$-\frac{da^*}{dx} = \mu a^* \tag{IV–10′}$$

The (total) transmitted activity A^* from a sample of cross-sectional area ℓ and thickness X is then obtained by integration from top to bottom of the sample as follows:

$$A^* = \int_0^X a_0^* \ell e^{-\mu x} dx = \frac{a_0^* \ell (1 - e^{-\mu x})}{\mu} = \frac{a_0^* (1 - e^{-\mu X})}{\mu X}$$

Finally, defining a self-absorption factor f as the ratio between observed (i.e. self-transmitted) activity and theoretical zero-absorption activity A_0^*, one has

$$A^*/A_0^* = f = \frac{(1 - e^{-\mu X})}{\mu X} \tag{IV–11}$$

where μ may be taken as the mass absorption coefficient (characteristic of $E_{\beta max}$ alone) and X as mass per unit area (mg/cm^2). The factor f may be used to correct mathematically observed count-rates for self-absorption.

When the self-geometry effect is negligible, a self-transmission curve based on increasingly thick samples of constant specific activity will approach (according to Eq. (IV–11)) asymptotically a constant value A^*_∞ as X approaches infinity. In practice A^*_{obs} becomes constant at a thickness equal to about half the *range* of the E_{max} of the beta particles. A sample of this, or greater, thickness is called *infinitely thick*. It is well known that the net count-rates of infinitely thick samples are proportional to the specific activities of the samples. This is merely a limiting case of the general Eq. (IV–8), in which the denominator, having become constant, may be disregarded (with respect to all infinitely thick samples).

In the first of two experiments, a series of sources will be prepared by precipitation of increasing weights of $CaCO_3$ from a solution containing ^{45}Ca. The self-weakening will be determined from the series of sources. In the second experiment, a self-transmission curve will be constructed for ^{14}C in $CaCO_3$ prepared from $^{14}CO_2$, and the corrected count-rates will be calculated from the theoretical equation above, with $\mu = 0.29$ cm^2/mg.

Experiment A. Self-weakening using $^{45}CaCO_3$

PROCEDURE

(1) Prepare 50 ml of a solution (solution A), 0.25M $CaCl_2$ and 0.1N HCl, containing 1 μCi ^{45}Ca (37 kBq ^{45}Ca).
(2) To prepare solution B, pipette 5 ml of solution A into a 50 ml volumetric flask, and make up with 0.1N HCl.
(3) Prepare about 50 ml of 5N ammonium hydroxide and 50 ml of 1M Na_2CO_3 solution.
(4) Pipette solutions A, B, water, ammonia and sodium carbonate in *Source No.* order according to Table IV–1.
(5) After precipitation, heat the precipitates on a hot-water bath or in a beaker with water on a hot plate. No not boil.
(6) Decant the supernatant, and wash twice with methanol (CH_3OH). Leave a $CaCO_3$/methanol slurry.
(7) Assemble the filtering apparatus (see Fig. IV–3) after the filter paper has been weighed carefully, and pour the $CaCO_3$ slurry into the glass cylinder. After it has settled for 2 to 3 min, initiate suction action *gently*, until the first 2 to 3 ml of methanol have come through. Then gradually apply full suction.
(8) When the filtration is complete, turn on the infra-red lamp, situated 10 to 15 cm above the glass cylinder, and keep it and the suction on for a few minutes until the $CaCO_3$ precipitate on the filter paper is dry.
(9) Remove the filter paper *at its $CaCO_3$-free edge* by means of a tweezer and transfer to a counting plate; weigh and count.

TABLE IV–1. SOLUTIONS IN $^{45}CaCO_3$ PROCEDURE

Source No.	Sol. A (ml)	Sol. B (ml)	Water (ml)	5N NH_4OH (ml)	1M Na_2CO_3 (ml)	
1	--	1	7	1	1	in 10 ml centrifuge tubes
2	--	2	6	1	1	
3	--	4	4	1	1	
4	--	6	2	1	1	in 40 ml centrifuge tubes
5	1	--	10	1	1	
6	1.5	--	10	2	2	
7	2	--	10	2	2	
8	3	--	25	2	2	in 50 ml beakers
9	4	--	25	2	2	
10	5	--	25	2	2	

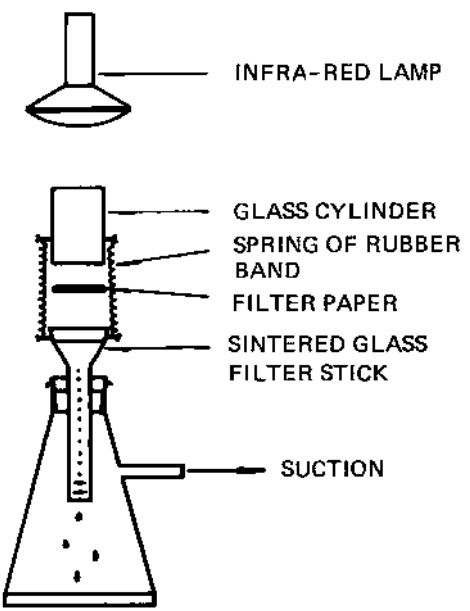

FIG.IV–3. Exploded view of filtering assembly.

(10) Plot the sample count-rate as a function of sample mass (mg).
(11) From this plot determine the correct specific activity (counts per second per milligram) taken as the slope of the initial straight part of the curve, where the effect of self-absorption is negligible. Determine for each sample the correct count-rate, which is equal to the correct specific activity (counts/s · mg) multiplied by sample mass (mg).
(12) Finally, determine the *self-weakening factor* (SWF) of each sample. The SWF is defined as the ratio of the correct count-rate to the observed count-rate. Plot the SWF as a function of sample mass.
(13) This last curve may be used to correct the count-rates of ^{45}Ca-labelled samples of $CaCO_3$ of known mass and the above cross-sectional area. The formula is simply:

$$\text{correct counts/s} = \text{observed counts/s} \times (\text{SWF}) \qquad \text{(IV–12)}$$

Experiment B. Self-transmission curve using $Ca^{14}CO_3$

PROCEDURE

This procedure includes the conversion of $Ba^{14}CO_3$ to $^{14}CO_2$, which is then precipitated as $Ca^{14}CO_3$. (There appear to be no obvious advantages to the precipitation of CO_3^{2-} as either the Ba or Ca salt for the purposes intended.) This complete system is normally not required; i.e. in practice the $^{14}CO_2$ evolved from a system may simply be caught in the observing system and precipitated. On the other hand, on occasion, contamination of the $CaCO_3$ or $BaCO_3$ may be suspected, for example with $BaSO_4$ (from SO_3) in the Van Slyke combustion procedure. Under these circumstances, the carbonate is purified simply by reconversion to the gas and reprecipitation as described. The reaction employed is

$$Ba^{14}CO_3 \xrightarrow{H^+} {}^{14}CO_2 \xrightarrow[H_2O]{Ca^{2+}} Ca^{14}CO_3$$

(1) Assemble an open system consisting of a reaction flask (main reaction flask plus funnel), connected to a gas-washing (CO_2-absorbing) bottle. The entire system is flushed with either N_2 or CO_2-free air (see Fig. IV–4).
(2) Put 0.1N CO_2-free NaOH in the gas-washing bottle and place in the main flask approximately 200 mg of $Ba^{14}CO_3$ containing about 0.2 μCi ^{14}C (7.4 kBq ^{14}C). Into the funnel of the reaction vessel place 2–3 ml of 10% $HClO_4$ (perchloric acid).
(3) Sweep the system with CO_2-free gas at such a rate that discrete bubbles are produced in the gas-washing bottle.
(4) Add the acid to the reaction vessel portion-wise, so that CO_2 is not formed at an excessive rate. Continue gas-sweeping for 10 minutes beyond the final addition of acid.

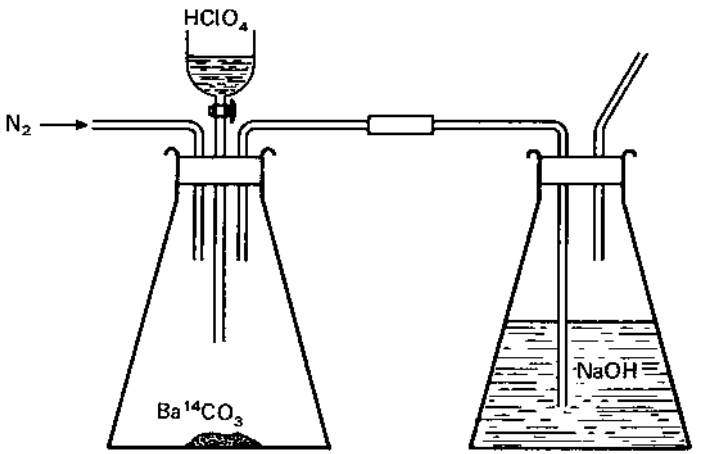

FIG.IV–4. Open assembly for conversion of $^{14}CO_2$.

(5) Remove the NaOH solution containing the $^{14}CO_3^{2-}$ to a measuring cylinder, and make up to 150 ml by washing of the CO_2-absorbing vessel with CO_2-free water. Divide the contents of the measuring cylinder into the following portions: 2 portions of 5 ml each, 2 of 10 ml each, 2 of 20 ml each, 1 of 30 ml and 1 of 50 ml, and place in 100 ml centrifuge tubes. Dilute each portion to 50 ml with CO_2-free water, and precipitate the $Ca^{14}CO_3$ in each by adding a few drops of saturated aqueous $CaCl_2$ solution.

(6) Centrifuge and test for completeness of precipitation by the addition of 2 to 3 drops of $CaCl_2$ solution *before* pouring off the supernatant. If further precipitate is obtained, recentrifuge and repeat the test. If no further precipitate is obtained, pour off the supernatant carefully, wash the precipitate with CO_2-free H_2O and recentrifuge. Again pour off the supernatant, resuspend the precipitate in absolute CH_3OH and centrifuge. Pour off the supernatant; suspend in CH_3OH and transfer to counting plates as in Experiment A.

(7) Count the plates, weigh and plot the sample count-rate as a function of mg/cm^2. Draw (through the origin) the best-fitting tangent to the first part of this self-transmission curve.

(8) Correct the observed count-rate of each plate using the theoretical self-absorption factor f given in Eq. (IV–11) by assuming $\mu = 0.29$ cm^2/mg. (Remember: correct count-rate = observed count-rate/f.)

(9) Plot these corrected count-rates on the same sheet as the self-transmission curve. Compare with the tangent drawn above.

IV–2. EXPERIMENTS WITH A SCINTILLATION COUNTER

IV–2.1. Crystal scintillation counting

When ionizing radiation enters a scintillator, a number of light photons (in the visible and ultra-violet wavelengths) are liberated. This number is proportional to the energy dissipated by the incident radiation. Many of these light photons hit the photocathode of the photomultiplier tube, which is optically linked to the scintillator, and a proportional number of electrons is liberated. Within the PM tube, a multiplication of electrons by a constant factor takes place. The whole sequence, from ionization onwards, gives rise to an output pulse which is *proportional* to the energy dissipated in the crystal by the primary ionizing event (see §I–2.3).

The substances used as scintillators (or phosphors) for the different types of radiation are basically:

Alpha radiation:	Zinc sulphide crystals spread thinly (10–20 mg/cm^2).
Beta radiation:	Anthracene, or naphthalene containing 0.1% of anthracene, in the form of a large crystal. Plastic scintillators are also used. For low-energy beta emitters, liquid scintillators are used.
Gamma radiation:	Sodium iodide, activated by about 1% of thallous iodide, in the form of a transparent, single crystal, cut to the required size.

Scintillation detectors have three advantages over GM tubes for counting gamma photons. These are (i) higher counting efficiencies (20 to 40 times), (ii) no significant resolving time corrections up to ~ 1700 counts/s (10^5 counts/min), and (iii) the output pulse height is proportional to the input photon energy.

The gamma crystal is hygroscopic and is therefore encased in an air-tight metal and glass holder, the end being in contact with the evacuated glass tube containing the photomultiplier system. The crystal and PM tube are housed in a light-tight metal barrel. The end of the barrel containing the crystal is surrounded by a *lead castle*. In a well-type crystal, the crystal is provided with a borehole sufficiently large to hold a test tube containing a solid or liquid sample of the gamma-emitting radioisotope. In spite of lead shielding, a scintillation detector will have a relatively high background (1–10 counts/s, i.e. 60–600 counts/min), some of which stems from *electronic noise* (which is lower the lower the temperature).

The electronic equipment connected to a scintillation tube is provided with an input discriminator, which is biased to prevent pulses below a certain voltage height from being registered. In this way unwanted small pulses may be rejected.

As opposed to a GM counter, which is operated at a chosen high voltage for all count-rates, the optimal working voltage of a scintillation counter depends on the ratio between sample activity and background. The length and slope of the *plateau* of a scintillation counter depend upon the source strength, the length decreasing and the slope increasing as the source strength decreases.

Close to background there is no real working plateau. Owing to the absence of a background plateau, the optimum high voltage setting is less obvious than in the case of the GM counter. Using the correct high-voltage and input bias voltage settings will result in a considerable saving of time at low count-rates.

Criteria for optimum operating conditions based on natural statistics are deduced in Part VI, Appendix VI-6. Sometimes, in practice, other considerations such as electronic stability outweigh those concerned only with natural counting statistics.

In the following experiment, the optimum working voltage (according to natural statistics) for a strong and a weak source will be determined at two bias voltages, using the criteria given in Part VI, Appendix VI-6.

PROCEDURE

The instructor must check whether 5 and 20 V are appropriate for the instrument being used, choosing other values, if necessary, to suit the needs of the experiment.

(1) Obtain a source containing about 0.2 μCi (7.4 kBq) of a gamma emitter such as ^{131}I, ^{137}Cs or ^{60}Co.
(2) Obtain a second source containing about 0.002 μCi (74 Bq) of the same isotope.
(3) Set the bias voltage at 5 V, and determine the count-rate of each sample at 50 V intervals of the high voltage setting (HV).
(4) Determine the background at 5 V bias and at 50 V intervals of the HV setting.
(5) Repeat the sample and background counts for 20 V bias.
(6) Plot $R^{\frac{1}{2}} - R_b^{\frac{1}{2}}$ as a function of the HV, and determine the optimum working voltage for both samples at biases of 5 and 20 V, respectively.
(7) Determine the maximum value of $(R - R_b)^2/R_b$ for both samples at biases of 5 and 20 V.
(8) Calculate the percentage natural standard deviation at which the samples have been counted, both at maximum $R^{\frac{1}{2}} - R_b^{\frac{1}{2}}$ and maximum $(R - R_b)^2/R_b$.

IV–2.2. Rapid radioactive decay

The primary purpose of this exercise is to investigate the general law of radioactive decay in one short laboratory period.

For this a short-lived radioisotope is used, and a secondary effect (of less importance) may be considered, since the determination of the activity of a

sample containing an isotope of short half-life becomes complex when the time which is required to obtain sufficient counts to give a desired accuracy is long compared with the half-life of the isotope. If the disintegration rate at a certain time t is called A_t^*, the count-rate, being proportional to the disintegration rate (i.e. activity) may be expressed as

$$R_t = \epsilon A_t^* \qquad \text{(IV–13)}$$

where ϵ is the counting yield.

The disintegration rate, however, changes with time according to:

$$A_t^* = A_0^* e^{-\lambda t} \qquad \text{(cf. Eq.(I–8))} \qquad \text{(IV–14)}$$

where A_0^* = the disintegration rate at zero time,
λ = disintegration constant = $0.693/T_{\frac{1}{2}}$,
t = time elapsed since zero time.

If the substance is decaying rapidly during the measurements, i.e. if the duration of counting time T is similar in magnitude to $T_{\frac{1}{2}}$, then the disintegration rate A_{t+T}^* at the end of the counting period will be significantly lower than at the beginning:

$$A_{t+T}^* = A_0^* e^{-\lambda(t+T)} \qquad \text{(IV–15)}$$

The decrease in activity of a sample (expressed, e.g., as number of radioactive atoms N) during the counting time T is shown in Fig.IV–5.

The observed count-rate $\bar{R}$ is the average over the counting period T. $\bar{R}$ and the sample activity at the beginning of the counting period R_t are related as follows:[2]

$$\bar{R} = \epsilon \bar{A}^* = \frac{\epsilon}{T}(N_t - N_{t+T}) = \frac{\epsilon}{T}[N_t(1 - e^{-\lambda T})] \qquad \text{(IV–16)}$$

since, from Eqs (IV–14 and 15):

$$N_{t+T} = N_t e^{-\lambda T} \qquad \text{(cf. Eq.(I–3))} \qquad \text{(IV–17)}$$

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[2] If the counting time T is not greater than $\frac{1}{2}T_{\frac{1}{2}}$, the relation $\bar{R}_t \triangleq R_{t+\frac{1}{2}T}$ (i.e. the average activity is about equal to the activity in the middle of the counting period) is correct within 1%. This approximation can be useful.

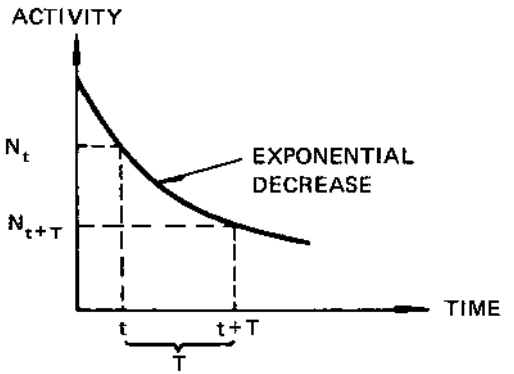

FIG.IV–5. Curve showing the exponential decay of the activity of a sample as a function of time.

Hence, as $A_t^* = \lambda N_t$:

$$\bar{R} = \frac{\epsilon}{T}\left(\frac{A_t^*}{\lambda}\right)(1 - e^{-\lambda T}) \qquad \text{(IV–18)}$$

Thus, using Eq. (IV–13):

$$\bar{R} = \frac{R_t(1 - e^{-\lambda T})}{\lambda T} \qquad \text{(IV–19)}$$

where

$$\lambda = \frac{0.693}{T_{\frac{1}{2}}}$$

and $T_{\frac{1}{2}}$ and T are in seconds.

With the aid of Eq. (IV–19) the ratio of R_t to $\bar{R}$ may be calculated for various durations of counting. If the duration of each count T is the same throughout a series of consecutive counts, then the ratio $R_t : \bar{R}$ is *constant*.

In the following experiment, Eq. (IV–19) will be used to correct the count-rate of $^{137}Ba^m$. Barium-137m is the metastable isomer of ^{137}Ba, and it emits 0.66 MeV gamma photons.

REAGENTS AND MATERIALS

(1) SO_3H type cation exchange resin (e.g. Amberlite IR-120 or Dowex 50).
(2) About 200 μCi (7.4 MBq) of ^{137}Cs.
(3) A conventional burette.
(4) EDTA solution (about 0.3% adjusted to pH 11–12 with NaOH).
(5) Plastic counting container.
(6) Stop-watch.

PROCEDURE

(1) Saturate 50 g of resin with Na^+ by leaving it overnight in 10% NaCl solution. Put a glass-wool plug at the bottom of the burette, and fill it half way up with resin. Run 1 litre of distilled water upwards through the resin to remove excess NaCl and air bubbles. Allow the resin to settle and wash with EDTA solution. *Never allow the surface of the EDTA solution to come below the top of the resin column.*
(2) Lower the surface of the EDTA solution to the top of the resin and apply the ^{137}Cs. Elute the column repeatedly with EDTA solution at 10 min intervals until the amount of $^{137}Ba^m$ coming through each time no longer increases. At each elution the $^{137}Ba^m$ concentration in the effluent will be maximum after about a half or a third of the resin-column volume of EDTA solution has run through (but the peak is not sharp).
(3) Take about 0.5 ml of the effluent rich in $^{137}Ba^m$ in a plastic counting container; start counting immediately, using a scintillation (well-type) detector.
(4) The counting should be carried out at 1 min intervals for a duration of 1 min counting time and a total running time of 30 min, without removing the sample container.
(5) At the end of 30 minutes from the start of counting, the $^{137}Ba^m$ remaining in the liquid will be much less than 1% of the original quantity, and most of the count-rate observed above empty-container background results from some ^{137}Cs leached by the EDTA effluent.
(6) Repeat counting for 1 min at 5 min intervals until the count-rate no longer decreases, and subtract the final count-rate from the observed count-rate and plot this net count-rate, from $^{137}Ba^m$, against time on log-linear graph paper. Deduce the half-life of $^{137}Ba^m$ from this plot.
(7) Use Eq. (IV–19) to obtain the net count-rates at the *beginning* of each counting period, and plot these corrected values of net count-rate against time on log-linear graph paper.

IV–2.3. Inverse-square-law effect and attenuation of gamma rays

The intensity of the rays emitted from a source of radiation can be reduced in the following two ways, (i) by increasing the distance from the source of radiation, and (ii) by increasing the attenuation in the path of the rays.

Experiment A. Inverse-square law

For a point source, the radiation intensity is inversely proportional to the square of the distance if no intervening matter (solid, liquid or gaseous) is present between the source and the target. This is usually referred to as the inverse-square-law effect. The intensity at a distance d from a point source (or other source whose dimensions are small in comparison with d) is thus given as

$$I_d = k/d^2$$

where I_d = intensity at d cm distance,
k = proportionality constant (see Lecture Material, §I–2.6).

In this exercise diminution by distance of gamma rays from a 5 μCi (185 kBq) ^{60}Co source is investigated. The crystal scintillation counter is used.

PROCEDURE

(1) Apply the operating voltage previously set in the experiment in §IV–2.1 for the scintillation counter.
(2) Determine the background count-rate.
(3) Determine the count-rate as a function of distance for d = 20, 40, 60, . . , 160 cm.
(4) Plot the count-rate resulting from sample ($R_s = R - R_b$) against the corresponding distance on log-log graph paper, and draw the best straight line through the points.
(5) Determine the slope of the line, and explain the reason for discrepancies, if any, between the result and the inverse-square law.

Experiment B. Attenuation

The attenuation by matter of a collimated beam of monoenergetic gamma photons is exponential. The attenuation of gamma rays from ^{60}Co which emits gamma photons of two characteristic energies (1.17 and 1.33 MeV) will be investigated.

PROCEDURE

(1) The previous experimental set-up is used except that the distance between the sample and counter is now kept fixed.
(2) Determine the count-rate of the sample without adding any absorber between source and counter.
(3) Determine the count-rate after placing one layer of lead absorber between source and counter, and keep on repeating with increasing the thickness of the absorber by adding more layers.
(4) Remove the source completely, and determine the background (with all layers of absorber in place).
(5) The net count-rate from the sample is taken as a measure of the radiation intensity, and this is plotted against linear absorber thickness on log-linear graph paper.
(6) Determine the half-thickness of lead for ^{60}Co gamma rays and compare with the table value (Lecture Material § I–3.2.1, Table I–3).
(7) Explain any discrepancy between the results and the simple exponential law (i.e. not a straight line when plotted on log-linear graph paper).

IV–2.4. Liquid scintillation counting

The principles of crystal scintillation counting of isotopes emitting gamma rays were dealt with in §IV–2.1 (see also §I–2.3).

For the assay of low-energy beta particles, liquid scintillation counting is often employed. This system involves many of the same principles as crystal counting; there are, however, a few differences. In the liquid scintillation system, the beta-emitting sample is mixed together with the scintillator (phosphor), which is in solution in a small (20 ml) counting vial. The vial is placed in a light-tight counting chamber in optical contact with a photomultiplier tube.

Sometimes two photomultiplier tubes are used simultaneously; a coincidence circuit is then employed, and only those events seen by both tubes are recorded (see Fig. I–7). This procedure reduces thermionic background enormously, but it introduces a tube-to-tube background effect. Placing the photomultiplier tube(s) and pre-amplifier(s) in a freezer chest reduces thermionic emission in the electronic components and thus reduces the background (electronic noise).

Variable-bias lower and upper discriminators are usually used with these systems so that counting in any desired pulse-height interval is possible.

One of the main sources of error in liquid scintillation counting (see §I–2.4) is *quenching*, which occurs because of the light-absorbing properties of many biological materials. When these substances are mixed with the scintillator solution, they may absorb or quench part of the light from the scintillations, which results in a lowered counting efficiency. Chemical reactions between the

sample and the scintillator may also result in quenching. The quenching problem can be resolved by the use of internal standards and suitable correction curves, or the samples can be converted to compounds which do not cause quenching, e.g. by combustion, filtering.

The following experiment is designed to introduce the student to the operation of the liquid scintillation counter.

PROCEDURE

(1) Obtain counting vials containing the following:

Solvent system

Dioxane	5 parts	417 ml	15 ml/vial
Ethyleneglycol monoethylether *(Cellosolve)*	1 part	83 ml	
PPO (2-5 diphenyloxazole)	1% wt/vol.	5 g	
Naphthalene	5% wt/vol.	25 g	
POPOP (1-4-bis-2-5-phenyloxazolyl)		0.25 g	
Benzene			

Labelled compounds

^{14}C-toluene, supplied as solution A
^{3}H-toluene, supplied as solution B

Solutions

A: 170 to 200 Bq, i.e. ~5 nCi/ml ^{14}C-toluene
B: 850 to 1000 Bq, i.e. ~25 nCi/ml ^{3}H-toluene

Remember, 1 Bq is 1 disintegration per second, i.e. 60 dis/min.

Make up counting vials as indicated in Table IV–2.

(2) Set lower and upper discriminator biases at 40 and 70 V, respectively, and determine the background rate R_b as a function of high voltage using intervals of 50 to 100 V and counting times of 2 min for expediency. Begin at about 600 V.
(3) Determine counting yield as a function of high voltage for ^{14}C (using vial 1) and ^{3}H (vial 6), and plot the curves of ϵ/R_b against HV.
(4) Count the different bottles at the HV that corresponds to maximum ϵ/R_b (see Part VI, Appendix VI–6) for ^{14}C and ^{3}H. Plot the activity curves.
(5) If time permits, repeat steps 2 to 4 for other bias settings.

TABLE IV–2. SOLUTIONS FOR COUNTING VIALS

Counting vial	Sol.A (ml)	Sol.B (ml)	Toluene (ml)	Solvent system (ml)
1	0.2		1.8	15
2	0.4		1.6	15
3	0.6		1.4	15
4	0.8		1.2	15
5	1.0		1.0	15
6		0.2	1.8	15
7		0.4	1.6	15
8		0.6	1.4	15
9		0.8	1.2	15
10		1.0	1.0	15
11			2.0	15

IV–3. CONTAMINATION AND DECONTAMINATION

In work with radioactive materials, it is always necessary to know if the operator is contaminating the apparatus with which he handles radioactive materials.

Often a fresh spill on a clean and polished surface can be washed without resultant detectable contamination. If it is allowed to react with the surface, however, drastic action would be required to remove residual radioactivity.

The methods of decontamination may be divided into physical and chemical. Physical methods include washing, vacuum cleaning and polishing, and steam or sand blasting. Chemical methods include the use of acids and alkali with or without carrier, detergents, complexing agents and ion exchange material.

This important experiment will consist of contaminating several different materials with various isotopes, followed by decontamination of the materials using chemical agents.

REAGENTS AND MATERIALS

(1) Radioactive solutions of ^{32}P, ^{45}Ca and ^{131}I
(2) Small pieces of the following materials: glass, lead, waxed linoleum, Perspex, stainless steel, painted wood and wood plus barrier cream (imitating skin plus barrier cream)
(3) *Washing solution:* 1% detergent solution + 0.3% EDTA + NaOH to give a pH-value of approximately 12, or, for example, Radiacwash
(4) 1% carrier solutions of phosphorus, calcium and iodine
(5) 2N HCl
(6) Acetone
(7) Handkerchief tissue or paper towels
(8) Barrier cream (e.g. ICI Savlon)

PROCEDURE

(1) Take a background reading of each material to be tested.
(2) Dry 10 μl of each radioactive solution on separate pieces of the material under test.
(3) Make a reading on each spot, and record as initial activity.
(4) Wipe the surface with a damp tissue paper, dry the sample, and measure the activity.
(5) For a second series, use a *washing solution* as decontaminating agent. Dry the sample, and measure the activity.
(6) Try all the relevant decontaminating agents in a similar manner. If necessary, as a final step with linoleum, try removing the wax with acetone.
(7) Record all the measured activities, and compare the effect of the different decontaminating agents upon various substances and radioactive isotopes.
(8) Discuss the results.

IV–4. EXERCISES ON BASIC UTILIZATION PRINCIPLES

IV–4.1. Combustion of carbon compounds

Determination of specific activity by persulphate oxidation of a compound

It is not always possible to compare specific activities of diverse labelled carbon compounds because of differences in crystal structure, ease of crystallization, etc. Consequently, it is necessary to convert all such compounds to a common one. For this purpose carbon dioxide has proved to be convenient, which, for practical purposes, is measured as the barium salt, $BaCO_3$.

Combustion may be performed in two main ways: *dry* combustion or *wet* combustion. In dry combustion the sample is burned in the usual manner with CuO for determination of formula composition, except that the CO_2 is caught quantitatively in an insoluble form, that is, either as the gas or as carbonate. In wet combustion the compound is dissolved in a solution which includes the oxidants. Two commonly used solutions, the Van Slyke combustion mixture (H_2SO_4, Cr_2O_7, KIO_3, H_3PO_4) and the persulphate mixture, differ sharply in their response according to the amount of water present. The utility of the former diminishes rapidly with increasing water content while the latter uses water as a solvent. The convenience of water as a solvent may well be an overriding factor, as is illustrated in this experiment.

Oxidation of an aqueous solution of a variety of organic compounds (organic acids, including acetate and succinate, glucose, acetone, etc.) may be accomplished quantitatively with potassium persulphate ($K_2S_2O_8$) at about 100°C. The CO_2 evolved is quantitative and is absorbed in a NaOH gas-washing bottle and converted to $BaCO_3$ for plating.

REAGENTS AND MATERIALS

(1) $CH_3{}^{14}COONa$
(2) 200 mg $K_2S_2O_8$
(3) 1 ml 5% $AgNO_3$
(4) 0.1N NaOH, CO_2-free
(5) H_2O, CO_2-free
(6) $BaCl_2$ (saturated aqueous solution)
(7) CH_3OH, absolute
(8) N_2 gas, or CO_2-free air
(9) Flow apparatus, consisting of a two-necked reaction flask, an H_2O condenser and a gas-washing tube
(10) Filter apparatus (chimney and filter-stick)
(11) Centrifuge
(12) Infra-red lamp
(13) Analytical balance
(14) Calibrated standard (i.e. infinitely thin ^{14}C source of usual sample area of known activity)

PROCEDURE

(1) Arrange the flow apparatus as shown in Fig. IV–6.
(2) Calculate the amount of active CH_3COONa needed to produce approximately 50 mg of $BaCO_3$, and place into the reaction chamber.
(3) Add about 20 ml H_2O, 200 mg $K_2S_2O_8$ and 1 ml 5% $AgNO_3$. (No reaction occurs at room temperature.)

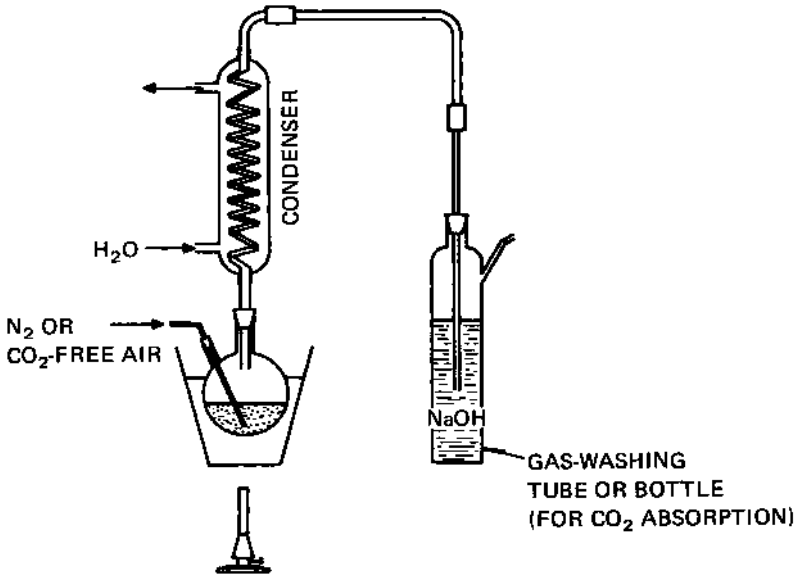

FIG.IV–6. Assembly for combustion of carbon compounds and conversion of CO_2 produced.

(4) Heat the reaction vessel to 70°C for 20 min; then increase the temperature slowly to boiling, and maintain until the solution becomes clear or the persulphate is dissolved.
(5) Simmer 10 min longer and sweep the system for an additional 10 min to remove all traces of $^{14}CO_2$.
(6) Wash the inlet of the gas-washing system with CO_2-free water, combining the washings with the 0.1N NaOH.
(7) Add sufficient saturated $BaCl_2$ solution to precipitate all the $^{14}CO_2$.
(8) Transfer quickly to a centrifuge tube, and centrifuge immediately (or stopper and centrifuge at will).
(9) Wash the precipitate once with CO_2-free water and then with absolute CH_3OH.
(10) Resuspend in CH_3OH, and filter in the filter apparatus.
(11) Place an infra-red lamp 4 to 5 cm above the plating apparatus, and continue drawing warmed air through the apparatus for 10 to 15 min, by which time the $BaCO_3$ plate will have dried.
(12) Weigh immediately.
(13) Count sample and standard.
(14) Calculate the absolute specific activity of sample (Bq per mmol), making corrections for self-absorption and taking the counting yield of the standard into account.

QUESTIONS

(1) State the conditions for preferring the persulphate to the Van Slyke oxidation procedure.
(2) What is the fate of the persulphate in the reaction?

IV–4.2. Isotope dilution chemistry

One of the important advantages of using a radioactive substance in quantitative analysis is that a quantitative isolation of the compound to be determined from a material is unnecessary. A simple isotopic dilution analysis of the phosphorus concentration in an unknown solution by comparison with a solution of known phosphorus concentration will be conducted in this experiment. The radioisotope technique illustrated by this experiment is advantageous in any situation where a normal quantitative determination of the test substance is not feasible for some reason.

REAGENTS AND MATERIALS

(1) Solution containing 0.20 mmol P/ml solution
(2) Unknown phosphorus solution (about 0.1M)
(3) Solution containing ^{32}P at an activity of about 0.1 μCi/ml, i.e. 3.7 kBq/ml (carrier-free or of known phosphorus concentration)
(4) Fiske's reagent (13 g MgO, 175 g citric acid, 330 ml 25% NH_4OH in water to give one litre solution)
(5) 25% NH_4OH

PROCEDURE

(1) Mark six 100 ml beakers as U_1, U_1', U_2, U_2', K and K', and pipette into them, respectively, the following aliquots:

U_1, U_1'	5 ml unknown
U_2, U_2'	20 ml unknown
K, K'	5 ml of 0.20M H_3PO_4 solution.

(2) Pipette accurately 1 ml of active phosphate solution into each beaker, and mix thoroughly.
(3) Add slowly 10 ml of Fiske's reagent and 10 ml of 25% NH_4OH while swirling.
(4) After 5 min decant the supernatant from the precipitates; wash three times with distilled water and once with methanol.

(5) Transfer the major part of the precipitates into weighed and marked counting cups. The amount of thick slurry of the precipitate transferred from the K beakers should be roughly mid-way between the amounts from the U_1 and U_2 beakers, respectively.
(6) Dry the thick slurry under an infra-red lamp, trying to make the surface even.
(7) After cooling, weigh the cups plus precipitates, and determine the weights of the precipitate alone.
(8) Count the activity, using a GM tube.
(9) Express the specific activities of phosphorus in the solid samples in counts/s per millimole.
(10) Calculate the molarity of the unknown phosphorus solution.

QUESTIONS

(1) Do the values obtained for U_1 and U_2 come out the same?
(2) What difference does it make to the calculation of unknown phosphorus concentration if the activities of the samples are expressed as counts/s *per milligram* of precipitate? Explain.
(3) Can the unknown phosphorus concentration be determined from the weights of the precipitates alone?

IV–4.3. Kinetics of exchange between ions in solution and those in solid form

It is often observed that, at equilibrium, the total concentrations of a substance distributed in two phases remain constant with respect to time. This, however, does not imply that the individual ionic or molecular species is restricted in one phase. Instead, dynamic exchange of ionic or molecular species between the two phases is continually taking place.

With the introduction of a radioactive label, it is possible to investigate the dynamic exchange of a species under equilibrium conditions.

If enough time is allowed for equilibration, the radioactive ions in solution will come to equilibrium with radioactive ions on the surface of a solid. Consider a schematic exchange reaction under conditions of chemical equilibrium (A in solution, B on solid surface) to which a radioactive label has been added (to the solution).

$$AX^* + BX^\circ \rightarrow AX^\circ + BX^*$$

where X^* represents a radioisotope of X and
X° represents a stable isotope of X.

By designating the rate constant for the above exchange reaction k_A (from solution to solid phase), and putting:

$$\left.\begin{aligned} (AX^*) + (AX^\circ) &= a \\ (BX^\circ) + (BX^*) &= b \\ (AX^*) &= x \\ (BX^*) &= y = c - x \end{aligned}\right\} \quad \text{expressed, for example, as molecules per ml solution}$$

where a, b and c are constant, and taking x as the variable, we find that the fractional rate of fall-off of AX^* towards equilibrium is given by:

$$-\frac{1}{a}\frac{dx}{dt} = k_A \frac{x}{a}\left(\frac{b-y}{b}\right) - k_A\left(\frac{a-x}{a}\right)\frac{y}{b} = k_A\frac{x}{a} - k_A\frac{y}{b} \qquad \text{(IV–20)}$$

Hence the equilibration of radioactivity (between liquid and solid) is a first-order reaction. After substitution of $y = c - x$, the differential equation becomes:

$$\frac{dx}{dt} = -\frac{a+b}{b}k_A x + \frac{a}{b}ck_A \qquad \text{(IV–21)}$$

and, on integrating, one obtains:

$$x = C_1 e^{-[(a+b)/b]k_A t} + \left(\frac{a}{a+b}\right)c \qquad \text{(IV–22)}$$

where C_1 is the integration constant.

For $t \to \infty$, we have $x \to x_\infty$, and $y \to y_\infty$ (i.e. equilibrium distribution of activity between liquid and solid is reached); then Eq. (IV–22) becomes:

$$x_\infty = \left(\frac{a}{a+b}\right)c; \quad y_\infty = c - x_\infty = \left(\frac{b}{a+b}\right)c \qquad \text{(IV–23)}$$

Hence:

$$\frac{x_\infty}{y_\infty} = \frac{a}{b} \qquad \text{(IV–24)}$$

which is the expected result, because this equation signifies that the specific activity is the same in both phases at equilibrium distribution of the label.

Since $x = c$ at $t = 0$, the integration constant C_1 is equal to $c - x_\infty$. After substitution of this relationship and x_∞ into Eq. (IV–22), the following final form is obtained:

$$x - x_\infty = (c - x_\infty)\, e^{-[(a+b)/b]k_A t} \qquad \text{(IV–25)}$$

Thus, if the activity concentration of the solution is x^* counts/s per millilitre, a plot of $\log (x^* - x^*_\infty)$ against t should give a straight line. The rate constant for net transfer of activity from solution to solid phase, i.e.:

$$k^*_A = \left(\frac{a+b}{b}\right) k_A \qquad \text{(IV–26)}$$

may be obtained from the slope of the line. Finally, the system discussed here should be compared with the generalized system given in Part I, Lecture Material, §I–4.3.3, bearing in mind that k_A is $k_{1,2}$, etc.

REAGENTS AND MATERIALS

(1) Cation exchange resin, Ca^{2+} saturated in solution (6)
(2) Anion exchange resin, $H_2PO_4^-$ saturated in solution (5)
(3) 5 μCi (185 kBq) ^{32}P
(4) 10 μCi (370 kBq) ^{45}Ca
(5) 10^{-3}M KH_2PO_4
(6) 10^{-3}M $CaCl_2$
(7) Stop-watch
(8) Two pipette tip caps

PROCEDURE

(1) Measure 2 ml of wet cation and 4 ml of wet anion exchange resin into two 150 ml beakers, separately.
(2) Add 100 ml each of phosphate and calcium solutions to the appropriately saturated resin. *Retain about 10 ml of each solution for steps (3) and (4).*
(3) Dilute the ^{32}P with some of the phosphate solution and add it to the phosphate-containing anion exchange resin while stirring, and simultaneously starting the stop-watch. Then take out 1 ml of the supernatant

solution[3] into a counting cup at the following times: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30 and 40 min after addition of radioactivity. Between sampling, the solution should be continuously stirred, in order to eliminate the activity concentration gradient near the resin particles.

(4) Using the ^{45}Ca and other appropriate solutions, repeat the procedures of step (3).

(5) Dry the samples under an infra-red lamp, and count the activity x*, using a thin-mica-window GM counter.

(6) Plot the activity difference $x^* - x^*_\infty$ on log scale against the time on linear scale.

(7) Obtain the slope from the graph, and calculate the rate constant k^*_A for the equilibration of activity in each of the solution-resin systems.

QUESTIONS

(1) How can the small discrepancy, if any, from a straight line be explained?

(2) On the basis of activity concepts alone, extrapolate each straight line to zero time; what does the ordinate intercept equal?

(3) On the basis of activity concepts alone, calculate the exchange rate constant k_A for each solution.

(4) On the basis of activity concepts alone, calculate the reverse exchange rate constant k_B for each resin.

(5) How many milliequivalents of ion are exchanged per minute per millilitre of solution in each system?

(6) As in (5), but per millilitre of wet resin.

(7) How great are the respective ion exchange capacities in milliequivalents per millilitre of wet resin (after saturation in 10^{-3}M solution)?

IV–4.4. Determination of copper in biological material by activation analysis

Because of the low copper content of biological materials, any chemical means to determine it quantitatively requires large amounts of the starting material.

Activating the copper present in biological material with neutrons has the advantage of requiring only a small sample for a quantitative determination.

Copper present in a tissue sample and in a standard is activated with neutrons to form ^{64}Cu, which has a half-life of 12.8 h and emits beta and gamma radiation

[3] It is important to avoid getting any resin particles into the pipette. The tip of the pipette must be covered with a fine-mesh cloth cap during filling. The cap is removed during emptying (and replaced before the next filling).

of 0.57 and 1.34 MeV, respectively. Activation for 13 h at a neutron flux of $10^{12}\ cm^{-2} \cdot s^{-1}$ gives a specific activity of 50 mCi/g (1.85 GBq/g). Simultaneously ^{66}Cu is formed with a half-life of 5.1 min. After tissue digestion, copper carrier is added and mixed with the active copper. Copper is then separated (non-quantitatively) by a chemical method, weighed and the ^{64}Cu is counted. The specific activity of the unknown separate is compared with that of the known standard separate, and the content of copper in the original tissue sample is thereby calculated.

REAGENTS

(1) 24N HNO_3
(2) 16N HNO_3
(3) 1N HNO_3
(4) 18N CH_3CO_2H
(5) 15N NH_3 solution
(6) 2N NH_3 solution
(7) 15% wt/vol. Na_2SO_3
(8) 20% wt/vol. KSCN
(9) 10% wt/vol. $Fe(NO_3)_3$
(10) 10% wt/vol. $NH_4H_2PO_4$
(11) 2% wt/vol. salicylaldoxime in C_2H_5OH
(12) Acetone
(13) SO_2 saturated water
(14) Cu carrier (20 mg Cu/ml): 6.28 g $Cu(CH_3CO_2)_2H_2O$ in 100 ml water.

PROCEDURE

Take about 0.05 g of tissue and 1 μg of copper standard sealed in polyethylene. Activate for 13 h.

(1) In a fume chamber transfer tissue and standard to 50 ml centrifuge tubes, and add 10 drops of 24N HNO_3. Boil until tissue has dissolved, and add 10 mg of Cu (0.5 ml of Cu carrier). Make up to 4 ml with water; add 1 ml of Na_2SO_3 and 1 ml of KSCN. Boil, and spin down CuSCN when it has settled, reject supernatant and wash precipitate with hot water saturated with SO_2.
(2) Dissolve precipitate in 0.5 ml of hot 16N HNO_3; add 5 drops of $Fe(NO_3)_3$ and 1 drop of $NH_4H_2PO_4$, then 15N NH_3 solution till dark brown. Boil, spin down $Fe(OH)_3$ precipitate and wash it once with 2N NH_3 solution.
(3) Combine supernatant and washings in a fresh tube, and acidify with CH_3CO_2H till pale blue. Then add 0.5 ml of 16N HNO_3, 1 ml of Na_2SO_3 and 1 ml of KSCN; boil and spin down CuSCN. Pour away supernatant and wash precipitate with hot water saturated with SO_2.

(4) Dissolve precipitate in 0.5 ml of 16N HNO_3; add 15N NH_3 until solution is deep blue and CH_3CO_2H until it is pale blue. Add 3 ml of salicylaldoxime, and boil for 3 min. Spin down precipitate, and wash it twice with water and once with acetone.
(5) Slurry precipitate with acetone onto a weighed aluminium counting tray; dry under a lamp, and count with an end-window GM counter. Correct counts for decay and self-absorption, and check the half-life of the separated copper-64.

The chemical steps take about 2 hours for eight samples. The chemical yield is about 75%.

CALCULATION

$$\mu g \text{ Cu in sample} = \frac{\text{counts/s sample}}{\text{counts/s standard}} \times \frac{\text{wt standard Cu-salicylaldoxime}}{\text{wt sample Cu-salicylaldoxime}}$$

RANGE AND ACCURACY

0.05 – 0.5 μg of copper is a convenient range for determination within an accuracy of 5%.

INTERFERENCES

Large amounts of zinc might interfere because of the reaction $^{64}Zn(n,p)^{64}Cu$. 1 μg of zinc in the sample yields ^{64}Cu equivalent to 7×10^{-4} μg of copper.

IV–4.5. Determination of phosphorus in biological material by activation analysis

Neutron activation of phosphorus gives rise to 14 day phosphorus-32, which emits beta particles of energy 1.71 MeV. Activation for 14 days at a neutron flux of 10^{12} $cm^{-2} \cdot s^{-1}$ gives a specific activity of 50 mCi/g (1.85 GBq/g).

REAGENTS

(1) 36N H_2SO_4
(2) 16N HNO_3
(3) 15N NH_3
(4) 50% wt/vol. $MgCl_2$
(5) 5% wt/vol. H_2SO_4 in diethyl ether
(6) Acetone
(7) Diethyl ether (dried over $CaCl_2$)
(8) Combined carrier (10 mg P/ml; 20 mg Na/ml; 20 mg K/ml), 37.1304 g $NH_4H_2PO_4$, 44.5652 g K_2SO_4 and 61.7689 g anhydrous Na_2SO_4 in one litre of water.

PROCEDURE

Take about 0.002 g of tissue and a 10 μg phosphorus standard. Activate for *at least* 15 h and preferably for 14 d.

(1) In a fume chamber transfer tissue and standard to 150 ml beakers. Add 1 ml of combined carrier and 1 ml of HNO_3. Heat to 180–200°C for 5 min.
(2) Cool, add 1 ml of H_2SO_4, and heat for 1 h.
(3) Cool, transfer to 50 ml centrifuge tube, add 15 ml of dry ether and agitate. Spin, pour ether into a fresh tube and wash residue with 4 ml of 5% H_2SO_4 in ether.
(4) Evaporate ether in supernatant in a current of air, and add NH_3 to residue to obtain pH 9. Cool and add 1 ml of $MgCl_2$; allow to stand for 10 min and spin. Wash $MgNH_4PO_4$ three times with 5 ml of water.
(5) Slurry $MgNH_4PO_4$ precipitate onto a weighed counting tray with acetone, dry under a lamp and count with an end-window GM counter. Self-absorption is negligible. Count again after 24 h to check the half-life of the phosphorus-32, or plot a beta-absorption curve.

The procedure takes about 2.5 h for eight samples (excluding irradiation), excluding *ashing* time. The chemical yield is about 90%.

CALCULATION

$$\mu g \text{ P in sample} = \frac{10 \times \text{counts/s sample}}{\text{counts/s standard}} \times \frac{\text{wt standard } MgNH_4PO_4}{\text{wt sample } MgNH_4PO_4}$$

RANGE AND ACCURACY

This depends on the activation time. With a 15 h activation period, 0.5 to 10 μg of phosphorus can be determined within ± 5%. If the activation period is extended to 14 days, this can be reduced to 0.02 μg of phosphorus.

INTERFERENCES

Large amounts of sulphur can interfere by the $^{32}S(n, p)^{32}P$ reaction. 1 mg of sulphur gives rise to phosphorus-32 equivalent to 55 μg of phosphorus. Chlorine could also interfere by the $^{35}Cl(n, \alpha)^{32}P$ reaction.

The percentage contamination of the $MgNH_4PO_4$ precipitate by four elements was found to be as follows: manganese, 0.35; potassium, < 0.07; sodium, < 0.07; sulphur, 1.0.

WORKING NOTES TO PART IV

NOTES (cont.)

NOTES (cont.)

NOTES (cont.)

NOTES (cont.)

PART V. APPLIED PART

PART V. APPLIED PART

V-1. RADIOISOTOPE UPTAKE AND EXCRETION PATHS THROUGH THE INSECT ORGANISM

In the Animal Kingdom, insects have the highest number of species. Because of transfer and accumulation of radioisotopes within different ecosystems, insects play a most important role as indicator organisms in radioecology. It is therefore extremely important to know exactly the various ways in which radioactive substances are taken up and excreted through the insect organism. Some of the experiments in this Training Manual are designed to elucidate these different pathways which are illustrated schematically in Fig.V-1. The possible pathways are as follows:

A. Uptake

λ_I Via mouth-parts and the gut system.

λ_{II} Through the cuticle. Sorption of tritiated water is also a cuticular uptake.

λ_{III} Transfer via sperm or auxiliary secretion during copulation with radioactively tagged males.

λ_{IV} Via the anus during rectal respiration and/or rectal osmo-regulation.

B. Elimination

λ_1 By regurgitation from the alimentary tract; in social insects, especially the crop.

λ_2 The tracer carried by the haemolymph enters the salivary (labial) glands (L) through their epithelium and is secreted with the saliva.

$\lambda_{3/4}$ The tracer is excreted during respiration ($^{14}CO_2$ given off) through the tracheal system (T) or during transpiration (tritiated water) through the cuticle (K), together with cuticular secretion or pheromones.

λ_5 The tracer is eliminated by females with the eggs, viviparous larvae or auxiliary secretion; likewise by males with sperm and secretion. The measurement of activity in sperm and secretion is in many cases the only definitive method for determining the competitiveness of sterile males in the sterile insect technique, particularly with species in which no spermatophores are deposited in the females.

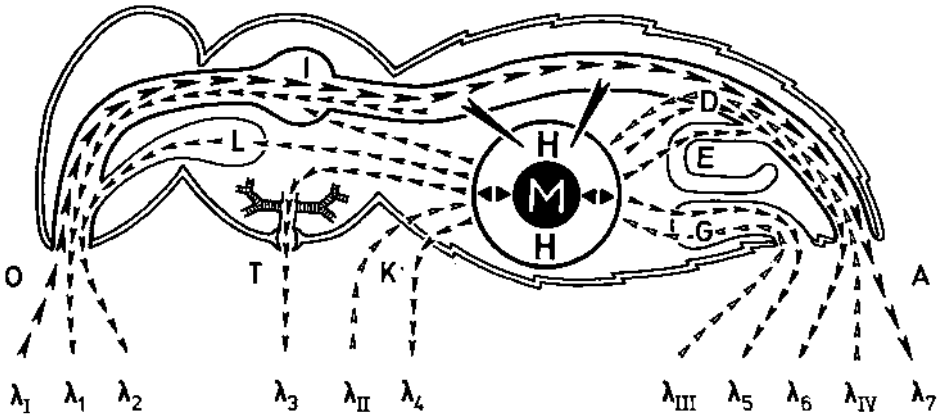

FIG. V–1. Pathways of radioisotope uptake (roman numerals) and excretion (arabic numerals) through the insect organism (unpublished original drawing by W.J. Kloft and E. Wolfram). O – os (mouth); L – labial (salivary) glands; I – ingluvies (crop); T – tracheal system; K – cuticle; H – haemolymph; M – metabolism; D – gut; E – excretion through Malpighian tubules; G – genital organs; A – anus. Further explanations are given in the text.

λ_6 The tracer is excreted via the Malpighian tubules (E) and the hind gut.

λ_7 The tracer is excreted with the faeces after normal passage through the alimentary tract.

Figure V-1 shows that the haemolymph (H) and the metabolism (M) are interconnected with all these processes.

V-2. PRINCIPLES OF INTERNAL AND EXTERNAL TAGGING OF INSECTS, WITH EMPHASIS ON THEIR PHYSIOLOGY AND ECOLOGY

When planning radioisotope experiments, the purpose of the experiment and the possibility of using other methods or a combination of other methods with radiotracers have to be considered first. The equipment available for detecting or measuring radioactivity in insects is very important. A basic problem is the selection of suitable radioisotopes. This selection depends on the following conditions:

(a) Physical: Type of emission
Radiation energy
Half-life

(b) Chemical: Formulation of the radioelement

(c) Physiological: Biological half-life (accumulation, retention) after internal application; penetration through the cuticle and internal contamination by the cleaning behaviour of the insects after external application; cuticular excretion and effective half-life; toxicity to the insect.

(d) Ecological: Habitat of the insects; methods of detection and recovery (baits, traps, etc.); duration of life cycle; food-chains and food-webs; danger of field contamination, etc.

V-2.1. Methods for determining the precise amount of radioisotope applied to individual insects

V-2.1.1. Internal application

A. Enteral application

(a) Force feeding using an oesophageal probe. This is especially useful for the application of substances with a repellent effect, such as pesticide solutions, and substances diluted in fatty oils.

(b) Voluntary feeding by oral application with graduated pipettes. Many insects, especially those with sucking or licking/sucking mouth-parts like honeybees, drink attractive fluids (sugar or honey solution) from the tip of pipettes. With the use of graduated pipettes the ingested volume can be measured. Even insects with chewing mouth-parts (like cockroaches) start sucking out of pipettes when recovering from CO_2 anaesthesia. (For further methods of application of measured amounts to plant feeding insects, see under B.)

(c) Radioisotope application by a rectal probe. Many substances, e.g. water, inorganic salts, organic compounds like carbohydrates of low molecular weight, amino acids, etc., are absorbed very fast by the rectal glands.

B. Parenteral application

It is advantageous to inject the radioactive solution via the intersegmental membrane of the abdomen into the haemolymph in the direction of the head. In the case of high haemolymph pressure, pierce the membrane at first with a pin (insect pin), allow the outflow of a droplet of haemolymph and start after

this with injection of the tracer. To prevent outflow of the injected tracer, go as far into the body as possible, but avoid damaging internal organs. Inject very slowly. Plasticine can be used for fixing and directing the micropipettes and needles while injecting. During injection, the insects should be kept under slight CO_2 narcosis. For special experiments, the insect can be restrained with double-sticking tape or with carboard (for larger insects) using Scotch tape or masking tape. The wounds close normally by haemolymph coagulation within a short time.

To prevent radioactive contamination by outflowing haemolymph, the wound can be covered with a glue which dries rapidly, forming a thin impervious film. If rapid circulation and distribution of the injected material is desired (which is necessary in some experiments), it is recommended to inject through the intersegmental membranes of the posterior tergites, just beside the dorsal vessel (heart), i.e. dorsolateral. Calibrated syringes (AGLA micrometer syringe, Hamilton syringe) are useful. Instead of steel injection needles, self-drawn glass capillaries can be fitted to the syringe. When very small amounts of radioactive solutions are used, better precision can be obtained by filling the syringe with a 'sham load' of a neutral (non-miscible) liquid (e.g. mineral oil in the case of aqueous radioactive solutions or aqueous solutions in the case of radioactively labelled fatty acids) thus increasing the volume. Normal uncalibrated or roughly calibrated syringes (disposable syringes) can be used to make self-calibrated capillaries. By coating the needles with silicon oil the adhesion and external flow-back of radioactive liquids at the needle point is prevented.

C. Spiracular or cuticular application

It is well known that various materials can penetrate the cuticle during transpiration or after condensing or subliming on its surface. Such substances include gaseous radioactive materials like $^{14}CO_2$, tritiated water vapour, toxic gases from evaporating organosynthetic pesticides, and certain pheromones. This is considered an internal application as the material enters the physiological system of the insect.

V-2.1.2. External application

A. Topical application

A precise volume of radioactive solution can be put on a selected spot of the cuticle. Usually we prefer the centre of the pronotum, a point which most insects cannot reach with their antennae or legs; thus the contamination of the insects while they clean themselves is prevented. It is advantageous to restrain

the insects, as previously described. Micropipettes, Hamilton syringes and micrometer syringes are used for topical application. The injection needle points should be sharpened and siliconized. A rectangular bending of the needle allows the syringe to be held in a horizontal position so that any outflow of the liquid is prevented. For example, $^{60}Co(NO_3)^2$ diluted in cellulose acetate with acetone can be applied in dots on the cuticular surface of insects. Since the acetone evaporates immediately, the dots are dry within a short time. For adult beetles, the solution can be applied on the lower surface of the elytrae (during narcosis beetles sometimes spread out the elytrae), thus totally preventing any mechanical rub-off.

B. Insertion of solid tracer material

Pieces of wire of radioactive metals like ^{60}Co, ^{226}Ra and ^{182}Ta can be inserted through the intersegmental membranes into the haemocoele of insects (see Fig.V-2(b)). To prevent surface corrosion of the tracer by the haemolymph, which would lead to radioactive excretion and possibly environmental contamination, the wire should be coated with gold, platinum or, as in medical applications, with synthetic materials, e.g. Teflon, PVC or rubber. Non-noble metals such as metallic cobalt or tantalum are corroded by insect haemolymph.

C. External application of solid tracer material

Pieces of wire, discs or strips of sheet metal (^{60}Co, ^{226}Ra, ^{182}Ta, etc.) can be fixed on the insect's surface with special glues (contact glue, spray glue, etc.) (see Fig.V-2(a)). The tracer should be 'imbedded' in the glue material so that it is coated. To reduce the radiation doses from handling of the tracer material, special applicator devices (of micromanipulator type) have been developed. Such applicators are helpful if larger numbers of insects have to be labelled.

D. External application of tracer materials suspended or diluted in cement, lacquer or similar sticking and dry-fixing substances

For application methods see Section V-2.1.2.A.

V-2.2. Further methods of tagging insects

In addition to the methods already described, several others can be used for marking groups of insects under laboratory conditions. In principle, most of these methods can be adapted to large-scale tagging under field conditions.

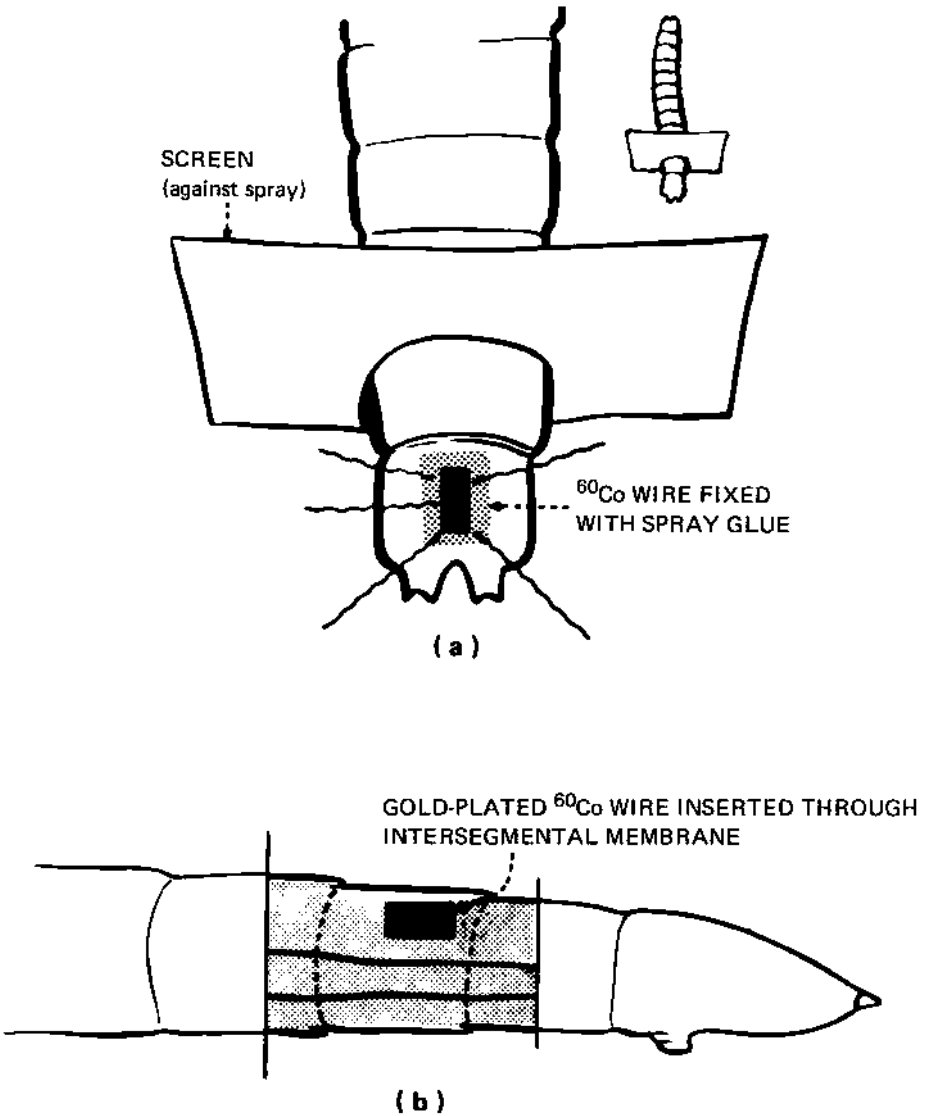

FIG. V–2. (a) External fixation of a ^{60}Co wire on the pygidium of a click beetle (Elateridae) larva with spray glue.

(b) Internal label with gold-plated ^{60}Co wire, inserted through an intersegmental membrane of a click beetle (Elateridae) larva.

V-2.2.1. External application

Spraying. One of the simplest techniques is spraying insects with radioactive solutions. This method cannot be recommended for marking insects for distribution, migration or population density studies, since this kind of labelling individuals is too uneven. However, for certain experimental purposes, e.g. checking the distribution, adhesion or penetration of labelled pesticides, the method is very useful if carefully applied.

Dipping. Dipping insects in aqueous solutions of radiotracers has often been used under field and laboratory conditions. To obtain a uniform contamination, wetting agents such as 20% ethanol or detergents (like Triton X 100®) should be added. The insects can be put into containers holding the liquid, which are then vigorously shaken. Another technique is to put the insects in a net or sieve, which is then repeatedly dipped into the liquid and finally kept for some time above the liquid to allow the excess fluid to drip back. After this procedure, the insects should be dried in an airflow.

Dipping of egg masses is particularly easy; in this way the eggs are contaminated externally and can be brought back into the ecosystem to identify egg predators [1]. This technique has often been used under field conditions. For example, in dispersal studies of cotton boll weevils, adult beetles have been dipped into $^{60}CoCl_2$ solutions with a specific activity of 0.05 mCi/ml (18.5 MBq/ml). Under field conditions, a simple radiation protection can be obtained by placing the containers in the soil. The insects can be dipped in nets hanging on poles or fishing tackles.

Pouring radioactive solutions over insects can be handled under laboratory as well as field conditions. Separating devices are available, making it possible to pour the liquid repeatedly over the insects and to recover it; the insects are then dried in an airflow. Such simple devices, which are available in every laboratory, are the separating funnel and the Buchner funnel. Smittle [2] has described such a technique for labelling lone star ticks *(Amblyomma americanum (*L.*)*). The ticks were placed in a Buchner funnel and exposed to a ferrous citrate (^{59}Fe) solution by pouring the liquid over them. One minute later, the excess liquid was drawn by vacuum into the vacuum flask beneath the funnel. The exposure was repeated three times with the same solution. Then the ticks were removed from the funnel, placed on absorbent paper and collected into a test tube with a suction device.

Dusting with radioactive powders is not very useful; it results in unequal marking, and the dust can get lost and contaminate the containers or the environment (see Section V-2.3).

Painting with radioactive tracers has the advantage of offering the possibility of double marking by mixing the radioactive materials with dyes.

Contamination of media is a method which is intermediate between external and internal labelling. Aquatic organisms like mosquito larvae have often been exposed to aqueous solutions of ^{131}I, ^{32}P, ^{89}Sr, ^{45}Ca and other radioisotopes. The tracers either adhere to the surface of the cuticle and penetrate through the cuticle or are ingested with soaked food or water. Such an experiment is described in Section V-3.4. This method can be used for tagging the pupae and emerging adults.

Submersed egg-masses can be tagged by adding radiotracers to the water medium. *Culicoides* species can be labelled by placing them in solutions containing 0.5–5μCi ^{32}P/ml (18.5–185 kBq ^{32}P/ml) and also by the application of tracers to the mud [3]. However, in this case it is hard to differentiate between medium contamination and food-chain contamination; both may be involved.

V-2.2.2. Internal application

Application via drinking water. Many terrestrial insects are used to drink water either normally or when dried out to get back their water balance. They all can easily be tagged internally by adding the tracer to the water. This method works for very different types of mouth-parts (for example, those of Hemiptera, house-flies, mosquitoes, honey-bees, beetles, Orthoptera, etc.). The uptake of water can be forced by maintaining the insects for some time under dry conditions. Crossley and Shanks [4] supplied $^{106}RuCl$ or $^{134}CsCl$ to cricket colonies *(Acheta domestica)* with drinking water.

Application via feeding. (a) Contamination of food mixtures. Many insects, such as flies, mosquitoes, Hymenoptera (bees, wasps, ants, parasitic Hymenoptera), Coleoptera, Lepidoptera, are attracted by sugar or honey solutions or fermented fruit juices. Tracer applications can be made using this feeding preference. In many cases, other media are more attractive. Mixing aqueous solutions of tracers with different media is a very convenient method of labelling many kinds of insect food. In the case of a feeding preference for fatty materials (oil baits, as for cotton weevils or fire ants), the tracer should be liposoluble. Thus ^{131}I-labelled oleic acid or triolein can be used successfully. When ^{14}C-labelled fatty acids are used, more specific equipment is needed, like liquid scintillation counters, for quantitative measurements.

Application via feeding. (b) Contamination of natural food sources (accumulation via food-chains). Using the accumulation of radiotracers by natural food-chains leads to higher rates of radioactivity in insects.

Aquatic insects foraging on algae can be successfully labelled by feeding them with radioactive algae (Chlorella can easily be cultured in ^{32}P-solution and accumulates high amounts of the tracer by light-dependent processes of phosphorylation).

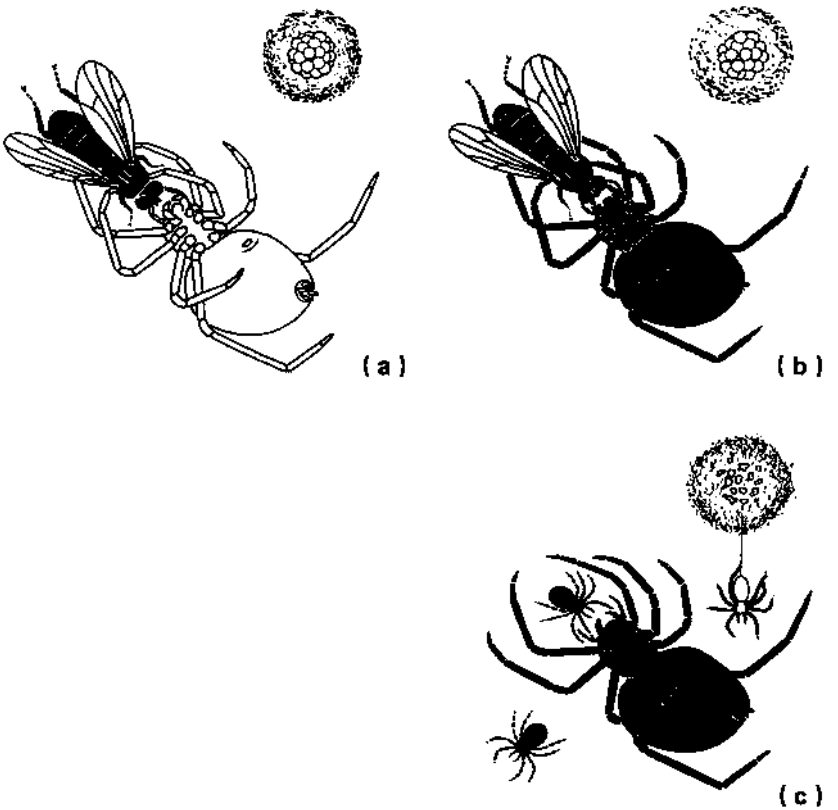

FIG. V–3. Labelling of a female spider and her newly hatched larvae by feeding the female spider with radioactive flies. This experiment demonstrates the social behaviour between mother and newly hatched larvae in spiders [7].

(a) A female spider (Araneae, Theridiidae) is fed with a radioactive (black) fly after production of its egg cocoon. (b) By feeding on the radioactive fly, the spider becomes radioactive (black) itself. (c) The newly hatched larvae are fed by maternal regurgitation thus becoming radioactive (black) in their turn.

Mycophagous microarthropods like Astigmata, Mesostigmata and Cryptostigmata can be supplied with ^{65}Zn via fungus mycelia [5].

Yeast feeding insects like many fruit flies are labelled within a short time by feeding them on yeast cultures grown on labelled media.

Bacteria assimilate many radiotracers and are therefore a good medium to label insects, especially scavengers.

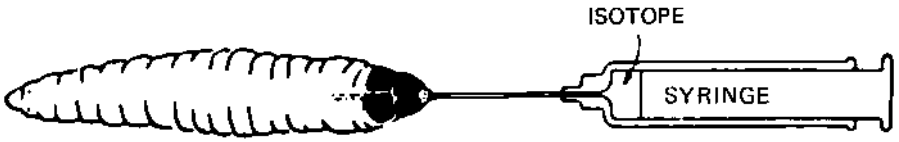

FIG. V–4. Injection of a radioisotope into an Ephestia *spp. caterpillar which will then serve as radioactive host for parasitic Hymenoptera (*Habrobracon *spp.)* [9].

Protozoa can accumulate radioisotopes. An experiment where newly hatched Odonata nymphs were supplied with ^{32}P-tagged *Paramecium* cultures is described in Ref.[6].

Predacious arthropods can be labelled by feeding them with radioactive prey [7]. Spiders can be fed with flies which had imbibed a ^{32}P sucrose solution (Fig.V-3); larvae of *Myrmeleontidae* (ant lions) can be labelled in the same way by feeding them with radioactive ants [8]. Using a natural food-chain, ladybird beetles can be labelled by feeding them with radioactive aphids, and ants are labelled by feeding them with the aphid's honey-dew. This can be done in the laboratory as well as in the field.

A very interesting method of labelling parasitic Hymenoptera via radioactive insect hosts was worked out by Steffensen and La Chance [9]. Since this method allows a separate marking of the sexes and, subsequently, of sperm or eggs, it is also of interest for experiments in genetics and radiobiology. *Habrobracon juglandis* can be bred in Petri dishes, and a stock colony of *Ephestia kuehniella* is used to supply the Hymenoptera with insect hosts. The caterpillars are supplied with the tracer by an oesophageal probe (Fig.V-4) (see Section V-2.1.1.A (a)).

Plant-sucking insects like phloem-feeding aphids and other Homoptera can be labelled via the host plant or artificial diets (see Section V-3.19). Other nutritional types such as 'local imbibers' [10] can be fed on leaf discs floating in radioactive solutions. For mass-rearing labelled crawlers (of the Coccid family Diaspididae), green melons can be tagged with $^{14}CO_2$ through photosynthesis or by injection of ^{14}C-glucose. The melons are more evenly labelled when $^{14}CO_2$ is used.

Woodboring beetles in living stems can be marked systemically via plant injection. Leaf-eating insects like saw-fly larvae, caterpillars, etc. can be labelled very easily on plant twigs dipped in radioactive solutions. For exact application (as regards time or amount), the sandwich method is convenient, using sandwiches made of leaf pieces, with the inner surface being 'smeared' with the radiotracer; also radioactive meridic diets are useful [11].

V-2.3. Non-radioactive methods for marking insects

In a book dealing with radiotracer methods, it may be surprising to find a short introduction into non-radioactive methods for marking insects. There are a number of reasons for including this material here. In many cases non-radioactive methods are easier to use and interpret than radioactive ones. They generally require much less equipment; the procedures are usually simple and allow marking individuals as well as populations. Non-radioactive procedures involve no health problems and (in field work) no problems of environmental pollution by radiotracers. Therefore one should always consider whether it is preferable to use a non-radioactive or a radioactive method. The choice depends not only on the actual problem and efficiency of the method, but also on equipment, costs, experience and licence to do field work with radiotracers, etc.

Moreover, non-radioactive methods can be of great help when combined with radiotracer techniques in double labelling, thus allowing differentiation between individual specimens, groups of insects or populations.

Methods of marking insects were briefly reviewed by Gangwere et al. [12]. Generally known techniques include: notching parts of the cuticle; clipping off single tarsae; marking with paint spots; applying numbered or coloured labels, bright aluminium foil or light-reflecting glass dust on the thorax. Recently, several methods were extensively developed because of the increased interest in ecological field studies. Thus fluorescent dyes were used by several authors for marking and recovering released insects or ticks (see Medley and Ahrens [13] and literature quoted by them). Dusts applied in adequate amounts to insects with abundant hair or setae may adhere long enough for later recognition. However, dusts tend to get lost and contaminate the environment; this is of no importance in field experiments but it is disadvantageous in experiments involving small areas or traps. Thus it is preferable to mark the insects or ticks individually on the dorsum with dyes dissolved in alcohol or acetone.

Soluble fluorescent dyes can be detected on whole insects under black light, provided sufficient dye adheres externally and self-fluorescence of the insect does not lead to errors. Portable ultra-violet lamps allow the detection of labelled specimens under field conditions. When the amount of fluorescent dye is so small that it can no longer be detected in this manner, we recommend putting the dead insects on white filter paper and dripping acetone over the bodies. Thus, even small amounts of any dye (whether fluorescent or not) can be seen on the filter paper, either directly as a coloured spot or, under black light, as a fluorescent spot. As a last check, the insects should be squashed on the filter paper. These techniques have been used very successfully in the Netherlands for labelling radiosterilized onion flies (*Hylemia antiqua* Meigen) [14]. These flies had been sterilized as pupae and transferred as such into the soil of an experimental field for 'self-release' after emergence. The distribution of these specimens after emergence was monitored using a 'self-marking' process:

the pupae were buried under a layer of sand over which a thin coating of dye was applied, followed by a second layer of sand and soil. Emerging flies had to bore their way up to the surface and thus some dye was deposited on the ptilinum. Retraction of the ptilinum into the head precluded the loss of the dye, which could later be detected by squashing the head on filter paper and dissolving the dye (fluorescent or not) with a few drops of acetone.

With the newly developed zinc or cadmium sulphide fluorescent dusts, which are neither water nor alcohol soluble, millions of insects can be marked at a time for field observation. In most cases, these compounds are superior to metallic powders and soluble dyes. There being no human safety problems with the fluorescent dusts, and the equipment for their detection being inexpensive, these materials can be very useful in entomological research. A major advantage of these dusts is that they will not be washed off when the specimens are immersed in water or preserved in alcohol. The insects remain marked even after digging in sandy soil for months. For information regarding manufacturers, detection, equipment, and application methods, see Ref.[15].

Liposoluble dyes like Calco® oil N-1700 were used successfully for internally mass-marking field populations of insects attracted by oil baits (e.g. boll weevils attracted by cotton-seed oil, fire ants by soybean oil; see Ref.[16]).

Genetic markers can be used in field studies of insect distribution and flight range. For example, Cordovan bees are used frequently because their distinctive body pigmentation permits of easy identification in the field [17]. A prerequisite is, of course, that the vitality of the genetically labelled strains is not less than that of the normal wild insects. Kloft (unpublished data) succeeded several times in estimating unknown population densities of *Drosophila* by means of a genetic marker. Also, in the laboratory, the same results were obtained with radioactively labelled specimens.

With honey-bees, a very interesting new capture/mark/recapture system was developed by Gary [18]. Ferrous labels are glued lightly to the abdomen of bees foraging in the field (these can be given individual numbers). When the bees thus marked return to the hive, their labels are taken off by magnetic traps as the bees pass the hive entrance, thus recording to which hive each individual bee has returned. Possibly this method could be adapted to other insects; however, its primary limitation is the improbability of the insect passing precisely within the recovery range of the magnets. It might be helpful to use a combination of attractive baits and magnetic traps for solitary insects.

V-3. EXPERIMENTS WITH INSECTS

V-3.1. Decontamination of insects, including live specimens

An undesired contamination of the external surface of insects occurs sometimes, e.g. after feeding on radioactive materials. If external transfer of

radioisotopes to other biological substances (nest mates in the case of social insects, see Section V-3.5; host plants in the case of plant-sucking insects, see Section V-3.2) is a possible source of error, it is necessary to decontaminate even live insects in the course of tracer experiments. The same holds true when measuring the biological half-life of radiotracers in insects. We have used with success the techniques described in Section V-3.2, Exp.C, viz. displacement or exchange with carrier solutions (i.e. chasers). For decontamination of live insects, we recommend the use of a detergent like Triton X 100, which is often used in entomology. For the decontamination of social insects like ants or honey-bees which have fed on radioactive sugar or honey solutions (tagged with radiophosphorus as sodium phosphate), proceed as follows.

After careful narcosis with CO_2, the anaesthetized insects are grasped with soft insect tweezers, and one by one dipped and waved for several seconds in the following fluids: 1. tap water with added detergent (Triton X 100); 2. decontamination fluid as described in the example below; 3. tap water without detergent to remove all chemicals. The insects should then be placed in a container with soft cellulose tissue to soak off the water. Insects recovering from the CO_2 anaesthetic should be dried after being allowed to move about a bit. The efficiency of this procedure can be checked experimentally. Thus, in a food distribution experiment with honey-bees, Kloft et al. [19] made sure that no transfer of radioactivity had taken place owing to squirting out and/or smearing of fed honey solution; each individual had to be decontaminated before measurement. The efficiency of the method was confirmed by taking at first whole-body counts of the bees and then preparing the guts and comparing the measurements with the readings of the alimentary tract. The results were similar (short-time experiments with counting before any resorption of the radiotracer into the haemolymph).

Example: 1. ^{32}P as $Na_2H^{32}PO_4$, and 2. ^{131}I as NaI are used in the laboratory for insect experiments.

The decontamination fluids should have the following formulations: 1. 3% Na_2HPO_4 as chaser in water with a small amount of wetting agent (detergent); 2. 3% NaI as chaser in water plus detergent.

V-3.2. Measurement of beta radiation from intact insects (absorption and backscattering)

Repeated radiation measurements on intact, live test insects for determining the sequence of physiological phenomena must take into account a series of factors which affect the readings. In addition to the physical constants, such as radiation energy and half-life, technical characteristics of the measuring apparatus must be allowed for. These include the geometrical arrangement of

the radioactive test insect and the GM tube, radiation absorption by air, and particle scattering by the chamber walls. These factors and the characteristic properties of the GM tube can, however, easily be maintained constant. On the other hand, measurements of beta particles are greatly affected by absorption and backscattering effects of body tissue. If, in addition, the tracer used participates in metabolic processes, the possibility of a further and rather important source of error arises, viz. cuticular excretion of the tracer. Measurements on ^{32}P-labelled insects are reported here with a view to studying such possible errors.

Radiation absorption by the body tissue must be taken into account, even when using a relatively hard beta source such as ^{32}P for small insects. Huot and Verly [20] measured the relationship between the radiation rates of live larvae and of incinerated larvae of *Tenebrio molitor* L. and developed a correction factor which is a function of the fresh weight. However, a total correction factor remains, which combines body absorption, backscattering of the animal and even particle scattering in the measuring cell designed for holding the live insect (see Section V-3.3). Another method is needed if the beta-particle absorption of single tissue layers, especially the cuticle, is to be measured. Radiation absorption constants are given in the literature, but it is almost impossible to interpolate from values in medical tables since insect tissue layers are very thin and furthermore non-homogeneous.

PURPOSE

To measure and calculate the absorption of beta particles by the insect body in order to demonstrate and estimate backscattering of beta particles by the cuticle.

PROCEDURE

Experiment A. Direct measurement of beta particle absorption by the insect body. The experiment described here (see Refs [21, 22]) can be carried out with ants (*Formica* spp.) fed on a ^{32}P-labelled aqueous solution of honey or 20% sucrose. If the insects are killed within 30 minutes after feeding, the total activity remains in the proventriculus, which neither digests nor absorbs the labelled food. The source of radiation is therefore localized in the abdomen of the insect in a spherical form, without contaminating the tissues.

After measuring the background of the counter, the freshly killed insect is fixed on a planchet with adhesive and positioned directly under the centre of the GM tube. The activity of the freshly killed insect is then measured over a sufficiently long interval to reduce the statistical error. Subsequently, the dorsal cuticle is carefully removed with fine dissection instruments. A further measurement is then taken which shows an increased count-rate owing to the

absence of cuticular absorption (Table V-1). These counts give values of I_{cu} and I_0, the count-rates with and without cuticle respectively. From these data, radiation absorption may be expressed as a percentage of I_0. More useful, however, is a calculation of the absorption coefficient μ (consisting of a real absorption value and a scattering value), given by the exponential equation for beta absorption:

$$I_{cu} = I_0 \times e^{-\mu d}$$

or

$$\mu = \frac{\ln (I_0/I_{cu})}{d}$$

or

$$\mu = \frac{2.303}{d} \log (I_0/I_{cu})$$

where d = thickness of the absorber, in this case the dorsal cuticle. This cuticle thickness was determined microscopically by means of median sections through entire ants *(Formica nigricans* Em.*)*, the average value being 0.020 mm. The calculated values of μ entered in Table V-1 apply to the cuticle and hypodermis. The latter always remains attached to the cuticle when the insect is dissected, and its value was always considered in conjunction with the cuticle. It must be stressed that the μ-values thus determined are dependent on the degree of activity and the particular measuring system used and must, therefore, be determined for any one equipment as well as for each insect species.

Experiment B. Indirect method of measuring beta particle absorption by the insect body (substituting aluminium filters for the excised tissue layers). In the direct radiation absorption measurements described above, the absorption coefficient was calculated for the dissected cuticle layer. For this purpose, the thickness of the layer had to be measured microscopically. The absorption value of the excised tissue may also be easily determined indirectly by substituting aluminium filters of known mass per unit area (mg/cm^2).

For this purpose calibrated filters can be used. (We have used a set of 30 calibrated filters, manufactured by Frieseke and Hoepfner, Erlangen, Federal Republic of Germany. The values for mass per unit area ranged from 1.3 to 65.8 mg/cm^2, corresponding to filter thicknesses of 0.0049–0.243 mm.) It is easy to prepare one's own filter set with household aluminium foil. The mass per unit area has to be determined carefully. The filters can be fixed (one or more on top of one another) between cardboard frames as used for photo slides; this gives a fine self-made set of calibrated aluminium filters.

TABLE V-1. EXAMPLES OF MEASUREMENTS TO DETERMINE THE BETA PARTICLE ABSORPTION OF THE TERGAL CUTICLE OF INSECTS [22]

Test insect No.	Measurements for Experiment A				Measurements for Experiment B			
	I_0 (counts/min)	I_{cu} (counts/min)	d_{cu} (mm)	$\mu = \frac{\ln (I_0/I_{cu})}{d}$	Filter No.	Mass per unit area (mg/cm^2)	d_{Al} (mm)	I_{Al} (counts/min)
1	10 509	9 928	0.020	1.23 mm^{-1}	F_2	3.4	0.0126	10 105
					F_3	6.2	0.023	10 023
					F_4	10.7	0.0396	9 758
2	6 357	5 903	0.020	1.47 mm^{-1}	F_2	3.4	0.0126	6 098
					F_3	6.2	0.023	5 996
					F_4	10.7	0.0396	5 834

μ = calculated absorption coefficient for the cuticle and hypodermis
I_0 = count-rate after dissection
I_{cu} = count-rate with cuticle
I_{Al} = count-rate after substituting aluminium filter
d_{cu} = cuticle thickness

In the experiments described by Kloft [21], these filters were fixed in the radiation path, 8 mm above the test insect, and the count-rate was measured after each filter substitution. Such an arrangement is also used in the present experiment. The purpose of the measurements is to find a filter reducing the count-rate I_0 to the original value I_{cu}. The mass per unit area in mg/cm² of the appropriate filter may then be considered equal to that of the cuticle. Similarly the next tissue layer, i.e. the fatty tissue lying between the digestive tract and the dorsal cuticle, has to be removed and its mass per unit area determined by substituting aluminium filters. In *Formica* spp., this value was a little above 3.4 mg/cm². The thickness of the layer, determined microscopically, gave an average value of 0.020 mm for worker ants *(Formica nigricans* Em.). A summation of the values for the individual layers gives the total radiation absorption by the layers (between the digestive tract and the outer surface) in mg/cm². If about 8 mg/cm² is taken to be the mass per unit area of the cuticle including the hypodermis, and 4 mg/cm² the value for the fat body between the cuticle and proventriculus, then the total amounts to approximately 12 mg/cm².

Discussion

The radiation absorption values of the excised tissue are expressed in terms of mass per unit area (mg/cm²); this is the quantity usually reported in handbooks. Knowing the specific gravity of the tissue concerned, it is possible to calculate those layer thicknesses which completely absorb the beta radiation of a certain energy (maximum range R) or reduce it to half the original energy (half-thickness R/2). R has been reported to be about 740 mg/cm² for ^{32}P. This value was checked by the author in a control experiment with aluminium as radiation absorber; R/2 was about 110 mg/cm². If the mass per unit area of the absorbing tissue layer is known, the important half-thickness can easily be determined. Three separate steps may now be taken:

(1) The mass per unit area of the cuticle, based on microscopic measurements of its thickness (0.020 mm for worker ants), may be determined and combined with the specific gravity reported in the literature (max. 1.3). From these data a mass per unit area of 2.6 mg/cm² can be deduced for the dorsal cuticle of the ant. Referred to R/2 = 110 mg/cm², based on the aluminium radiation filter, the calculated half-thickness of the ant cuticle is 0.84 mm.

(2) The mass per unit area may be determined by the substitution method. A value of about 8 mg/cm² can thus be obtained. Using the known layer thickness of 0.020 mm and R/2 = 110 mg/cm², a value for the half-thickness of only about 0.275 mm is calculated for chitinous insect cuticle.

(3) Once the absorption coefficient, μ, has been determined for the excised tissue, the layer thickness which lowers the radiation to the half-value level, $I_0/2$, may be calculated from the equation

$$\mu = \frac{\ln (I_0/I_{cu})}{d}$$

Hence, for the material under consideration, the value corresponding to half-thickness, $d(I_0/2)$, is given by

$$d(I_0/2) = (\ln 2)/\mu = 0.245 \text{ mm}$$

In close agreement with direct measurement, the substitution method shows a greater reduction of radiation intensity in the cuticle than would be expected from calculation. This discrepancy may be due to the biconvex form of the dorsal cuticle producing a higher backscattering effect, or even to the difference in material (aluminium being used as filter). The effective layer thickness may also be greater than the value measured microscopically since many rays pass through at an angle to the perpendicular. Cuticle thicknesses of 0.2–0.8 mm are found in large, well armoured, sclerotized forms, especially beetles. In such cases, a reduction of I_{cu} to $I_0/2$ might be expected. The real activity I_0 in the insect may easily be calculated from the reduction coefficient μ and the microscopically measured thickness d of the cuticle. The following equation is valid:

$$\ln I_0 = d \times \mu + \ln I_{cu}$$

Thus, correct values for $\ln I_0$ may be obtained without dissection by only adding the value $(d \times \mu)$ to the externally measured count-rate.

Experiment C. Backscattering effect of the insect cuticle (including radiation absorption). The scattering of beta particles is a very complex process and plays an important part in radiation absorption by the body. Backscattering at 180° of particles emitted downward from the source is of particular interest, since these will cause an increase in count-rate (see Fig.V-5). It is known that scattering increases with the square of the atomic number of the scattering material [23]. When a beta source is mounted on a solid backing material (such as slides), an increase in the count-rate owing to the backscattered particles entering the tube has to be taken into account – the so-called backscattering effect. This factor can be maintained constant, insofar as it is dependent on the substrate, by always using the same material. If a very thin membrane is used, this factor can be discounted in practice.

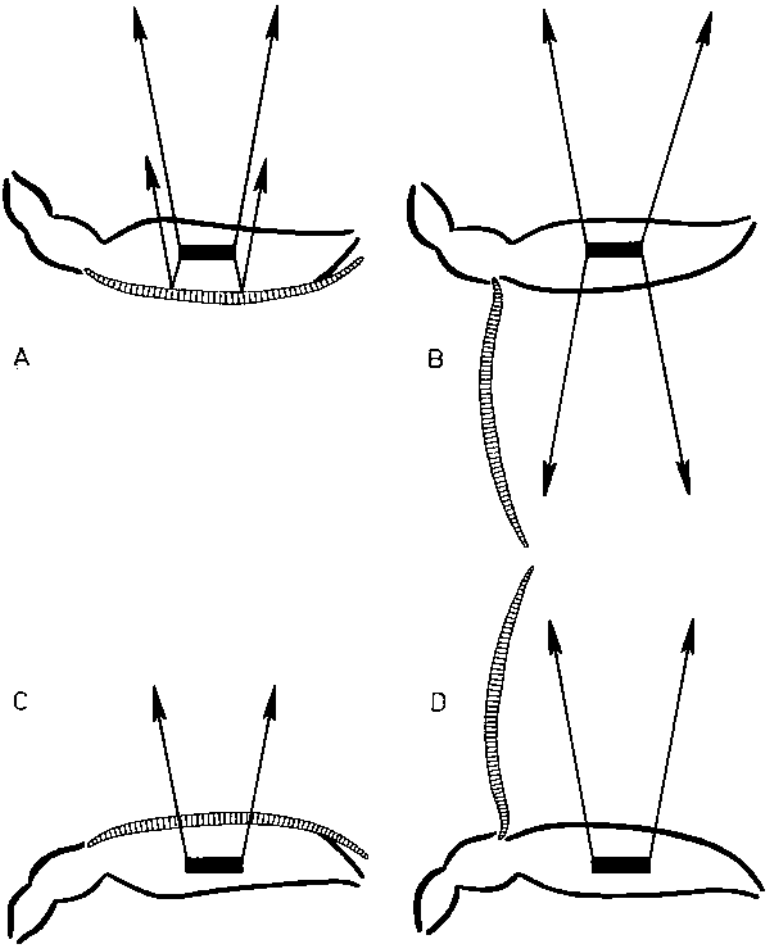

*FIG. V–5. Arrangement by which the backscattering effect and radiation absorption by the elytra of a beetle (*Tenebrio molitor *L.) may be measured. The vectors in Position A indicate the backscattering effect, the shorter length of the vectors in Position C indicates a decrease of beta radiation owing to higher absorption by the elytra. (Drawing by E. Wolfram.)*

Therefore, always place your samples on the same material when making measurements which should be comparable. The same holds true for small containers used to enclose live insects during measurements (see Section V-3.3). With these, additional complications are involved, such as backscattering and side-scattering effects from the walls of the container.

This experiment is suitable to demonstrate in a very simple way and within a short time both the backscattering effect and the radiation absorption by the insect cuticle.

An enclosed ^{32}P source is placed in the digestive tract of freshly killed (using chloroform) adult beetles. Either a small piece of a microcapillary or a small strip of filter paper (1 mm × 3 mm) should be soaked with ^{32}P solution and carefully wrapped and sealed with parafilm. The source must be tight, in order to prevent any outflow of radioactive material, and should give a value of 10 000–30 000 counts/min in the detector system. With the help of sharp-pointed tweezers or an eye dropper, it is possible to introduce the source through the anus and rectum into the middle of the alimentary tract. In a series of experiments [22] the common household beetle bred in most entomological laboratories, *Tenebrio molitor* L., proved to be suitable for this purpose. In Florida we sometimes used sugar beetles (bessbugs, betsy-beetles, Passalidae).

For this experiment, the beetle must be mounted on a perforated metal slide so that the upper and lower surfaces remain exposed. Backscattering owing to the substrate (not that from air, of course) is thus avoided. The membranous hind wings are removed to simplify the procedure. The insect is measured in the following positions (see Fig. V-5):

Position A – upside down, with elytra
Position B – upside down, without elytra
Position C – upright, with elytra
Position D – upright, without elytra

During counting in positions A and B (backscattering) or C and D (absorption), the geometry for each effect should be exactly the same.

Backscattering of beta particles by the elytra increases the count-rate, and absorption of beta particles by the elytra decreases it. Calculate both effects in per cent and record the values in tabular form.

Position	Counting time[a]	Counts	CPM ± .... (counts/min)	Per cent increase
A				CPM Pos. A > CPM Pos. B
B				$(CPM_A/CPM_B) \times 100 = ...\%$ (Backscattering effect)
C				CPM Pos. D > CPM Pos. C
D				$(CPM_D/CPM_C) \times 100 = ...\%$ (Absorption effect)

[a] 10 minutes counting time are recommended to reduce the statistical error.

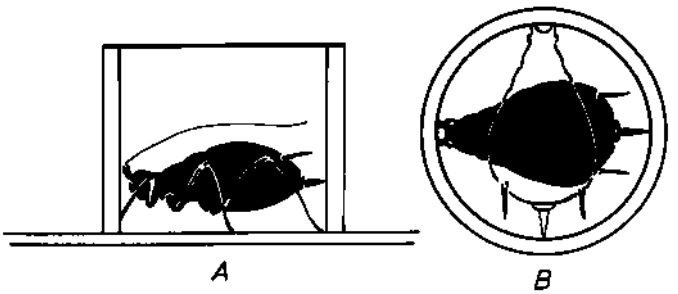

FIG. V–6. Cage for measuring insects (here aphids), consisting of a ring of glass (or plastic tube), covered by a thin membrane [22]. *A – profile view; B – view from above.*

V-3.3. Use of small containers for measuring radioactivity in live insects

In many experiments it is important to measure exactly the total radioactivity in or on a live insect. The insects can be enclosed in small containers; the form and size of these depend on the type and size of the insects. Modern consumer industry produces a series of disposable containers adaptable to our experimental use.

PURPOSE

To demonstrate how the readings differ as a result of backscattering and side-scattering, limited and varied mobility of the insect, etc. Backscattering and side-scattering are influenced by the kind of material (aluminium, glass, plastic of different types) and by its thickness and form.

MATERIALS

(1) Containers of different types, partly open or closed with Parafilm® membranes (see, for example, Fig.V-6).
(2) A number of ant workers, fed ^{32}P in sugar solution with a total activity of roughly 0.1 mCi/ml (3.7 MBq/ml).
(3) Talcum powder.

PROCEDURE

Ants (any species) are fed on ^{32}P sugar solution shortly before the experiments and then externally decontaminated (see Section V-3.1). Subsequently they are enclosed in different containers. All counts must be taken one after

another, with the same end-window type GM tube, at the same working voltage and at the same distance between the bottom of the container and the tube window, always using the same sliding sheet as support. Background readings should be taken carefully and subtracted from each individual value. The walls of the containers should be powdered with talcum to prevent the ants from climbing upward.

V-3.4. Tagging insects with radioisotopes

The tagging of insects with radioactive markers has been used in studying dispersion, speed of flight, total number of a natural insect population in an area, and in other biological investigations. Phosphorus-32 is a useful tagging agent, but the short half-life of 14.3 days precludes its use for studies extending for more than a few weeks.

PURPOSE

It is necessary to be familiar with handling mosquitoes and tagging them in the laboratory before field dispersal studies are undertaken. The purpose of this short experiment is to serve as an introduction to such a type of work.

The best way to obtain tagged adult mosquitoes is the application of the tracer to the larvae via water. Suitable radioactive substances like ^{32}P as phosphate or phosphoric acid are absorbed through the thin larval cuticle, taken up with food, and imbibed with water. These processes ensure good internal labelling of the adults emerging from the pupae.

MATERIALS

(1) Pans or glass-ware, holding from 0.5 to 1 litre of water, suitable for rearing mosquito larvae
(2) Phosphorus-32 as phosphoric acid or a phosphate
(3) Insect emergence cages
(4) Pipettes and other laboratory glass-ware
(5) Counting equipment
(6) Mosquito larvae
(In Gainesville, Florida, for example, we used *Culex pipiens quinquefasciatus* Florida, USDA laboratory strain, in their late second or early third instar: 200 individuals in a normal rearing pan of 100 ml H_2O.)

PROCEDURE

To reduce the amount of radioactive solution, we use only 0.1 litre salt-water (sea salt solution) to which 0.05 mCi ^{32}P is added and thoroughly mixed. The larvae are fed the normal artificial diet. The pan must be covered with glass to avoid water loss (which would change the specific activity) in a laboratory without controlled air humidity.

After 24 hours, take about half of the larvae out of the radioactive medium; after washing them in salt-water, transfer them to another pan containing inactive salt-water and normal larval food (at a ratio of 1/2: 1). For the rest of the larvae, follow the same procedure after 48 hours.

After pupation, take out the pupae, wash them briefly, and transfer them to waxed paper cups containing inactive salt-water. Put the cups into a hatching cage.

Measurements. The adults must be measured for radioactivity for a period of 2 days. In both time groups, record separately the males and females. Check larval radioactivity at intervals (from the second day).

QUESTIONS

(1) Which sex has the greatest radioactivity?
(2) How does the uptake of radioactivity compare in the 24 and 48 hour treatments?

V-3.5. Demonstration of food exchange among social insects

Social insects like ants, bees and termites have a highly developed behaviour of social food exchange between the different individuals of the community. With the help of radioisotopes, it is possible to demonstrate this behaviour in laboratory experiments with ants over a short period. Using ants of different subfamilies, one can demonstrate certain differences between them, which have to be taken into consideration in tracer field experiments. The time required for this experiment is from 4 to 8 hours, after completion of preparatory work with equipment and insects.

(For literature see Refs [19, 24, 25].)

PURPOSE

To demonstrate uptake and distribution of food from ant to ant or caste to caste.

MATERIALS

(1) Glass containers, 10 cm X 10 cm X 5 cm, with covers
(2) Insect tweezers
(3) Glass slides and cover slips
(4) Chloroform or ether
(5) Honey or sucrose solution (20%)
(6) 0.5–1.0 mCi (18.5–37 MBq) ^{32}P in water, with a specific activity of about 0.5–1.0 mCi/ml (18.5–37 MBq/ml)
(7) Hollow plastic stoppers for holding the ants during measurement (containers), with a diameter corresponding to the size of the ants; talcum powder
(8) Non-radioactive phosphorus of the same composition as under (6)
(9) Vials, bottles, ordinary laboratory glass-ware, plastic squeeze bottles
(10) Radiation measuring equipment
(11) 500 ants from the subfamily *Camponotinae* and 500 ants from the subfamily *Myrmicinae*
(12) Decontamination solution: 1% sodium phosphate plus a small amount of detergent such as Triton X-100 or Tween 20

PROCEDURE

Experiment A. Rate of direct food uptake by ants. Prepare liquid radioactive food for the ants by mixing two parts of a 30% solution of honey in water with one part of ^{32}P solution. The specific activity of the food should be 0.5–1.0 mCi/ml (18.5–37 MBq/ml).

Ant species:
Caste:
Composition of food:
Mode of radioisotope application:
Air temperature:
Date of collection:
Specific activity:
Time at beginning of count:
Time at end of count:

Measurement: GM tube No.: Working voltage:

Ant No.	Counting time	Counts	CPM (counts/min)	Background (counts/min)	CPM minus background (counts/min)

Put 10–15 specimens of each species into separate jars. Feed the ants by putting a drop of the prepared radioactive food on cover-slips and placing these in the jars. Record the starting time, duration of feeding and air temperature. After careful decontamination, the ants should be measured individually. For records, use a form of the type shown at the foot of page 164.

Experiment B. Social food distribution among ants. Individual ants with the highest radioactivity are now used as donors. Mark the donors carefully with a colour spot and put each donor in a jar containing 30 hungry worker ants (unfed for 2–3 days) of the same species and from the same colony. Prepare three jars of each species.

Using chloroform or ether, kill the ants in one jar after 4 hours, those in the second after 20 hours, and those in the third after 40 hours.

Measure and record the radioactivity of each ant. Group the number of specimens in each jar in a category of radioactivity, i.e. 0–50, 50–200, 200–500, 500–2000, and over 2000 counts/min. Compare the food distribution of the two species on a time basis.

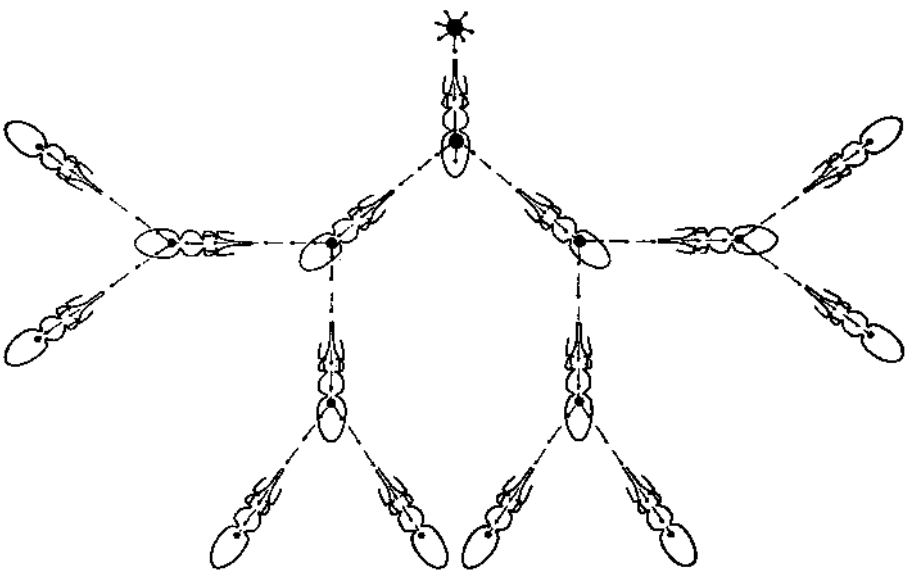

FIG. V–7. Social food distribution in honey-bees. The donor bee (top) imbibes a radioactive sugar solution, which is distributed by multiple regurgitation to other members of the hive. With a single donor bee fed 50 µl radioactive sucrose solution and kept at 31°C, we recorded a maximum crop-content distribution: 349 individuals (these results vary among races of the western honey-bee Apis mellifica *L., see Refs* [19, 24]).

Experiment C. Social food exchange among honey-bees (Fig.V-7). Put about 250 honey-bees in two small wire screen cages; keep them in the dark and without food for about 2 hours (at 30°C).

Take out one worker bee from each cage and feed it with labelled honey solution (about 0.5 mCi/ml (18.5 MBq/ml)) through a pipette. Check the radioactivity (counts/min) of the labelled individuals; put them back into the cages.

Supply the insects of cage (a) with water but not with additional food. Supply the insects of cage (b) with additional food and water. Keep both cages dark (at 30°C) for 4 hours; finish the experiment by very slow and careful anaesthetization with CO_2, followed by killing with chloroform. Measure each individual for radioactivity (counts/min).

QUESTIONS

(1) What time was required for maximum distribution of food to be obtained?
(2) Which ant subfamily accomplished the distribution first?
(3) Are there any differences in the honey-bee experiments with and without food supply? What are the sources of error?

V-3.6. Demonstration of time required for secretion via saliva of perorally ingested ^{32}P

As illustrated in Fig.V-1, perorally ingested radiotracers absorbed through the gut are transported with the haemolymph to the salivary glands. The cells of the gland epithelium absorb the radiotracer from the haemolymph and secrete it with the saliva. Flies are ideal for the experimental demonstration of the time required for secretion of perorally ingested inorganic ^{32}P via the saliva. This turnover time includes: uptake; transport into the gut; absorption by the haemolymph; transport to the salivary glands; uptake by the gland cells; and secretion via the saliva.

PURPOSE

To demonstrate the turnover of perorally ingested ^{32}P to the salivary glands and the time it requires, and thus a way by which pathogenic microorganisms may be transmitted from insect vectors to plants and animals.

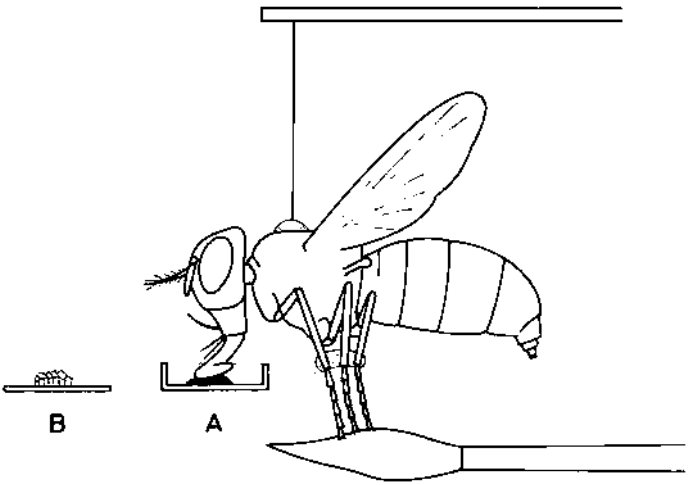

FIG. V--8. Experimental arrangement for working out exactly the time required for secretion via saliva of perorally ingested ^{32}P (after Ref.[26]*). For explanations see text.*

MATERIALS

(1) Flies of different species
(2) Device to fix the flies as for suspended flight
(3) Glue for gluing together the legs
(4) Solution of 20% sucrose with ^{32}P, specific activity ca. 0.5 mCi/ml (18.5 MBq/ml)
(5) Painting brush, non-radioactive sucrose solution
(6) Sugar granules to check for secretion of the tracer; the sugar should be glued on double-sticking Scotch tape on slides

PROCEDURE

Flies of different species, including *Musca domestica* L., are mounted as for 'suspended flight'. The legs are glued together to prevent contamination (Fig. V-8, Ref.[26]). The flies are fed with radioactively labelled sucrose (for activity see Table V-2). The 'tarsal reflex' (extension of the proboscis including the labellum) is elicited by touching the tarsa with a paint brush, moistened with non-radioactive sucrose solution (Fig. V-8 A). Thus the time of uptake of the radioactive food can be determined very exactly. By releasing the reflex in the same way, the labellum can be decontaminated carefully.

TABLE V-2. SECRETION OF PERORALLY INGESTED ^{32}P WITH THE SALIVA IN DIFFERENT SPECIES OF FLIES [27]

Species	Temperature (°C)	Composition of liquid food	Amount of ingested food	First radioactive secretion after:	No. of measured flies	Last radioactive secretion after:	No. of measured flies
Musca domestica L.	20–23	20% sucrose solution labelled with ^{32}P at a total specific activity of 0.3–0.5 mCi/ml (11.1–18.5 MBq/ml)	0.01–0.03 ml per fly	7 h	98	21 h	78
Calliphora vicina (R.-D.)	20–23			6 h	100	24 h	94
Callitroga macellaria (Fabr.)	28–30			8 h	96	20 h	81
Lucilia cuprina (Wiedemann)	28–30			7 h	93	21 h	79

Relative air humidity: 70% for all experiments.

After a certain time it is possible to check whether or not the flies already secrete radiophosphorus via the saliva. To dilute dry food, the flies secrete saliva; this behaviour is elicited by offering them sugar granules (Fig.V-8 B) which are withdrawn after a short time and checked for radioactivity. The tarsal reflex is released by touching again the tarsae with a paint brush moistened with inactive sugar solution. The results of a series of experiments are given in Table V-2 [27].

QUESTIONS

(1) Which are the steps in the turnover of radiotracers from peroral uptake to secretion with the saliva?
(2) What is the main object of this type of experiment?

V-3.7. Topical application and injection of aqueous ^{32}P phosphate or radioiodine for testing the penetration and excretion through the cuticle of insects

The cuticle of insects seems to allow the passage of substances from the inside to the outside (cuticular excretion) as well as from the outside to the inside. Advantage is taken of the latter property in the application of organosynthetic insecticides; these are soluble in the lipoids of the outer layers of the cuticle, which facilitates their penetration. From tracer experiments with aquatic or semi-liquid media (soils) we know that the cuticle of living insects is also permeable for inorganic substances. This is important for the osmoregulation of aquatic insects.

(For literature, see Ref.[28].)

PURPOSE

To study the permeability of the cuticle of non-aquatic insects to externally administered radioactive ^{32}P (as phosphate).

MATERIALS

(1) American cockroaches at different stages
(2) Cardboard, scissors, Scotch tape
(3) Calibrated syringe with a hypodermic needle for topical application
(4) ^{32}P as phosphate in aqueous solution, specific activity 0.1–0.5 mCi/ml (3.7–18.5 MBq/ml)
(5) Glass jars
(6) Microcapillaries

PROCEDURE

(a) Mounting of the cockroaches. Legs and antennae of the cockroaches are fixed on cardboard strips with Scotch tape. The insects remain in this position until the end of the experiment so that contamination of the cuticle is prevented.

(b) Topical application of the tracer. Avoid any other contamination of the cockroaches. In the middle of the pronotum apply a 3 μl drop (corresponding to .... mCi or .... MBq ^{32}P)[1] of the radioactive solution. Note the exact time and temperature.

After tracer application, the mounted cockroaches are placed in containers with a high relative humidity (provided by wet cellulose layers on the inside of the cover of the container).

(c) Checking of cuticle penetration of the tracer. After various time intervals (24 h, 48 h, 72 h, etc.) specimens of each series are checked for cuticle penetration of the tracer. If penetration occurs, we can expect the tracer to circulate with the haemolymph. Therefore, we pierce the cuticle between the coxae of the hind-legs with a calibrated capillary tube and soak up 2 μl haemolymph. Before this operation, the background activities of the capillary tube and the planchet have to be counted for 10 minutes. After soaking up the haemolymph, we measure again (for 10 minutes) for detection of radioactivity in the blood. Of course, the same experiment can be carried out with radioiodine or other tracers.

The experiment can also be modified for the detection of cuticular excretion, since radioactive substances which have been absorbed are excreted after some time. To prove this, the abdomen is wiped gently with soft cellulose which is then checked for radioactivity. The best way to check cuticular excretion is to inject some tracer solution into the haemolymph.

QUESTIONS

(1) What is the ecophysiological significance of the permeability of the cuticle to inorganic materials?
(2) By which experimental procedures can this permeability be demonstrated?

V-3.8. Scintillation detection of insecticide penetration through and into the cuticle of insects

Scintillation counters enable the scientist to selectively detect gamma, beta and alpha radiation and fast neutrons, even in the presence of heavy background

[1] Calculate the ^{32}P activity corresponding to the actual specific activity of the 3 μl drop administered.

counts. These instruments also give information on the energy spectrum of the radiation and operate effectively at extremely high count-rates. Liquid scintillation counting equipment is used. These two experiments require 4 to 6 hours each to complete.

PURPOSE

To detect the penetration of ^{3}H-labelled insecticide through and into the cuticle of insects.

MATERIALS

(1) ^{3}H-labelled insecticide in acetone solution
(2) Microapplicator
(3) Cockroaches
(4) Hot plate
(5) Beakers
(6) Test tubes
(7) Pipettes
(8) Dioxane cocktail
(9) Toluene cocktail
(10) 1.5M tris buffer (tris(hydroxymethyl)aminomethane)
(11) Colourless concentrated nitric acid
(12) Forceps and scissors
(13) Log-linear graph paper
(14) Scintillation counter

PROCEDURE

Experiment A. Penetration through the cuticle

Apply 1 μl of the labelled insecticide in acetone solution to the pronotum of each of 15 cockroaches. Remove the pronota from three cockroaches each at the following times after treatment: 1 min, 5 min, 30 min, 1 h and 2 h. When the pronotum has been excised, the inner thoracic tissue should be thoroughly removed and the pronotum dropped into a test tube containing 1 ml of nitric acid; place the test tube in boiling water on a hot plate. When the pronotum is dissolved, add 9 ml of water and allow to cool. After cooling remove 1 ml and add this to 18 ml of dioxane cocktail. To this add 1 ml of 1.5M tris buffer and count.

For controls (zero time) remove three pronota and proceed as above, except that 1 μl of ^{3}H-insecticide solution is added to the pronotum immediately before digestion. Count.

For the blank use 1 ml 10% nitric acid plus 18 ml of dioxane cocktail plus 1 ml 1.5M tris buffer.

When all counts are completed, plot the percentage of the insecticide remaining on the cuticle on the y-axis and the time on the x-axis of graph paper.

Experiment B. Penetration into the cuticle

Use the same dosage of ^{3}H-labelled insecticide solution, the same number of insects and the same time intervals as in Experiment A. Instead of removing the pronotum, carefully wash the pronotum into a test tube with 2 ml acetone, then add 18 ml of toluene cocktail and count.

For controls add 1 μl of ^{3}H-labelled insecticide to 2 ml of acetone and 1 ml of toluene cocktail and count.

For the blank add 2 ml of acetone to 18 ml of toluene cocktail and count. Plot on graph paper as in Experiment A.

QUESTIONS

(1) Is insecticide penetration through insect cuticle linear or exponential with respect to time?
(2) Do your data from each experiment support each other?
(3) What percentage of penetration could you expect after 12 and 24 hours by extrapolating your graphs?

V-3.9. Use of radiolabelled insecticides in studies of their metabolic fate

Numerous economically important insect pests are resistant to DDT and other insecticides. Studies on the absorption and metabolism of insecticides of various insects will help to understand some of the problems related to resistance. The time required to conduct this experiment is 4 to 6 hours.

PURPOSE

To study the metabolism of DDT in house-flies by means of paper and thin-layer chromatography.

MATERIALS

(1) ^{14}C-DDT, ^{14}C-aldrin and ^{14}C-dieldrin, with high activity
(2) Acetone
(3) Paper chromatography scanner

(4) Mechanical shaker
(5) Counting equipment
(6) Usual laboratory glass-ware
(7) Glass columns
(8) Glass wool
(9) Celite 545 (Johns Manville)
(10) Concentrated sulphuric acid
(11) Fuming sulphuric acid
(12) Carbon tetrachloride
(13) Sodium sulphate, anhydrous
(14) Diethyl ether
(15) Ethyl alcohol
(16) DDT resistant and susceptible house-flies
(17) Whatman No.1 filter paper; equipment for thin-layer chromatography
(18) Benzene
(19) Mortar and pestle
(20) N,N-Dimethylformamide
(21) Mineral oil
(22) Isooctane
(23) Soxhlet apparatus
(24) Micrometer-driven syringe

PROCEDURE

Experiment A. ^{14}*C-DDT*

Prepare a stock solution of ^{14}C-DDT in benzene or acetone and make proper dilutions as desired.

Apply topically to individual male or female flies the desired amount of insecticide in 1 μl of solvent.

At predetermined intervals, rinse the flies with three 15-ml portions of acetone to remove the unabsorbed DDT. Combine the rinses.

Grind the flies in a mortar with anhydrous sodium sulphate until a dry powder is obtained.

Extract the brei with diethyl ether, preferably in a Soxhlet apparatus for 3–4 hours.

Concentrate the extract in a water bath at 50–60°C, and prepare for radioassay and paper chromatography.

The extract is purified as follows. For each 1 g of flies (approximately 50 flies) weigh 5 g of Celite. Mix the Celite with 1.5 ml of concentrated H_2SO_4. Add CCl_4 and triturate until a homogeneous slurry is obtained. Place the slurry in a column in small portions and pack it tightly with a tamping rod. Adjust the flow rate to approximately 120 drops/min. Gently pour the concentrated

extract (in CCl_4) back into the column and allow the upper level to settle to within 2 mm of the absorbent. Then pour in CCl_4 until 100 ml has been collected in the receiving flask. The eluate can be concentrated and adjusted to volume for various analytical procedures.

Analysis

Paper chromatography [29]. Paper chromatography is the simplest, most rapid and cheapest method of estimating and identifying pesticides and their metabolites. However, it is neither the most sensitive nor the most precise.

For the analysis of DDT and DDT-metabolites in house-flies the following systems may be used (see also Ref.[30]):

	Solvent system I (mobile phase aqueous)	Solvent system II (non-aqueous)
Stationary phase	4% mineral oil in ether	35% dimethylformamide in ether
Mobile phase	3:1 ratio by volume of acetone: H_2O	Redistilled isooctane

Place a few microlitres of the radioactive extract in spots on strips or sheets of Whatman No.1 filter paper (or the equivalent). Make the spots as small as possible; allow to dry.

Dip the paper in stationary-phase liquid in such a manner that the liquid will not touch the spots of radioactive extract. Allow to dry for a few minutes. Place the paper in a chromatography chamber and allow the mobile-phase liquid to move up the paper to within 1–2 cm of the top. Remove the paper and allow to dry.

Cut the paper into 2.5 cm wide strips and scan in a radioactive scanner with recording attachment.

Determine R_f values and compare with known standards treated similarly.

$$R_f = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

DDT and DDT-metabolites may also be determined by thin-layer chromatography, as described below.

Experiment B. ^{14}C-aldrin

Proceed as outlined in Experiment A, up to the purification of the extract. Then continue as follows:

Purification of aldrin and dieldrin extracts

Method (1). Evaporate the extract to dryness in a round bottom flask. Add 3 ml of 50% aqueous KOH and 12 ml of 95% ethyl alcohol. Reflux for 1 hour. Cool to room temperature and transfer to a separatory funnel. Add 10 ml of distilled water and 50 ml of hexane and shake for 2–3 minutes. Allow the layers to separate.

Discard the aqueous phase. Wash the hexane with distilled water until neutral and discard the water. Wash with 10 ml of saturated NaCl or Na_2SO_4 to dry the hexane and discard the aqueous phase. Concentrate the hexane and prepare for chromatography.

Method (2). If the extract contains only small amounts of impurities, the latter may be removed by column chromatography.

To a glass column add 10 g of a mixture of 2 parts magnesite[2] and 1 part Celite-545 by weight. Saturate the absorbent with redistilled n-hexane. Add concentrated extract and elute with 150 ml of redistilled n-hexane. Concentrate the solvent and prepare for chromatography.

Analysis of ^{14}C-aldrin and ^{14}C-dieldrin

The same system of paper chromatography as outlined for ^{14}C-DDT may also be applied to aldrin and dieldrin.

Thin-layer chromatography. Prepare glass plates with Silica gel G or with Camag D-5 aluminium oxide. It is simpler to use commercially prepared Eastman Chromagram polyester sheets (Eastman Type K301 R_2, Silica gel G) or preparations of the same type or of a different one from other sources [31].

Apply spots of radioactive extract on the sheets, as for paper chromatography, and develop with redistilled n-hexane for the Eastman Chromagram. Determine R_f values as in Experiment A.

Thin-layer chromatography is easier and more convenient to use than paper chromatography since it requires no treatment with a stationary phase.

QUESTION

Why are the extracts purified by passing them through the Celite column before chromatography?

[2] Magnesite is a brand of magnesia (magnesium oxide) specially prepared for chromatography.

V-3.10. Metabolism of ^{14}C-malathion in the house-fly

The toxicity of insecticides to insects is controlled by several factors. Among these is the inherent toxicity of the particular compound. Important modifying factors are the rate of absorption of the compound by the insects and the insects' ability to defend themselves against the poison, i.e. the rate at which insects detoxify the material. Differences in the rates of absorption and degradation of a compound often account for differences in toxicity to different species or strains within a species. Experiments A, B and C are closely related and should be done simultaneously. The experiments will require 7 to 9 hours of work over a 2-day period.

PURPOSE

To study the metabolism of ^{14}C-malathion in malathion-susceptible and malathion-resistant house-flies. To measure the absorption rate and the rates of detoxification of malathion in vivo and in vitro, in flies treated with malathion alone and in flies pretreated with a malathion synergist.

MATERIALS

(1) ^{14}C-malathion with high activity
(2) DEF (S,S,S-tributylphosphorotrithioate), a malathion synergist
(3) A supply of adult female house-flies, 2–6 days old, of a malathion-susceptible and a malathion-resistant strain
(4) Micrometer-driven syringe (or other dosing device) equipped with 27- to 30-gauge needles for treating individual insects
(5) Mortar and pestle for grinding insects
(6) Chloroform
(7) Acetone
(8) Distilled water
(9) A clinical centrifuge equipped with 15-ml tubes
(10) Glass-ware
(11) Counting equipment
(12) Sand washed with acetone
(13) Filter paper

PROCEDURE

Experiment A. Absorption of malathion in the house-fly. Treat six groups of five flies each of both strains topically with 1 μg of malathion dissolved in acetone (concentration: 1 μg/μl). Use a 27-gauge needle. At 0, 5, 10,

20, 40 and 80 minutes after treatment, rinse a group of the treated flies with 5 ml of acetone. Count two 1-ml aliquots from each sample. Use the means of the counts from the two aliquots to plot absorption according to time.

Experiment B. Detoxification of malathion in vivo

(a) Treat 25 flies of each of both strains topically with 1 μg of DEF (concentration 1 μg/μl). One hour later inject 1 μg of ^{14}C-malathion into each of the flies, using a 30-gauge needle. At 0, 5, 10, 20 and 40 minutes after injection, grind groups of five flies in sand with a mortar and pestle and put them into a centrifuge tube. Add 5 ml of water and 5 ml of chloroform. Shake, centrifuge, and count two 1-ml aliquots of both the chloroform and the water by using a gas flow counter or dioxane cocktail and liquid scintillation counting equipment.

(b) Inject 1 μg of ^{14}C-malathion into 25 flies of each of both strains. At 0, 5, 10, 20 and 40 minutes after injection, grind the flies as above. Add chloroform and water, shake, centrifuge, and count two 1-ml aliquots of the chloroform and water layers as above.

Experiment C. Detoxification of malathion in vitro. Grind 10 flies of each strain with a mortar and pestle. Add 10 ml of water and filter. Pipette 1-ml aliquots of each strain into each of two centrifuge tubes containing 1 μg of ^{14}C-malathion. Add 5 ml of chloroform and 4 ml of water to one tube after 15 minutes and the same to the other tube after 30 minutes.

Pipette 2-ml aliquots of each strain into separate centrifuge tubes containing 2 μg of DEF. After 15 minutes pipette 1 ml from each tube into tubes containing 1 μg of ^{14}C-malathion. After further 15 minutes add 4 ml of water and 5 ml of chloroform.

To two centrifuge tubes containing 1 μg of ^{14}C-malathion add 5 ml of chloroform, 1 ml of fly extract and 4 ml of water.

Shake all tubes, centrifuge, remove two 1-ml samples from each of the chloroform and water layers, and count as in Experiment A.

QUESTIONS

(1) How long in minutes does it take for flies of the two strains to absorb half of the topically applied malathion?
(2) Do flies from each strain detoxify malathion in vivo? If so, what are the differences in detoxification rates and what is the effect of the malathion synergist? What practical advantage might this have?
(3) Compare the detoxification rates in vitro between the strains. Is there a difference? What is the effect of the malathion synergist?

V-3.11. Effect of temperature on the absorption of ^{14}C-DDT

It has been known for many years that house-flies will be killed faster by DDT when they are held at low temperatures than at high temperatures for several hours after treatment. It has also been shown that less DDT is actually absorbed at lower temperatures than at higher temperatures (21°C versus 32°C). This experiment requires 3-4 hours on each of two consecutive days to complete.

PURPOSE

To determine the amount of ^{14}C-DDT absorbed when house-flies are held at 21°C and 32°C after treatment.

MATERIALS

(1) ^{14}C-DDT with high activity
(2) House-flies for which approximately 5 μg of DDT is required to cause a 40–80% mortality
(3) Micrometer-driven syringe
(4) Twelve pint or quart jars fitted with screen-wire tops
(5) Two constant-temperature cabinets adjusted for temperatures of 21°C and 32°C respectively
(6) Ordinary insect cages and laboratory glass-ware
(7) Counting equipment

PROCEDURE

If the dosage of DDT necessary to kill approximately 50% of the flies is unknown, run a series of tests with non-radioactive DDT to determine this dosage. Prepare an acetone solution of ^{14}C-DDT so that each microlitre contains the LD_{50} dosage of DDT.

Treat 40 flies with an LD_{50} dosage of ^{14}C-DDT. Immediately after treatment place half of them (20) in a quart or pint screen-capped jar, which is then placed in a constant-temperature cabinet operating at 21°C.

Handle the other flies in the same manner but place the jar in a 32°C cabinet. At the same time discharge 1 μg of ^{14}C-DDT solution on each of three planchets, dry, and measure the count-rate (counts/min).

After 20–24 hours, separate live and dead flies from the jars held at the two different temperatures. Kill the live flies with chloroform. Keep the different lots of flies separate and well marked.

Rinse the external DDT from the lots of five flies from each of the four groups in 2–3 ml of acetone in a vial or test tube. Pour the acetone onto

planchets, mark, dry, and count. Macerate the five flies from each group in a mortar with 5 ml of acetone to extract the ^{14}C-DDT. Decant the acetone onto planchets and count.

Tabulate the amount of radioactive DDT absorbed in living and dead flies at each temperature.

QUESTIONS

(1) Is more DDT found in flies held at 32°C than in those held at 21°C? If so, explain why.
(2) How does the mortality of the flies compare at the two temperatures?

V-3.12. Radioassay of excised leaves to study the translocation of a systemic insecticide

This experiment demonstrates how a systemic insecticide can be studied by using radiolabelled material. It has been designed so that an insect bioassay can be made in conjunction with the radioassay. It can also be expanded to obtain precise metabolism data if techniques are available for separation of the metabolites. The experiment will require about 4 hours to complete.

PURPOSE

To measure the extent of translocation of a topically applied systemic insecticide in leaves.

MATERIALS

(1) ^{32}P-labelled systemic insecticides, such as Bidrin or Phosphamidon, preferably with a specific activity greater than 5 mCi/mmol (185 MBq/mmol)
(2) Potted plants (beans, peas, cotton, etc.) with fully expanded leaves
(3) Micropipettes or other apparatus for accurately measuring 5–10 μl
(4) Radioassay equipment
(5) Aphids and aphid leaf-cages

PROCEDURE

Prepare a water solution or an emulsion of the labelled insecticide so that 1 μl of the final solution contains 1 μg of the insecticide. Apply 5–15 μl of the solution or emulsion to a 1–2 cm^2 spot between the major leaf veins of a leaf. Treat several leaves. Retain the plant under normal growing conditions for 2–4 days.

To estimate the amount of translocation, either an autoradiograph may be prepared or direct radioassay may be used. For the autoradiograph remove the treated leaf from the plant and expose it to X-ray film for several days in a deep-freezer. For direct radioassay, cut out the treated area of the leaf and several equal-size parts round the treated area. Radioassay these pieces of leaf directly. Draw a picture of the leaf and record on the picture the radioactivity associated with each radioassayed piece.

An insect bioassay can be made in conjunction with the radioassay. Phloem-sucking aphids *(Aphididae)* can be enclosed in leaf-cages at different spots. Check for toxic effects and for radioactivity ingested with the labelled insecticide.

QUESTIONS

(1) What path has the systemic insecticide followed in the leaf?
(2) How far was the insecticide translocated from the point of application? Assuming proportionality, what is the rate of translocation per unit time?

V-3.13. Radioassay of leaf extracts to study the metabolism of a systemic insecticide

This experiment demonstrates how the metabolism of a systemic insecticide can be studied by using radiolabelled material. It has been designed so that an insect bioassay can be made in conjunction with the radioassay. It can be expanded to obtain precise metabolism data if techniques are available for separation of the metabolites. The experiment requires several hours over a 7-day period to complete.

PURPOSE

To study the metabolism of a systemic insecticide in plant tissue.

MATERIALS

(1) ^{32}P-labelled systemic insecticides, such as Bidrin or Phosphamidon, preferably with a specific activity greater than 5 mCi/mmol (185 MBq/mmol)
(2) Radioassay equipment
(3) Plants (beans, peas, cotton, etc.) with fully expanded leaves
(4) Small vials or glass tubing sealed at one end, about 4 mm inside diameter and 5 cm long
(5) Mortar and pestle
(6) Clean, washed sand

(7) Chloroform
(8) Separatory funnel
(9) Centrifuge and centrifuge tubes

PROCEDURE

Prepare a water solution or an emulsion of the labelled insecticide so that 1 μl of the final solution contains 1 μg of the insecticide. Place 100 μl of this solution in each of the small vials. Cut several leaves from the plant, keeping the petioles under water. The petioles should be about 2 cm longer than the vials. Place the petioles of these leaves in the vials containing the solution of labelled insecticide. Place the leaves under a lamp so that they take up the solution. After all the solution has been taken up, transfer the leaves to clean water in a beaker or Erlenmeyer flask.

At 0, 1, 2, 4 and 7 days after treatment, grind one or two leaves with sand and water in the mortar. Place the homogenates in a separatory funnel with equal volumes of chloroform and water. Shake for 1–2 minutes. Allow the water and chloroform to separate. If they do not separate readily, place the liquid in centrifuge tubes and centrifuge until separation is achieved.

Radioassay aliquots of water and chloroform phases after separation. Usually, the water phase will contain the non-toxic hydrolytic metabolites and the chloroform will contain the parent compound and toxic metabolites. Loss from volatilization may be reduced by adding 10–25 mg of cooking oil to each planchet.

Draw a curve showing the decomposition of the parent compound to non-toxic hydrolytic products.

QUESTIONS

(1) What sort of curve (exponential, linear) is obtained? Explain why.
(2) Is the decomposition rate greatest during any period? If so, to what part of the curve does that period correspond?. Or is the decomposition rate constant?

V-3.14. Comparative study of absorption and translocation of systemic insecticides by plants following soil application

This experiment demonstrates how absorption and translocation of two or more labelled systemic insecticides may be studied simultaneously in plants. It has been designed so that an insect bioassay can be made in conjunction with

the radioassay. It can be expanded to obtain precise metabolism data if techniques are available for separation of the metabolites. Each part of the experiment can be completed in several hours over a 7-day period.

PURPOSE

To compare the systemic action of two or more systemic insecticides applied to the soil.

MATERIALS

(1) Two or more ^{32}P-labelled systemic insecticides, such as Bidrin and Phosphamidon, with specific activities of 1 mCi/mmol (37 MBq/mmol) or higher.
(2) Potted plants (beans, peas, cotton, etc.) of uniform size growing in soil
(3) Oven
(4) Laboratory grinder or mortar and pestle

PROCEDURE

Prepare water solutions or emulsions of each labelled insecticide. Either pour the desired amount on top of the soil around each plant or use a hypodermic syringe to inject the materials below the soil surface. Place the potted plants in a glass tray, pie plate or some other container so that leakage will not contaminate the laboratory or greenhouse.

One, 2, 4 and 7 days after treatment, harvest at least three plants treated with each insecticide. Remove all leaves from each plant, weigh, place in a small paper sack and dry at 40--50°C for 24—48 hours. Keep the leaves from each plant separate. Obtain the dry weight.

After all plants have been harvested and dried, grind the leaf tissue in a laboratory grinder or in a mortar. Weigh two 75—125 mg aliquots of each dried, ground leaf tissue sample and radioassay.

Express the results as follows:

(a) In counts/min per milligram dry weight
(b) In counts/min per milligram fresh weight
(c) In μg-equivalents per milligram dry weight
(d) In μg-equivalents per milligram fresh weight
(e) In ppm (μg-equivalents per gram fresh weight)
(f) In percentage of applied dose recovered

QUESTIONS

(1) For any of the insecticides, which measurement is more meaningful from the practical standpoint, in counts/min per dry weight or in counts/min per fresh weight, and why?
(2) Also, which measurement is more meaningful, in μg-equivalents per milligram dry weight or μg-equivalents per milligram fresh weight?
(3) What is the relationship between the above two kinds of measurements?
(4) Which of the absorbed insecticides takes longest to reach the 50% degradation level?

V-3.15. Speed of absorption and elimination of labelled (^{32}P) food in larvae of *Trichoplusia ni* (Hbn.) (Lepidoptera, Noctuidae)

The speed of food passage through the insect's alimentary tract can be measured very well with radiolabelled food. In this regard there are big differences between the different nutritional types of insects. The so-called continuous feeders, e.g. caterpillars of the cabbage looper *Trichoplusia ni* (Hbn.) (Lepidoptera, Noctuidae), have a high speed of food passage. These larvae are becoming more and more preferred objects of research in insect physiology. One of the main reasons for this is that they can be bred on semi-artificial diets.

PURPOSE

To demonstrate the speed of normal food passage through the gut (see Fig.V-1, λ_7) and especially to check the transfer of the food through quick absorption into the haemolymph to the Malpighian tubules and the excretion through these (compare Fig.V-1, λ_6).

MATERIALS

(1) Cabbage looper larvae
(2) Cabbage looper diet such as dry powder (for composition, see Ref. [11])
(3) Small plastic beakers; stand to hang these over a rotating disc
(4) Synchronous clock
(5) Cardboard disc
(6) Tangle-foot® glue
(7) Radiophosphate
(8) Decontamination fluids
(9) Several jewellers' forceps for preparation
(10) Capillary tubes for haemolymph samples
(11) Laboratory glass-ware
(12) Graph paper
(13) Stop-watch

PROCEDURE

The cabbage looper diet contains crushed pinto beans, dried Torula yeast, wheat-germ, ascorbic acid, methyl-p-hydroxy-benzoate, sorbic acid, H.W.G.[3], formaldehyde 40%, vitamin mixture, tetracycline, water. The cabbage looper diet must be mixed and 'cooked'. While putting some of the hot prepared diet into a beaker, a small amount of radiophosphate is added and thoroughly mixed with the diet. The beaker is fixed, hanging (upside-down) over a prepared disc, divided into time sectors and smeared with 'tangle-foot' glue. The caterpillar is fixed with the prolegs on a horizontal insect pin or piece of wire. The caterpillar remains only one single minute feeding on the radioactive diet (record the time carefully, using a stop-watch) and is then transferred to non-radioactive food. Note the temperature. To demonstrate the fast absorption of the food (70 s were recorded by Ru and Kloft [11]), haemolymph samples are taken and checked for radioactivity. Plot the count-rate (counts/min) of the faecal pellets against time in minutes.

QUESTIONS

(1) How many minutes after feeding started were the first radioactive pellets dropped?
(2) How can we distinguish between the first elimination of ^{32}P via excretion through the Malpighian tubules and the radioactivity eliminated after normal passage through the gut?
(3) After what time is the first radioactivity found circulating in the haemolymph?
(4) What time is required to eliminate most of the ingested radioactivity?

V-3.16. Determination of the speed of haemolymph circulation in insects by injected tracers

The circulation rate of body fluid in insects and the time needed for an introduced material to become homogeneously mixed with it are important factors in certain studies of insect physiology and toxicology. Since insects have an open circulatory system, the time required for uniform mixing of an introduced substance may be a more appropriate term than the time for a complete circuit of any portion of the haemolymph. The introduction of labelled phosphorus allows to determine with simple methods this mixing time, even in small insects.

[3] H.W.G. is a protein formula which is used in US laboratories like the USDA.

PURPOSE

To demonstrate haemolymph circulation in insects by the use of radioactive tracers.

MATERIALS

(1) Adult American cockroaches, 2—3 specimens per group
(2) Insect Ringer solution; special composition for cockroaches:

NaCl	4 g
KCl	0.7 g
$CaCl_2$	0.7 g
H_2O	500 ml

(3) Add to a small amount of Ringer solution (enough for filling the microsyringe) ^{32}P to obtain a specific activity of 0.05 and 0.1 mCi/ml (1.85 and 3.7 MBq/ml)
(4) Microsyringe with hypodermic needle No.27, silane (or any other silicon) for preventing adhesion of watery fluids to the injection needle
(5) Carbon dioxide for immobilizing the cockroaches during injection
(6) Cardboard; Scotch tape
(7) Lead sheet pieces, 2 mm thick, or any other shielding

PROCEDURE

(a) Mounting the cockroaches. The cockroaches are fixed on cardboard strips with Scotch tape. They remain in this position until the end of the experiment.

(b) Injection of ^{32}P and measurement. The labelled Ringer solution is injected through the dorsal side of the abdomen, near the posterior end of the heart (dorsal vessel). The tracer can be expected to reach first the wings, then the antennae, the mouth-parts and last the thoracic legs in their order, i.e. the first, the second and then the third pair. If we protect the injected insect body with lead sheet (absorbing totally the beta particles of ^{32}P) and fit only one unprotected appendage (antenna, leg) under a detecting system (GM tube with rate meter and recorder), we can record directly, by a more or less sharp peak, the time when the tracer reaches the appendage. The time until the activity decreases to a constant is the time needed for uniform mixing of the injected tracer with the haemolymph (a double peak can be expected).

If we have no self-recording system, we can take digital counts for one minute every third minute after injection (fore-wing or antenna). Note the count-rate carefully and plot it on graph paper in counts/min against time after injection in minutes.

Another method for a rough estimation of these data is that of Craig and Olson [32]. At various times after injection, an appendage (leg, antenna or wing) is cut off and the amount of radiophosphorus present in it is determined with a GM counter. After a *long time,* to ensure that complete mixing of radiophosphorus in the blood has taken place, the corresponding appendage on the other side of the insect is removed and its radiophosphorus content determined. If the first appendage of a pair has less radiophosphorus than the second, the former is presumed to have been cut off before the injected solution was evenly mixed with the body fluid. If an equal amount of radioactivity is found in the two appendages, the distribution of the radiophosphorus is considered to have been completed before the first appendage was removed. It is shown that the radiophosphorus reaches corresponding appendages on each side of the insect at essentially the same time.

QUESTION

How can we find out if the injected tracer is completely mixed with the haemolymph?

V-3.17. Measurement of the biological half-life of radiotracers in insects

The biological half-life is the time required for the loss of half the amount of an ingested substance. It is primarily dependent on the rate of excretion. The biological half-life (T_{biol}) cannot be measured directly. For a radioactive substance it can be computed by measuring the effective half-life (T_{eff}), i.e. the effective decrease of the count-rate of a radioactive substance (in a time unit) owing to both biological and physical decay of radioactivity (Fig.V-9). This can be expressed as:

$$\frac{1}{T_{eff}} = \frac{1}{T_{phys}} + \frac{1}{T_{biol}}$$

The effective decrease in the activity of a radioactive substance follows an exponential law, as does the physical and biological decrease of the count-rate. Since the physical half-life of a radioisotope is known, the biological half-life can be calculated after having graphically obtained the effective half-life (T_{eff}) from the following formula:

$$T_{biol} = \frac{1}{\frac{1}{T_{eff}} - \frac{1}{T_{phys}}} \quad \text{or} \quad T_{biol} = \frac{T_{eff} \times T_{phys}}{T_{phys} - T_{eff}}$$

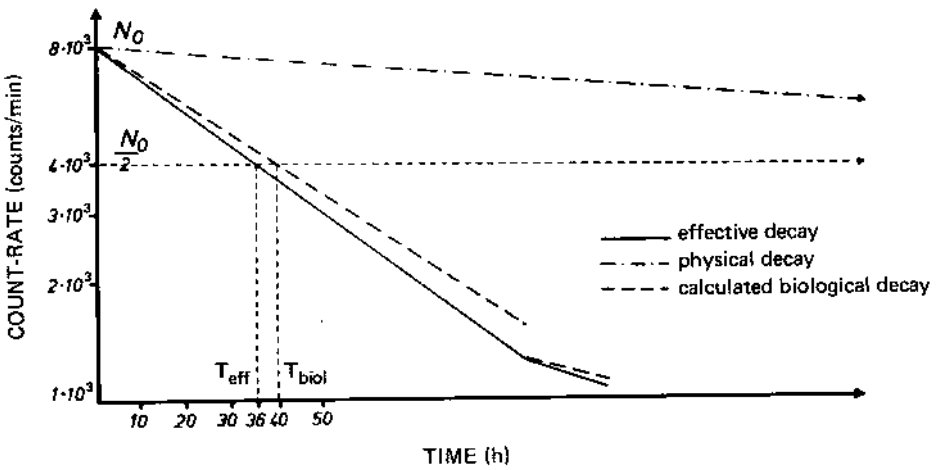

FIG. V–9. Relationship between effective, physical and biological decay of radioactivity in an imago of Megoura viciae *Buckt.* [22]. *The half-lives,* T_{eff} *and* T_{biol}, *were found graphically.* N_0 = *initial activity of the aphid.*

Excretion being a physiological process, the biological half-life of radionuclides depends on the temperature and follows the Q_{10}-law (except for some substances which are stored, e.g. radioiodine, some of which remains in the cuticle, especially of newly moulted individuals). Effective and biological half-lives can have different phases (Fig. V-10).

For literature see Ref. [33].

PURPOSE

To determine the biological half-life of ^{32}P and ^{131}I in cockroaches with emphasis on temperature dependence.

MATERIALS

(1) Medicine droppers
(2) Pipettes
(3) 1/4-ml syringe
(4) ^{32}P
(5) ^{131}I
(6) Petri dishes

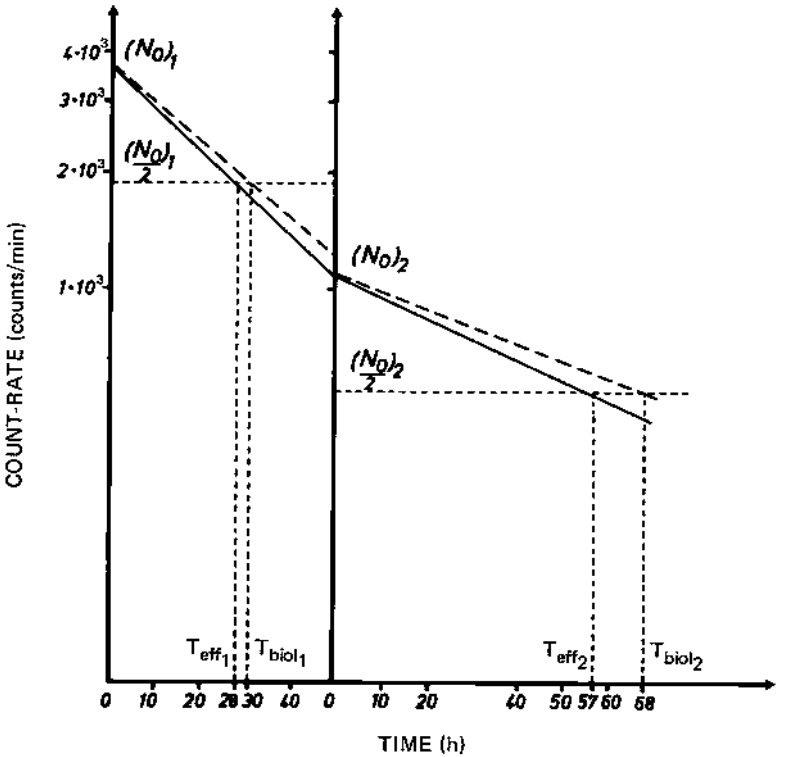

FIG. V–10. Change in the biological half-life of ^{32}P in a larva of Megoura viciae *Buckt. The effective and biological half-lives change, owing to the rapid elimination of radioactivity through the gut in the first phase* [23].
$(N_0)_1$ – initial activity for the first phase, $(N_0)_2$ – initial activity for the second phase.

(7) Gelatin capsules to hold cockroaches firmly
(8) Sucrose solution
(9) Counting equipment
(10) Glass slides
(11) Ordinary laboratory glass-ware
(12) Carbon dioxide for anaesthetizing insects
(13) Sodium phosphate
(14) Incubators set at two different temperatures
(15) Log-linear graph paper

PROCEDURE

Anaesthetize the cockroaches with CO_2 and fix them on glass slides with the dorsum next to the glass. Scotch tape is satisfactory for this purpose. As the cockroaches are recovering from the CO_2, feed them ^{32}P or ^{131}I in sucrose solution with a medicine dropper with a fine tip. The insects will readily imbibe sufficient amounts of the solution. The specific activity of both preparations should be about 0.5 mCi/ml (18.5 MBq/ml). The cockroaches may become contaminated externally during the feeding process and must be decontaminated. Decontaminate by washing freely, first with a 1% solution of sodium phosphate in water containing a detergent, and then with pure water. Allow the insects to dry.

Radiation measurements should be taken soon after feeding and repeated 2, 24, 48, 72 and 96 hours later. Measure ^{32}P with a GM tube and ^{131}I with scintillation counters. Each measurement must be taken under the same geometric and physical conditions. Confining the cockroaches in a small gelatine capsule is a good way to maintain the same geometry for different measurements.

Keep the cockroaches isolated in Petri dishes within incubators set at different temperatures (if possible, with a difference of 10 deg C). Renew the filter paper in the dishes daily and provide food and water. The excrements also have to be measured for radioactivity to obtain values for passage through the alimentary canal. Wipe the pronota after 2–3 days with filter paper and measure for cuticular excretion.

Plot the results on log-linear graph paper (log of count-rate (counts/min) against time in days); determine T_{eff} graphically. Calculate T_{biol} for the different time units.

QUESTIONS

(1) Are there any differences in the biological half-lives of the ^{32}P and ^{131}I solutions?
(2) If there are differences, to what can they be attributed?
(3) If there is a significant temperature dependence of the biological half-life, what can be concluded?

V-3.18. Autoradiography and scintigraphy of insects

Photographic emulsions are acted upon by radiation in a manner similar to light and forming a similar latent image. Ionizing radiation interacts with the photographic emulsion to provide electrons, leading to the reduction of silver halides to metallic silver, which acts as a catalyst for further reduction of silver halides in its immediate vicinity during development.

In autoradiography the tissue section chromatograph or any other biological sample containing a radioactive substance is placed in close contact with a photographic emulsion. After a given period of exposure the film is developed, and the precise location of radioactivity in the sample can be determined by the darkening pattern on the film. According to Smittle and Labreque [34], Polaroid film can be used under field conditions; in this case there is no need for any developing equipment.

PURPOSE

To illustrate by two different laboratory experiments the use of autoradiography as an entomological technique.

In experiment B the distribution of ^{32}P in the wings of cockroaches is measured using a scanner for the beta emissions. After that the wings are exposed to X-ray film and both results compared.

MATERIALS

(1) A water-soluble compound containing a beta-emitting isotope (preferably $H_3{}^{32}PO_4$)
(2) Two lots of about 500 adult house-flies that have been starved for 10—12 hours
(3) Sucrose solution (about 10%)
(4) X-ray sensitive film (Eastman, no-screen)
(5) Glass-ware
(6) Cages
(7) Cellulose tape
(8) Glass plates of the same size as X-ray film or slightly larger
(9) Forceps
(10) Adult cockroaches, e.g. *Blaberus trapezoideus* Burm.
(11) Cardboard, household wrap, Scotch tape, contact glue
(12) Micrometer syringe
(13) CO_2, chloroform
(14) Scanner (for example Berthold-scanner II) with printer, paper, etc.
(15) Incubator

PROCEDURE

Experiment A. Add the labelled compound to the sucrose solution in an amount to give 10^6 counts/min per ml. (Approximately 1 ml for each batch of 100 flies will be required.)

Place radioactive food in cages for 1 hour. At the end of the exposure period remove the food from both cages. Kill the flies in one cage by freezing and place 10% sucrose in the other cage.

Place eight lots of 25 flies each, with the dorsum down, on the sticky surface of a cellulose tape. Arrange the flies so that they will contact the X-ray sensitive film. Fix the flies' sternum to the film with tape. Place a glass plate upon the flies to assure good contact. Do not move the film or glass plate.

At the end of 1, 3, 6 and 24 hours remove two lots of flies from the contact with the X-ray film. Develop the film as convenient.

Kill the flies of the second cage 24 hours after exposure and repeat the exposure technique as above.

Experiment B. Fix the cockroaches on cardboard strips as described in Section V-3.7. Inject 2 μl ^{32}P (specific activity 5 mCi/ml or 185 MBq/ml) into the abdomen. Incubate at 20°C for 15 hours. Kill the cockroaches with chloroform, cut the four wings and mount these on foil. For one hour the wings should undergo thermal coagulation of the haemolymph and drying at 60°C. Subsequently they are covered with a thin household wrap and scanned. After obtaining the scintigram, the wings are put on X-ray film (three hours for the amount of radiophosphorus mentioned). Finally, process and dry. The times indicated are tested several times for the specific activity mentioned.

QUESTIONS

(1) Which exposure period gave the clearest resolution (Experiment A)?
(2) Was the darkening of the film linear or exponential with respect to time?
(3) Had the cockroaches fed uniformly?
(4) Did the cockroaches that were killed 24 hours after feeding on radioactive food have as much radioactivity as those killed immediately? Why? Which cockroaches had the best distribution of radioactivity?
(5) Compare the scintigram with the autoradiogram: which procedure gives better, faster and more distinct results?
(6) Are there any differences in radioactivity distribution between fore-wings and hind-wings?

V-3.19. Tracer experiments with aphids

Plant-sucking insects, especially aphids feeding on phloem sap, are of great importance in plant pathology. A basic knowledge on the nutritional physiology of aphids is necessary to learn more about the host-plant/insect relationships,

the mode of virus vectoring and control with systemic insecticides. Considering the small size of most of the species and the small amounts of food they take up and saliva they inject, the use of radiotracers is of great advantage.

PURPOSE

A. Demonstration of the time required by aphids to reach the phloem and start feeding.
B. Differentiation between host and non-host plants.
C. Deposition of saliva into the host plant during piercing and sucking.
D. Demonstration of food uptake through artificial membranes and of feeding preferences for different diets.
E. Determination of the beginning of food uptake of a phloem-feeding aphid using a radioactive host plant.
F. Use of double membranes to check the repellent effect of an insecticide and the attractant or repellent effects of some plant surface substances.
G. Indirect transfer of substances from aphid to aphid via the host plant.
H. Transfer of ingested radioactively labelled substances from virgin females to their larvae.

MATERIALS

(1) Two species of aphids (Aphididae): a good mass rearing with predominantly apterous virgins (keep host plant under long-day conditions, i.e. 13 hours light or more). *Myzus persicae* Sulz. (if available) and *Aphis craccivora* Koch (the 'black cowpea aphid') are suitable
(2) Small cages (glass rings) for artificial feeding
(3) Parafilm (product of Marathon, Inc., Menasha, Wisconsin, USA, or Lindsay and Williams, Ltd., London, UK) for making artificial membranes.
(4) ^{32}P as phosphate
(5) Chemicals for artificial diet (see Experiment D)
(6) Fine paint brushes
(7) Thin cellophane wrap
(8) Scissors, tweezers, glass-ware

PROCEDURE

The various methods used in radioisotope research with aphids are shown in Fig. V-11 [35].

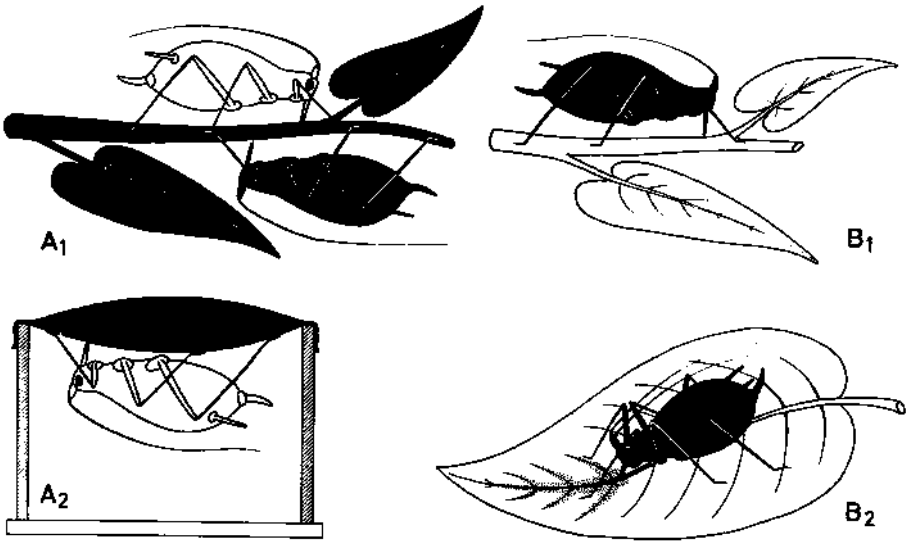

FIG. V–11. Schematic summary of radioisotope methods used [35].
A_1 – Non-labelled aphids (white) are transferred to a radioactive plant (black).
A_2 – Aphids feeding on an artificial diet: the radioactively labelled diet (black) is enclosed between two membranes of Parafilm®, stretched over a glass ring to form an aphid cage.
B_1 – Labelled aphid (black) is transferred to a non-radioactive plant (white).
B_2 – Radioactive aphid injects labelled saliva into a non-radioactive leaf. The radioactivity disperses in the leaf (dotted area), primarily along the veins.

Experiment A. Time required for food uptake. About 10–20 aphids are transferred with a fine camel-hair brush to a young sprout or seedling of tobacco (for *M. persicae*), or of cowpea (for *A. craccivora*); the plants should be radioactive for 24 hours. Check the aphids individually for radioactivity, after 30 minutes (first group), after 1 hour (second group), after 3 hours (third group), and after 6 hours (fourth group). Plot the number of radioactive individuals against time.

To save radioactivity, the same radioactive plant is used by the different groups.

Experiment B. Differentiation between host and non-host plants. Use the same radioactive plants as in Experiment A. Put at the same time and on the same leaf of tobacco or cowpea plants 5–10 individuals of both aphid species.

Observe the behaviour of the aphids. After 3 hours, check individually the radioactivity of the specimens and compare both groups. According to data from several species, aphids have first to obtain a good 'gulp' of the sieve-tube sap before they can differentiate between host and non-host plants. This means that, at first, radioactivity reaches about the same level in both groups, but later it increases in the species feeding on its host plant. (See also Ref.[36].)

Experiment C. Deposition of saliva. Aphids fed for about 20–24 hours on radioactive plants are transferred to non-radioactive host plants. For external decontamination ('self-cleaning') of the mouth-parts, induce the aphids to pierce 1–2 times the non-radioactive plants before the salivation experiments are started. Put the radioactive aphids on new leaves. Observe exactly the piercing behaviour, note the time and course of piercing and mark the piercing point by a colour spot on the opposite side of the leaf. Avoid contamination by honeydew (observation). After one or more hours, remove the aphids.

There are two possibilities of showing the deposition of radioactive saliva: 1. Cut out the part of the leaf on which the aphid has settled down (use a cork borer). Put the pieces in water in liquid scintillation counting vials. Measure the 'Cerenkov effect' (to save chemicals!) using the ^{3}H-channel of Tricarb. 2. Cut off the whole leaves immediately (or after some time, thus making it possible to observe saliva translocation); freeze-dry them (or use the quicker and cheaper, but rough, method of heat-fixation at 60°C for one hour) and expose for 14 days on X-ray film for autoradiography. In each case use only highly radioactive aphids. For checking, they can be fixed for a short time in a small droplet of water.

(See also Refs [37, 38].)

Experiment D. Food uptake through artificial membranes.

1. Preparation of artificial diet:

(a)	**Amino acids**	
	Alanine	100 mg
	Asparagine	300 mg
	Glutamine	600 mg
	Leucine	200 mg
	Methionine	100 mg
	Valine	200 mg
(b)	**Sucrose**	20 g
(c)	**Water**	100 ml

2. Preparation of feeding cages. Parafilm is stretched and fixed over a cover ring. In the middle of this membrane drop a little radioactive 'diet' (about 0.15 ml/ring). Cover this membrane carefully with a second membrane of stretched Parafilm thus spreading out the fluid. Make the fluid-containing

'pillow' tight by pressing the membranes with warm fingers along the periphery of the ring. Outflow of liquid and contamination must be avoided (try the technique first with non-radioactive fluids).

3. Transfer of aphids and measurement. Line the bottom of each of three feeding cages with a disc of cellophane to collect radioactive honeydew. Put 20–30 aphids inside the cages with (a) complete diet, (b) 20% aqueous sucrose (for comparison), and (c) plain water. Note the time and temperature.

Measure the radioactivity of the living aphids individually, and compare the results for the three different 'diets'. The aphids can be fixed during the measuring time in a droplet of water in the centre of the planchet (transfer gently with the wet tip of a camel hair brush, laying the insects on the dorsum). Good results can be obtained by lightly squeezing the aphids in water and measuring with a liquid scintillation counter (Cerenkov effect!).

The rates of radioactivity measured in parallel for the various diets at suitable time intervals give an information on feeding preference. As can be seen, in short-time experiments there is no difference between the full diet and the aqueous sucrose [38].

The first detection of radioactivity from honeydew on the cellophane discs determines the passage time through the alimentary tract as well as the food uptake.

Experiment E. Determination of the beginning of food uptake of phloem-feeding aphids using a radioactive host plant. Because of the function of the stylets and the anatomy of the host plant, some time is needed before the aphids reach the phloem and can begin with food uptake. The tracer method is excellent for monitoring this phenomenon. In our experience (Ehrhardt [36, 39]; Kloft and Ehrhardt [23]; Kunkel [40]), the minimum time required is 10 minutes. However, about six hours are needed for 100% of the aphids to reach the phloem and become radioactive.

Put a large group of aphids on a radioactive host plant and check after different time intervals (5, 10, 15, 20, 40, 60, 80 min, etc.) whether or not they have become radioactive. Plot the number (or percentage) of radioactive aphids against time (in minutes) elapsed since externally visible feeding started. Note the temperature and air humidity.

Experiment F. Use of double membranes to check the repellent effect of an insecticide and the attractant or repellent effects of some plant surface substances. Artificial diets are advantageous for checking the aphicidal effect of insecticides, since no plant metabolic effects are involved. The addition of a tracer or the use of a radioactively labelled insecticide allows to work out relations between dose (concentration), amount of ingested fluid and insecticidal effect. A double labelling technique (carbon-14 labelled insecticide, tritiated water) proved excellent for estimating the absolute uptake of insecticides and liquids by aphids [41].

Two-compartment experiments with double stretched Parafilm® membranes are particularly valuable. Prepare at first a normal sachet of two membranes, filled with diet. After that, put on the upper (outer) membrane another drop of liquid diet and stretch over this a third membrane. Thus we get two sachets which may contain different diets. However, the diets should have the same pH-value to avoid pH-effects. One diet may contain an attractant or a repellent. The sequence (upper or lower sachet) of the different diets may be changed from one experiment to another, but the upper sachet is always the one labelled with ^{32}P. Thus, the aphids feeding underneath can become radioactive only if they pierce through the lower sachet and reach the upper one containing the radiotracer.

Experiment G. Indirect transfer of substances from aphid to aphid via the host plant. Aphids can inject radioactive saliva into the plants (see Experiment C). It is possible that initially non-radioactive aphids ingest from the host plant radiotracers deposited by radioactive aphids [42].

Ten highly radioactive (^{32}P) aphids (check individually) are enclosed in a leaf cage; another cage contains non-radioactive aphids. The two cages are placed either on opposite sides of a plant or at some distance from one another. After a certain time (2—4 days) the unlabelled aphids are checked. The use of a liquid scintillation spectrometer provides a higher efficiency.

Experiment H. Transfer of ingested radioactively labelled substances from virgin females to their larvae. Radioactive substances ingested by virginoparous females are transferred to their larvae and these are radioactive when born (see Fig. V-1, λ_5). It is even possible to demonstrate the very high speed of radioactivity transfer within the ovary to unborn embryos (only 1 hour after the Virginopara start feeding on radioactive ^{32}P-diet).

Put adult virgins on radioactive diet and record their whole-body activity after 1 hour feeding time (in other cases after 2, 4, etc. hours). Immediately thereafter, extract with sharp-pointed jewellers' forceps the first unborn larva from its mother's abdomen. Decontaminate the extracted larva carefully from outside (see Section V-3.1) and measure its activity. After 2, 4, etc. hours, proceed likewise with the remaining larvae. By this technique, the radioactivity of the unborn larvae can be related to the original total radioactivity of their mother.

For literature see also Refs [43, 44].

QUESTIONS

(1) How can one demonstrate the time required by aphids to reach the phloem?
(2) Why do phloem-sucking aphids need a certain time to reach the phloem?
(3) How can aphids discriminate between host and non-host plants?
(4) What is the phytopathological significance of the deposition of saliva while piercing and feeding?

(5) How can attractant or repellent effects be demonstrated in two-compartment experiments?
(6) At what rate do aphids transfer organic substances to unborn embryos? Of what importance is this in determining the various aphid morphological types?

V-3.20. Laboratory-simulated radioecological experiments – Determination of nectar preference of mosquitoes

It is well known that most mosquitoes, and particularly the males, require the nectar or exudates of plants to sustain life. The knowledge of host preferences for a certain species of mosquito could lead to the control of this species by controlling its host plant. Radioisotopes provide an excellent tool to determine the preference of a certain species of mosquito for certain plant species.

PURPOSE

To determine the host preference for any locally available species of mosquito.

MATERIALS

(1) Several cages of 12 in × 12 in × 12 in (~ 30 cm cube) or larger
(2) Wild or laboratory-cultured species of mosquito (newly emerged unfed adults)
(3) Several species of wild flowering plants
(4) Several beakers or jars
(5) ^{32}P-phosphoric acid (or radiophosphate)
(6) Radiation counter or radiographic film

PROCEDURE

Twenty-four hours before the start of the actual experiment, each species of potential host plant should be placed in a dilute solution of $H_3{}^{32}PO_4$. Provision should be made to prevent mosquitoes from coming in contact with the ^{32}P solution.

After the 24-hour period the potential host plants are placed in cages containing 100 or more mosquitoes. Water should be provided for the mosquitoes. After a further 18–24 hours the potential host plants should be removed and the mosquitoes killed by heat or cold. The mosquitoes should then be exposed to a laboratory counter or radiographic film. If radioactivity is detected, the mosquitoes should be grouped by sex and the radioactivity determined for each sex. If no radioactivity is detected, the mosquitoes have not fed on the host plant.

QUESTIONS

(1) What conclusion can be reached from this experiment?
(2) Were both sexes radioactive?
(3) Were all specimens of either sex radioactive?

V-3.21. Laboratory-simulated radioecological experiments — Determination of movement of subterranean insects

Radioisotopes provide the easiest and the most accurate method of detecting and measuring the movement of insects which spend relatively long periods completely below the soil surface. The determination of the movement of these insects is important for the better understanding of their ecology and could provide useful information for the control of subterranean insects of economic importance.

PURPOSE

To determine the movements of mole crickets (or other species such as wireworms, corn rootworms, etc.), and to plot these movements on graph paper.

MATERIALS

(1) One large shallow tray, 18 in X 30 in X 3 in (~ 45 cm X 75 cm X 7.5 cm), of wood or metal
(2) Good-quality top soil with high moisture content
(3) Fine ^{60}Co wire or a water-soluble ^{60}Co salt; the activity of ^{60}Co should be sufficient to penetrate 3 in (7.6 cm) of soil, i.e. about 0.05 mCi (1.85 MBq) per millimetre of wire
(4) Cellulose tape
(5) Ball of string or fine wire
(6) Portable counter
(7) Graph paper
(8) Small beakers, pipettes
(9) Forceps, scissors, etc.

PROCEDURE

Fill the tray with soil and make a grid over the tray with either string or wire. Place ^{60}Co wire into the abdominal cavity of the test insect (see Fig.V-2(b))

or put a drop of ^{60}Co salt solution on the adhesive side of a small piece of cellulose tape and allow the solvent to evaporate. Place the tape under the wing of the mole cricket.

The tagged insect should then be placed on the surface of the soil at any particular location relative to the grid. The location should be marked on graph paper with the same number of grid spaces as the tray.

The position of the insect should be located periodically with the counter. Active insects should be located at 5-minute intervals. The highest activity will be at a point immediately above the insect. Record the new position of the insect on the graph paper and connect the various points with lines.

QUESTIONS

(1) Were the movements of the insect random?
(2) What type of movement could be expected in soil containing a host of the insect?
(3) How far did the insect travel per unit of time?

V-3.22. Laboratory-simulated radioecological experiments — Tagging adult house-flies for estimating population density by the isotope dilution method

Estimates of insect populations in certain areas are important since they allow the exact planning of activities for insect control, ecological studies, etc. Tagging large numbers of individual flies with a radioactive tracer and employing a marking/recapture method for estimating the density is a method by which many problems can be solved.

PURPOSE

To provide training in tagging techniques and in handling of large numbers of insects and special equipment by simulated field experiments under laboratory conditions instead of a field experiment requiring much preparation and field work. Adult house-flies are convenient to use because they ensure a good mixture of tagged and untagged insects through their activity.

MATERIALS

(1) Cage for holding 500 house-flies
(2) Large cubical fly cage (about 3 ft X 3 ft X 3 ft, i.e. ~ 1 m cube)

(3) About 500 newly hatched adult house-flies of mixed sexes, unfed, with water supply. Count the flies to determine the exact number
(4) Two days later, about 5000 newly hatched house-flies of mixed sexes (they need not be counted exactly)
(5) Milk and sucrose solution
(6) About 1 mCi (37 MBq) of ^{32}P as sodium phosphate
(7) Tweezers
(8) Waxed cardboard cups
(9) Filter paper
(10) Small fly traps

PROCEDURE

(a) Tagging of house-flies. An exactly known number of house-flies (500) are put in a small cage (with filter paper on the bottom) and provided with radioactive food. Milk, sugar solution and ^{32}P (total amount circa 25 ml) are well mixed in a cardboard cup, and small pieces of styrofoam are placed in the feeding solution to prevent the insects from drowning.

After consumption of the feeding solution, add normal milk or sugar solution to the remainder of the radioactive solution. Thus more ^{32}P is available to the flies.

After 24 hours, check several captured individuals under the scaler for radioactivity (counts/min).

Remove the contaminated feeding cup and also the filter paper that may have been contaminated by the excrements, etc., of the flies. This is necessary in order to prevent later contamination of the population of unknown density.

(b) Release of the labelled individuals in a population of unknown density. Check the small cage for dead flies, take these out and subtract their number and that of the flies which had been taken out for informal measurements from the total number of tagged flies. The small cage is then introduced into the big one, which contains about 5000 newly hatched flies.

Cut with scissors several openings in the cloth of the small cage. Be sure that there is a thorough mixing of the tagged and untagged flies.

(c) Recaptures and measurements. The first samples of flies should be recaptured with traps or a mechanical aspirator or by hand, after 4–5 hours, about 200 specimens for each sample. Check the flies individually for radioactivity.

The last samples of flies (2 × 200 specimens) should be recaptured after 72 hours and checked individually for radioactivity.

(d) Calculations of population density. The population density is calculated using the formula of Bailey [45]:

$$N = \frac{T(n+1)}{t+1}$$

where N = total number of the whole population
T = number of introduced tagged individuals
n = number of recaptured specimens
t = number of marked individuals in the recaptured sample
(In calculations of the final recapture remember to subtract from T the number of recaptured marked individuals of the first recapture.)

Variance is calculated as follows:

$$V = N^2 \frac{n-t}{(n+t)(t+1)}$$

The calculation after Jenkins [46] is simpler:

$$\text{Total population} = \frac{\text{number marked released} \times \text{total number recaptured}}{\text{number marked recaptured}}$$

(e) Final examination of the method. To obtain an idea of the efficacy of the method, count by hand the population remaining in the cage (after killing with chloroform). To this number add the number of the recaptured flies.

QUESTIONS

(1) What are limiting factors in this technique?
(2) What are advantages of this technique over other marking techniques?
(3) What was the maximum variation between replicates?

Introduction to V-3.23. – V-3.29.
Insect sterilization using ^{60}Co and chemosterilants

The use of sexually sterile males in insect control is an exceptionally promising technique for controlling certain destructive pests. Accordingly, these laboratory exercises are designed to acquaint the student with some of the basic procedures necessary to initiate these investigations.

The following seven experiments require approximately 24 days to complete when starting with insect eggs. When adult insects of the right stage and age are ready, approximately 10 days are required, with certain manipulations to be done every day. Each single experiment requires about 10 days to complete,

but not day-to-day attention. It is essential to organize the programme carefully and to spend a full 4-hour laboratory period each day during the 10-day period if all experiments are done at one time.

MATERIALS

The materials needed for all seven experiments are given below; the methods and procedures are given separately for each experiment.

(1) House-fly and mosquito colonies
(2) Small cages and all equipment necessary for handling flies
(3) ^{60}Co source
(4) Chemosterilants
(5) Usual laboratory glass-ware and other equipment
(6) Micro applicator
(7) House-fly saline: 14.52 g NaCl, 0.36 g KCl, 0.29 g $CaCl_2$ and 2.5 g dextrose per litre of solution and 25 ml of 0.04M phosphate buffer of pH 7.0
(8) Acetone
(9) Ringer's solution: 0.1 g potassium chloride (KCl)
0.0135 g calcium chloride ($CaCl_2$)
0.0120 g sodium bicarbonate ($NaHCO_2$)
0.75 g sodium chloride (NaCl)
Dissolve in distilled water to make 100 ml of solution.

V-3.23. Radiation dosage required to produce sterility in the house-fly

PURPOSE

To determine which dosage of gamma radiation produces 100% sterility in male and female house-flies, as measured by dominant lethals induced in the sperm or eggs produced, and also whether infecundity in the females is obtained when the house-flies are irradiated in the pupal stage.

PROCEDURE

Expose several hundred house-fly pupae, between 2 to 3 days old, to each of the following dosages of radiation in a ^{60}Co source: 500 (0.13), 1000 (0.26), 2000 (0.52), 3000 (0.77), 6000 (1.55) and 9000 R (2.32 C/kg). Place 100 pupae from each dose level in emergence cages. Allow the adults to emerge and separate the sexes after a period of 12 hours. When three days old, place 30 irradiated males from each group in cages containing 25 virgin females. Also place

25 irradiated females from each group in cages with 30 three-day-old virgin males. A control cage consists of 30 three-day-old virgin males and 25 three-day-old virgin females. The five additional males in each cage are included to ensure that the males will not have to mate twice. Supply the cages with food and water. Check adult mortality daily. The females are allowed to oviposit for a period of several hours on the seventh day. Float the eggs from each group. Place about 300–400 eggs each in Petri dishes on moist, black cloth until all the eggs are plated out. Record the number of eggs in each dish. Determine the percentage of hatch after 24 hours. To be truly precise, the females should be egged individually, but this is tedious. The above method will give reliable data.

After the females are egged, it is important to check them for insemination as this may alter the results; unmated females will often lay a few eggs. The procedure is relatively simple. The anaesthetized female is placed on her back, the abdomen is depressed with a blunt probe and the extruded ovipositor is gently pulled off with jewellers' forceps. In most instances, the spermathecae and other reproductive glands remain with the ovipositor. The severed tissue is then placed in a drop of modified Ringer's solution on a slide, and the spermathecae and ducts are dissected from the adjoining tissue under X10 to X15 magnification. The spermathecae and ducts are then placed in another drop of Ringer's solution on a slide, covered with a cover slip and examined with a compound microscope. If the spermathecae are still whole, firm pressure on the cover slip with the tip of the probe will rupture them and any sperm present can be easily seen.

As in all experiments of this type, a control is run concurrently. Plot the lethality percentage obtained, as reflected in the hatch data, against the dose for both sexes.

SCHEDULE

Day 1 – a.m. Irradiate the pupae.
p.m. Place 100 pupae from each dose level into emergence cages.
Day 2 – a.m. Separate the flies by sex and place them in separate cages.
Day 4 – Set up the mating cages for each group.
Day 7 – Collect and count the eggs. Determine insemination rate.
Day 9 – Determine hatch and plot data.
Day 2 through Day 7 – Check adult mortality in each cage.

QUESTIONS

(1) What effect did the irradiation have on the females? Explain.
(2) At what dosage was 100% dominant lethality produced in the males?
(3) Was there any effect on longevity at the higher dosages? If so, explain.

V-3.24. Effect of radiation on sexual competitiveness of male house-flies

PURPOSE

To determine whether irradiation with 6000 R (1.55 C/kg) produced any deleterious effect in male house-flies that would prevent them from being sexually competitive with non-irradiated males.

PROCEDURE

House-fly pupae, 65–72 hours old, are exposed to a sterilizing dosage of 6000 R (1.55 C/kg). Newly emerged adult males and females are isolated before mating. Irradiated males, non-irradiated males and non-irradiated females at ratios of 1:1:1, 2:1:1 and 5:1:1 are placed in cages containing food and water and allowed to mate. To determine whether the males are sterile, a check-cross of irradiated males and non-irradiated females is run, and a cross of non-irradiated males and females is run concurrently to establish a natural-sterility base-line. The crosses are made as follows:

Irradiated males	Non-irradiated males	Non-irradiated females	Ratio
–	5	5	0:1:1
5	5	5	1:1:1
10	5	5	2:1:1
25	5	5	5:1:1
5	–	5	1:0:1

The females are introduced into the mating cages 24 hours after the males.

The adults are allowed to mate; several days later, CSMA® larval medium[4] is placed in the cages and the females are allowed to oviposit. All eggs from each individual cage are floated in water, and 100 eggs, randomly selected, are placed on a square of moistened cloth. The cloth is placed in a Petri dish and 24 hours later the percentage of hatch determined. The eggs from each cage are evaluated as described in the schedule.

[4] Standard house-fly larval medium manufactured by the Rolston Turina Company, USA.

SCHEDULE

Day 1 – a.m. House-fly pupae are irradiated at 6000 R (1.55 C/kg). This is done by the instructor.
Day 2 – a.m. All flies that have emerged overnight are discarded because mating may have occurred. The irradiated and normal males and females that emerge in the morning are isolated by the instructor before copulation.
Day 2 – p.m. Correct ratios of virgin irradiated and virgin non-irradiated males are placed in competition cages with adequate food and water. The females are held separate for 24 hours to allow all males to become oriented in the cages.
Day 3 – p.m. Introduce the females into the competition cages.
Day 8 – p.m. Collect and count random samples of 100 eggs from each cage.
Day 9 – p.m. Observe the eggs with a dissecting microscope to determine the number of hatched eggs. Calculate the percentage of sterility.

QUESTION

Is a dosage of 6000 R (1.55 C/kg) effective for sterilization?

V-3.25. Effect of a chemosterilant on the reproductive potential of the house-fly

PURPOSE

To determine the effect of various concentrations of a chemosterilant, in this case tepa, on male and female house-flies. The insects are treated in various ways – topically, by injection or through residues – and the efficacy of the various treatments is compared.

PROCEDURE

(a) Topical application. Groups of 30 one-day-old unmated males and 25 one-day-old unmated females are treated topically by means of a micro-applicator at the following concentrations of the chemosterilant tepa in an acetone solution: 0.1, 0.2, 0.4, 0.6 and 0.8 $\mu g/\mu l$. The control group is treated with the solvent only. After treatment the flies are placed in holding cages containing food and water. Twenty-four hours later, 25 virgin females are added to the cages containing the treated males, and 30 unmated males are added to the cages containing the treated females. When the females are 6 days old, they

are allowed to oviposit over a period of 3 hours. The checks for egg hatch and for insemination are carried out in the same way as described in Section V-3.23. Plot the percentage of lethality, as indicated by the hatch, versus the concentration of the chemosterilant for both sexes.

(b) Injection. By means of a microapplicator the same numbers of male and female flies are injected with the chemosterilant tepa, dissolved in a modified Ringer's saline solution at the following concentrations: 0.1, 0.2, 0.4, 0.6 and 0.8 $\mu g/\mu l$. In this case, two controls are used: one in which only the males are injected with the modified saline solution and one in which the females are also injected with it. The rest of the test is carried out in exactly the same way as in *(a)* above.

(c) Residue application. Pint jars are treated on their interior with 5, 10, 25, 50 and 100 mg/ft^2 of tepa and kept for 24 hours. Groups of flies, as described under *(a)*, are anaesthetized, placed in Petri dishes and covered with a card. When the flies have recovered from the carbon dioxide treatment, they are allowed to ascend into the inverted jars by removing the card. After an exposure period of 1 hour, the flies are permitted to escape from the jars into holding cages containing food and water. On the third day, 30 virgin males and 25 virgin females are introduced into the respective cages for the crosses. The rest of the experiment is handled in the same way as in *(a)*.

QUESTIONS

(1) Compare the percentage of dominant lethals obtained by the different application methods of the chemosterilant. Explain any differences observed. Which treatment is the most effective?
(2) What effect has the sex of the fly on the results of the treatment? Why does it have this effect?

V-3.26. Effect of a chemosterilant on sexual competitiveness of male house-flies

PURPOSE

To determine whether any deleterious effect on sexual competitiveness is produced by the chemosterilant in the males, thereby reducing the impact of introducing such sterilized males into a natural population.

PROCEDURE

Newly enclosed adult male flies are offered fly food, containing 0.25% tepa, for 3 days. The treated males are confined, together with normal males, in ratios of 1:1, 2:1 and 5:1. Five females are introduced into each of the mating cages 1 hour later. To determine if the males are sterile, a cross of treated males and normal females is run. A cross of normal males and normal females is run concurrently to establish a natural-sterility base-line. Two replicate experiments for each ratio are set up using the following number of flies:

Sterile males	Normal males	Normal females
--	5	5
5	—	5
5	5	5
10	5	5
25	5	5

The flies are allowed to mate; three days later, moistened CSMA® larval medium is placed in the cages and the females are allowed to oviposit. The eggs from the individual cages are floated in water and 100-egg samples from each cage, selected randomly, are placed on moistened black cloth patches. On the following day the number of hatched eggs is determined.

SCHEDULE

Day 1 — a.m. The instructor prepares the treated diet. Tepa is incorporated in the fly diet as acetone solution, the diet is left to dry overnight in the hood and then repulverized.
Day 2 — a.m. The instructor separates the flies by sex upon emergence to assure that no mating occurs.
Day 2 — p.m. Students set up the cages with treated or untreated food and introduce the separated flies.
Day 5 — p.m. Students remove the flies from the cages and make proper crosses.
Day 9 — a.m. The instructor initiates the egging procedure for competition tests.
Day 9 — p.m. Students collect the eggs and prepare 100-egg samples from each test cage.
Day 10 — p.m. Students check the hatched eggs collected on the previous day and calculate the percentage of sterility.

QUESTIONS

(1) What is the effect of increasing the ratio of treated males to normal males?
(2) Is the effect greater or smaller than the theoretical? Explain.

V-3.27. Effect of a chemosterilant on sexual competitiveness of male mosquitoes

PURPOSE

To determine if male *Aedes aegypti* mosquitoes sterilized by contact with a chemosterilant are sexually competitive with non-sterile mosquitoes.

PROCEDURE

One-day-old adults are confined for 4 hours in a tepa-treated glass jar. After exposure, the treated males, normal males, and normal females are confined together in ratios of 1:1:1, 2:1:1, and 5:1:1. To determine if the males are sterile, a cross of treated males and normal females is run. A cross of normal males and normal females is run concurrently to establish a natural-sterility base-line. Two replicate experiments for each ratio are set up using the following numbers of mosquitoes:

Sterile males	Normal males	Normal females
–	25	25
25	–	25
25	25	25
50	25	25
125	25	25

The mosquitoes are allowed to mate for 4 days, after which time guinea pigs are offered to the females for blood feeding. The guinea pigs are placed in the mosquito cages for 1 hour. Two days later, pint jars, lined with blotting paper and containing 250 ml of tap water, are placed inside the cages for oviposition. One week later the jars are removed and samples of at least 300 eggs are cut from the paper and flooded; then the hatching ratio is determined.

SCHEDULE

Because the experiment is of such a prolonged nature, the instructor will perform the initial phase at the laboratory.

Day 1 -- a.m. The instructor sets eggs in order to obtain adults of the proper age.

Day 8 – Day 9 a.m. The instructor separates pupae from larvae and later separates the newly emerged adults by sex to ensure that no mating occurs.

Day 10 – a.m. The instructor exposes virgin adults to tepa-treated jars for 4 hours.

Day 10 – p.m. The instructor makes the necessary mating crosses.

Day 14 – a.m. Students feed the mosquitoes on guinea pigs since a blood meal is necessary for oviposition.

Day 16 – p.m. Students collect the mosquito eggs.

Day 23 – p.m. Students collect egg samples and check the hatching ratios.

QUESTIONS

(1) What is the effect of increasing the ratio of treated males to normal males?
(2) How does this effect compare with the results obtained in Experiment V-3.26 with house-flies?

V-3.28. Effect of sterilizing treatments on the reproductive functions of the house-fly

PURPOSE

To determine whether a female has become inseminated, to determine the insemination rate of a sterile male compared with a normal insect, and to determine whether the sperm from a sterilized male remains motile in the female.

PROCEDURE

The female house-fly is anaesthetized with carbon dioxide. It is then immobilized by placing the dorsum on a strip of masking tape fastened on a card. The abdomen is depressed with a blunt probe and the extruded ovipositor is slowly pulled off with jewellers' forceps. In most instances, the spermathecae and other reproductive glands remain with the ovipositor.

The severed tissue is then placed in modified Ringer's solution on a depression slide, and the spermathecae and ducts are dissected from the adjoining tissue under X10 to X15 magnification. The spermathecae and ducts are then placed in a drop of Ringer's solution on a slide, covered with a cover slip and examined with a compound microscope. If the spermathecae are still whole, firm pressure on the cover slip with the tip of a probe will rupture them and any sperm present will be readily visible in the ruptured area.

This experiment is particularly useful in determining the number of females inseminated in a wild population, as well as in determining the number of times a male is capable of inseminating a female.

V-3.29. Effect of sterilizing treatments on the chromosomes of the house-fly

PURPOSE

To determine whether irradiation or treatment with a chemosterilant has any effect on the chromosomes. For this purpose, a simple technique has been developed whereby consistently good chromosome preparations from gonads of adult female house-flies can be obtained.

PROCEDURE

(1) Tissue of the reproductive organs is dissected and placed in 1.0% hypotonic solution of sodium citrate for 10 minutes.
(2) The tissue is soaked in modified Carnoy's fixative for 5 minutes.
(3) The tissue is briefly washed in 45% glacial acetic acid.
(4) The tissue is placed in a drop of 45% glacial acetic acid on a glass slide.
(5) The tissue is covered with a siliconized cover slip and a piece of filter paper.
(6) Gentle pressure is applied to the filter paper to squash the tissue; then the cover slip is gently tapped.
(7) The slide is placed on dry ice for 30 minutes.
(8) The cover slip is removed with a chilled razor blade.
(9) The slide is immersed in 95% enthyl alcohol in a coplin jar for 5 minutes.
(10) A drop of Gurr's Natural Orcein and fast green stain is placed on the tissue while the slide is still damp.
(11) The tissue is covered with a siliconized cover slip.
(12) Excess stain is removed with filter paper.
(13) The preparation is now temporary but it will last for months if the cover slip is ringed with warm balsam-parafilm. To make the slide permanent, ignore step No.13 and proceed to step No.14.

(14) The slide is placed on dry ice again for 1 to 8 hours
(15) The cover slip is removed with a chilled razor blade.
(16) The slide is bathed in 95% ethyl alcohol for 5 minutes and excess alcohol allowed to drain.
(17) The slide is transferred to absolute ethyl alcohol for 1 minute and excess alcohol allowed to drain.
(18) A drop of euparal is placed on the tissue.
(19) A non-siliconized cover slip is applied to the tissue and the excess euparal is absorbed with filter paper.
(20) The preparation is allowed to dry for 14 hours before use.

V-3.30. Effect of anoxia on radiation sterilization of insects

Inadequate supply of oxygen during radiation sterilization of insects frequently results in incomplete sterilization and in unpredictably erratic sterilization. Anoxia can occur as a result of overcrowding in air-tight containers for relatively long periods of time. When the condition of anoxia exists, cell division in proliferating tissues slows down or comes to a halt, a state which tends to nullify the sterilizing effects of radiation.

PURPOSE

To show that for insects in the state of anoxia larger dosages of gamma radiation are necessary to cause sterility than for insects with an adequate supply of oxygen.

MATERIALS

(1) House-fly pupae (7500), one day from emergence
(2) Gas-tight containers (canisters, small jars, plastic bags)
(3) Cylinders of carbon dioxide, nitrogen and oxygen
(4) Cages (15).
(5) Adult-fly food and water
(6) Petri dishes (15)
(7) Filter paper of the same size as the Petri dishes
(8) Three binocular microscopes
(9) Three lengths of rubber tubing for gas
(10) Plastic bags
(11) Pressure-reducing valves

PROCEDURE

Divide the house-fly pupae into 15 lots of 500 flies each. Make three groups of 5 lots each, one group for each type of gas. Work with one gas at a time. Put the pupae in unsealed gas-tight containers and put the containers in a plastic bag. Fill the bag with gas (5 litres/min for 5 minutes, and then 1 litre/min for 25 minutes). Remove the gas hose and close the bag opening. Place the lids on the gas-tight containers without disturbing the gas concentration and use different exposures for the five lots within a group, as shown below:

Group 1 Lot (pupae)	Group 2 Lot (pupae)	Group 3 Lot (pupae)	Irradiation (R)	Irradiation (C/kg)
1	1	1	1000	0.26
2	2	2	2000	0.52
3	3	3	3000	0.77
4	4	4	4000	1.03
5	5	5	5000	1.29

After irradiation, place the pupae in cages with food and water and a properly prepared label, stating the kind of gas used and the radiation dose. Five days after irradiation the emerged flies should be egged. Place the eggs on moist filter paper in Petri dishes and label. Twenty-four hours after incubation count the hatched and unhatched eggs and determine the percentage of hatch.

QUESTIONS

(1) Was the dose response linear or exponential for each of the various gases?
(2) Which gas caused the greatest depression of fertility? Which gas caused the least?
(3) What are some ways to prevent anoxia when large numbers of insects are being irradiated?

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WORKING NOTES TO PART V

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PART VI. APPENDIXES

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Appendix VI–1

HOW TO PUT ON AND TAKE OFF RUBBER GLOVES[1]

The technique employed in this procedure is such that the inside of a glove is not touched by its outer side or the outside of the other glove, nor is any part of the outside of a glove allowed to come into contact with the bare skin. The procedure is as follows:

Putting on the gloves

(1) The gloves should be dusted internally with talcum powder.
(2) The cuff of each glove should be folded over, outwards, for about 5 cm.
(3) Put one glove on by grasping only the internal folded-back part with the other hand.
(4) Put the second glove on by holding it with the fingers of the gloved hand tucked in the fold and only touching the outside of the glove.
(5) Unfold the gloves (cuffs) by manipulating the fingers inside the fold.

Taking off the gloves

(6) In taking off the gloves, seize the fingers of one glove by the other gloved hand and pull free.
(7) Take off the other glove by manipulating the fingers of the free hand under the cuff of the glove and fold it back so that an internal part is exposed which may be seized, and the remaining hand freed.

It is a great advantage if the inside and the outside of the gloves are distinctly different, for example in colour or texture.

[1] **Adapted from BOURSNELL, J.C., Safety Techniques for Radioactive Tracers, Cambridge University Press, London (1958).**

Appendix VI–2

RADIOACTIVE WASTE CONTROL AND DISPOSAL[2]

VI–2.1. WASTE COLLECTION

Suitable receptacles should be available in all working places where radioactive waste may originate.

Solid waste should be deposited in refuse bins with foot-operated lids. The bins should be lined with removable plastic or paper bags to facilitate removal of the waste without contamination.

If no other facilities for liquid-waste disposal exist, liquid waste should be collected in bottles kept in pails or trays designed to retain all their contents in the event of a breakage. Containers are available for liquid waste, which are provided with a suitable absorbent so that the waste is held in a solid form for subsequent storage or disposal.

All receptacles for radioactive waste should be clearly identified. In general, it will be desirable to classify radioactive waste according to methods of storage or disposal, and to provide separate containers for the various classes of waste. Depending upon the needs of the installation, one or more of the following bases for classifying waste may be found desirable:

(a) Gamma radiation levels (high, low);
(b) Total activity (high, medium, low);
(c) Half-life (long, short);
(d) Combustible, non-combustible.

For convenient and positive identification, it may be desirable to use both colour coding and wording.

Shielded containers should be used when necessary (e.g. for gamma emitters).

It is generally desirable to maintain an approximate record of the quantities of radioactive waste released to drainage systems or to sewers, of for burial. This may be particularly important in the case of long-lived radioisotopes. For this purpose it may be desirable or necessary to maintain a record of estimated quantities of radioactivity deposited in various receptacles, particularly for high levels of activity or long-lived radionuclides. Depending upon the system of control used by the installation, it may be desirable to provide for the receptacle to be marked or tagged with a statement of its contents.

Radioactive waste should be removed from working places by designated personnel under competent supervision.

VI–2.2. WASTE STORAGE

All waste which cannot be immediately disposed of in conformity with the requirements of the competent authority must be placed in suitable storage.

Storage may be temporary or indefinite. Temporary storage is used to allow for decrease of activity, to permit regulation of the rate of release of activity, to permit monitoring of materials of unknown degree of hazard or to await the availability of suitable transport. Indefinite centralized storage in special places must be provided by the competent authority for the more hazardous waste for which no ultimate disposal method is available to the particular user.

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[2] Based on §8 of Safe Handling of Radionuclides, 1973 Edition, Safety Series No.1, IAEA, Vienna (1973). See also: The Management of Radioactive Wastes Produced by Radioisotope Users, Safety Series No.12, IAEA, Vienna (1965); The Management of Radioactive Wastes Produced by Radioisotope Users: Technical Addendum, Safety Series No.19, IAEA, Vienna (1966).

Storage conditions should meet the safety requirements for storage of sources, as set forth in the IAEA's Safety Series No.1, §4. [3]

The storage site should not be accessible to unauthorized personnel. (Control of animals should not be overlooked.)

The method of storage should prevent accidental release to the surroundings.

Appropriate records should be kept of the storage.

VI 2.3. EFFLUENT RELEASE TO THE ENVIRONMENT

VI–2.3.1. General considerations

Radioactive effluent releases to the environment should be carried out in accordance with conditions established by the radiological health and safety officer and by the competent authority.

The ways in which radioactive materials may affect the environment should be carefully examined in relation to any proposed method of effluent release.

The capacity of any route of disposal to accept radioactive effluent safely depends on the evaluation of a number of factors, many of which depend on the particular local situation. By assuming unfavourable conditions with respect to all factors, it is possible to set a permissible level for effluent release which will be safe under all circumstances. This usually provides a very considerable safety factor. The real capacity of a particular route of effluent release can only be found from a lengthy study by experts.

The small user should first try to work within restrictive limits which are accepted as being safe and which will usually provide a workable solution to the problem of effluent release. Such a restrictive safe limit may be arrived at by identifying:

(a) The critical radionuclide;
(b) The critical pathway to man;
(c) The critical group of the population concerned;
(d) The critical organ.

Thereafter it will be possible to calculate the amount and rate of radioactivity that may safely be released at a given point in the environment, taking into account any alternative requirements the competent authority may impose if local studies by experts provide reasonable justification for other levels. More detailed guidelines for this exercise can be found in the appropriate Safety Series publications of the International Atomic Energy Agency. In practice, however, the small user seldom requires to go into such detailed considerations since the type of work and the amount and nature of the radionuclide involved would not in all probability pose a significant environmental problem.

VI–2.3.2. Effluent release to drains and sewers

The release of radioactive effluent into drains does not usually need to be considered as a direct release into the environment. Hence, a restrictive safe limit will usually be provided if the concentration of radioactive effluent, based on the total available flow of water in the system averaged over a moderate period (a day or a month), does not lead to exposure of individual members of the public to doses in excess of the dose limits prescribed in the Agency's Basic Safety Standards for Radiation Protection[4]. In arriving at the dose rates and amounts of discharge the factors summarized in the last paragraph of the previous section may need to be considered in some cases. Finally, before release of radioactive

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[3] INTERNATIONAL ATOMIC ENERGY AGENCY, Safe Handling of Radionuclides, 1973 Edition, Safety Series No.1, IAEA, Vienna (1973).

[4] INTERNATIONAL ATOMIC ENERGY AGENCY, Basic Safety Standards for Radiation Protection, 1976 Edition, Safety Series No.9, IAEA, Vienna (1967).

effluent to public drains, sewers or rivers, the competent authority should be consulted to ascertain that no other radioactive effluent is being released in such a way that the accumulated releases will create a hazardous situation.

Radioactive release to drains should be readily soluble or dispersible in water. Account should be taken of possible changes of pH-value due to dilution or other physico-chemical factors, which might lead to precipitation or vaporization of diluted materials.

In general, the excreta of persons being treated by radioisotopes do not call for special consideration.

Wastes should be flushed down the pipe by a copious stream of water.

The dilution of radioactive effluent by the addition of stable isotopes of the radioactive elements present in the effluent may be considered.

Maintenance work on active drains within an establishment should only be carried out with the knowledge of the radiological health and safety officer and under competent supervision. Special attention should be given to the possibility that small sources may have been dropped into sinks and retained in traps or catchment basins.

The release of radioactive effluent to sewers should be done in such a manner as not to require protective measures during maintenance work on sewers outside the establishment, unless other agreement has been reached with the authority in charge of those sewers. This authority should be informed of the release of radioactive effluent into the sewer system; mutual discussion of the technical aspects of the waste disposal problem is desirable to provide protection, and to avoid unnecessary anxiety.

VI–2.3.3. Effluent release to the atmosphere

Any release of radioactive effluent in the form of gases or aerosols into the atmosphere should conform with the requirements of the competent authority.

If protection is based on an elevated release point from a stack, levels of release should only be set after examination of local conditions by an expert.

The need for filtration of gases or aerosols before release as waste should be assessed.

Used filters should be handled as solid waste.

VI–2.3.4. Burial of waste

Burial of waste in soil sometimes provides a measure of protection not obtained if the waste is released directly to the environment. The possibilities of safe burial of waste should always be appraised by an expert.

Burial under a suitable depth of soil (about one metre) provides economical protection from the external radiation of the accumulated deposit.

A burial site should be under the control of the user, and adequate steps to exclude the public from it should be taken.

A record should be kept of disposals into the ground.

VI–2.3.5. Incineration of waste

If solid waste is incinerated to reduce the bulk to manageable proportions, adequate precautions should be taken.

The incineration of active waste should only be carried out in equipment embodying such features for filtration and scrubbing as may be necessary for the levels of activity to be disposed of.

Residual ashes should be prevented from becoming a dust hazard, for example by damping them with water, and should be properly dealt with as active waste.

Appendix VI–3

DERIVED LIMITS FOR CONTROLLABLE EXPOSURE[5]

The limits of doses for controllable exposure are given in detail in the Agency's Basic Safety Standards for Radiation Protection (1967 Edition)[6]. To ensure, in the case of internal radiation exposure, that the maximum permissible doses and dose limits are not exceeded, derived limits are available for practical application. The more appropriate derived limit for this purpose would be the body or critical organ content of the radionuclides giving the maximum permissible dose rate. However, in practice, the use of this derived limit is not easy and therefore another derived limit which may be used is the maximum permissible annual intake in air or water, subject to the qualifications indicated in the Basic Safety Standards[5]. The intakes are given in Tables IIA and IIB in the Basic Safety Standards. However, the estimation of intakes is not always easy and, for practical control of internal exposure, a third derived standard is used, i.e. the derived concentration limit (DCL), formerly known as maximum permissible concentration. The derived concentration limit is not given in the Basic Safety Standards, though a method of computing is indicated. For the convenience of the user, DCL values for occupational exposure are given in the following Table. Derived air concentration (DAC) and derived water concentration (DWC) are also given for practical convenience. For control purposes, keeping within the DCL will ensure that the dose equivalent to the worker does not exceed the maximum permissible dose for occupational exposure, provided there is no external exposure. It should be noted that the DCL values in this Table apply to workers only. Furthermore, in practice, the DWC values should normally be of technical interest only, since workers should not, at their working place, consume water containing radioactivity at higher concentrations than in public water supplies.

Table of derived concentration limits commences overleaf.

[5] INTERNATIONAL ATOMIC ENERGY AGENCY, Safe Handling of Radionuclides, 1973 Edition, Safety Series No.1, IAEA, Vienna (1973), Table A1–IIa.

[6] INTERNATIONAL ATOMIC ENERGY AGENCY, Basic Safety Standards for Radiation Protection, 1967 Edition, Safety Series No.9, IAEA, Vienna (1967).

DERIVED CONCENTRATION LIMITS OF RADIONUCLIDES IN AIR AND WATER FOR OCCUPATIONAL EXPOSURE (40 h/week)

These tables give curies as units of radioactivity; the data for μCi (or μCi/cm^3) should be multiplied by 37 to obtain data in kBq (or kBq/cm^3).

Radionuclide		Critical organ[a]	Organ content giving maximum permissible dose rate (μCi)	Derived air concentration (μCi/cm^3)	Derived water concentration[b] (ingestion) (μCi/cm^3)
$^{3}_{1}$H (HTO or $^{3}H_2O$)	(soluble)	Body tissue Total body	1.2×10^{3}	5×10^{-6}	0.1
($^{3}H_2$)	(submersion)	Skin		2×10^{-3}	
$^{7}_{4}$Be	(soluble)	GI (LLI) Total body	5.6×10^{2}	6×10^{-6}	0.05
	(insoluble)	Lung GI (LLI)	52	10^{-5}	0.05
$^{14}_{6}$C (CO_2)	(soluble)	Fat	1.6×10^{2}	4×10^{-6}	0.02
	(submersion)	Total body		5×10^{-5}	
$^{18}_{9}$F	(soluble)	GI (SI)		5×10^{-6}	0.02
	(insoluble)	GI (ULI)		3×10^{-6}	0.01
$^{22}_{11}$Na	(soluble)	Total body	12	2×10^{-7}	10^{-3}
	(insoluble)	Lung GI (LLI)	1	9×10^{-9}	9×10^{-4}
$^{24}_{11}$Na	(soluble)	GI (SI)		10^{-6}	6×10^{-3}
	(insoluble)	GI (LLI)		10^{-7}	8×10^{-4}
$^{31}_{14}$Si	(soluble)	GI (S)		6×10^{-6}	0.03
	(insoluble)	GI (ULI)		10^{-6}	6×10^{-3}
$^{32}_{15}$P	(soluble)	Bone	3.1	7×10^{-8}	5×10^{-4}
	(insoluble)	Lung GI (LLI)	1.2	8×10^{-8}	7×10^{-4}
$^{35}_{16}$S	(soluble)	Testis	0.2	3×10^{-7}	2×10^{-3}
	(insoluble)	Lung GI (LLI)	15	3×10^{-7}	8×10^{-3}
$^{36}_{17}$Cl	(soluble)	Total body	75	4×10^{-7}	2×10^{-3}
	(insoluble)	Lung GI (LLI)	3.2	2×10^{-8}	2×10^{-3}
$^{38}_{17}$Cl	(soluble)	GI (S)		3×10^{-6}	0.01
	(insoluble)	GI (S)		2×10^{-6}	0.01
$^{37}_{18}$Ar	(submersion)	Skin		6×10^{-6}	
$^{41}_{18}$Ar	(submersion)	Total body		2×10^{-6}	

[a] The abbreviations GI, S, SI, ULI, and LLI refer to gastro-intestinal tract, stomach, small intestine, upper large intestine, and lower large intestine, respectively.

[b] The derived water concentration values should not be interpreted to mean that the worker is liable, at his working place, to drink water containing radioactivity at higher concentrations than in public water supplies.

These tables give curies as units of radioactivity; the data for μCi (or μCi/cm³) should be multiplied by 37 to obtain data in kBq (or kBq/cm³).

Radionuclide		Critical organ	Organ content giving maximum permissible dose rate (μCi)	Derived air concentration (μCi/cm³)	Derived water concentration (ingestion) (μCi/cm³)
$^{42}_{19}$K	(soluble)	GI (S)		2×10^{-6}	9×10^{-3}
	(insoluble)	GI (LLI)		10^{-7}	6×10^{-4}
$^{45}_{20}$Ca	(soluble)	Bone	26	3×10^{-8}	3×10^{-4}
	(insoluble)	Lung GI (LLI)	9.7	10^{-7}	5×10^{-3}
$^{47}_{20}$Ca	(soluble)	Bone	4.2	2×10^{-7}	10^{-3}
	(insoluble)	GI (LLI) Lung	1	2×10^{-7} 2×10^{-7}	10^{-3}
$^{46}_{21}$Sc	(soluble)	GI (LLI) Liver	2.2	2×10^{-7} 2×10^{-7}	10^{-3}
	(insoluble)	Lung GI (LLI)	1.3	2×10^{-6}	10^{-3}
$^{47}_{21}$Sc	(soluble)	GI (LLI)		6×10^{-7}	3×10^{-3}
	(insoluble)	GI (LLI)		5×10^{-7}	3×10^{-3}
$^{48}_{21}$Sc	(soluble)	GI (LLI)		2×10^{-7}	8×10^{-4}
	(insoluble)	GI (LLI)		10^{-7}	8×10^{-4}
$^{48}_{23}$V	(soluble	GI (LLI)		2×10^{-7}	9×10^{-4}
	(insoluble)	Lung GI (LLI)	0.93	6×10^{-8}	8×10^{-4}
$^{51}_{24}$Cr	(soluble)	GI (LLI) Total body	780	10^{-5} 10^{-5}	0.05
	(insoluble)	Lung GI (LLI)	60	2×10^{-6}	0.05
$^{52}_{25}$Mn	(soluble)	GI (LLI)		2×10^{-7}	10^{-3}
	(insoluble)	Lung GI (LLI)	0.87	10^{-7}	9×10^{-4}
$^{54}_{25}$Mn	(soluble)	GI (LLI) Liver	6.2	4×10^{-7}	4×10^{-3}
	(insoluble)	Lung GI (LLI)	3.6	4×10^{-8}	3×10^{-3}
$^{56}_{25}$Mn	(soluble)	GI (LLI)		8×10^{-7}	4×10^{-3}
	(insoluble)	GI (LLI)		5×10^{-7}	3×10^{-3}
$^{55}_{26}$Fe	(soluble)	Spleen	19	9×10^{-7}	0.02
	(insoluble)	Lung GI (LLI)	130	10^{-6}	0.07

These tables give curies as units of radioactivity; the data for μCi (or μCi/cm^3) should be multiplied by 37 to obtain data in kBq (or kBq/cm^3).

Radionuclide		Critical organ	Organ content giving maximum permissible dose rate (μCi)	Derived air concentration (μCi/cm^3)	Derived water concentration (ingestion) (μCi/cm^3)
$^{59}_{26}$Fe	(soluble)	GI (LLI) Spleen	 0.37	 10^{-7}	2×10^{-3}
	(insoluble)	Lung GI (LLI)	2	5×10^{-8}	2×10^{-3}
$^{57}_{27}$Co	(soluble)	GI (LLI)		3×10^{-6}	0.02
	(insoluble)	Lung GI (LLI)	16	2×10^{-7}	0.01
$^{58m}_{27}$Co^{m}	(soluble)	GI (LLI)		2×10^{-5}	0.08
	(insoluble)	Lung GI (LLI)	4.2	9×10^{-6}	0.06
$^{58}_{27}$Co	(soluble)	GI (LLI) Total body	 32	8×10^{-7}	4×10^{-3}
	(insoluble)	Lung GI (LLI)	3	5×10^{-8}	3×10^{-3}
$^{60}_{27}$Co	(soluble)	GI (LLI) Total body	 13	3×10^{-7}	10^{-3}
	(insoluble)	Lung GI (LLI)	1.2	9×10^{-9}	10^{-3}
$^{59}_{28}$Ni	(soluble)	Bone	1400	5×10^{-7}	6×10^{-3}
	(insoluble)	Lung GI (LLI)	110	8×10^{-7}	0.06
$^{63}_{28}$Ni	(soluble)	Bone	100	6×10^{-6}	8×10^{-4}
	(insoluble)	Lung GI (LLI)	40	3×10^{-7}	0.02
$^{65}_{28}$Ni	(soluble)	GI (ULI)		9×10^{-7}	4×10^{-3}
	(insoluble)	GI (ULI)		5×10^{-7}	3×10^{-3}
$^{64}_{29}$Cu	(soluble)	GI (LLI)		2×10^{-6}	0.01
	(insoluble)	GI (LLI)		10^{-6}	6×10^{-3}
$^{65}_{30}$Zn	(soluble)	Total body Prostate Liver	61 0.1 9.5	10^{-7} 10^{-7} 10^{-7}	3×10^{-3}
	(insoluble)	Lung GI (LLI)	5.6	6×10^{-9}	5×10^{-3}
$^{69}_{30}$Zn^{m}	(soluble)	GI (LLI) Prostate	 0.013	4×10^{-7} 4×10^{-7}	2×10^{-3}
	(insoluble)	GI (LLI)		3×10^{-7}	2×10^{-3}

These tables give curies as units of radioactivity; the data for μCi (or μCi/cm^3) should be multiplied by 37 to obtain data in kBq (or kBq/cm^3).

Radionuclide		Critical organ	Organ content giving maximum permissible dose rate (μCi)	Derived air concentration (μCi/cm^3)	Derived water concentration (ingestion) (μCi/cm^3)
$^{69}_{30}$Zn	(soluble)	GI (S) Prostate	0.015	7×10^{-6}	0.05
	(insoluble)	GI (S)		9×10^{-6}	0.05
$^{72}_{31}$Ga	(soluble)	GI (LLI)		2×10^{-7}	10^{-3}
	(insoluble)	GI (LLI)		2×10^{-7}	10^{-3}
$^{71}_{32}$Ge	(soluble)	GI (LLI)		10^{-5}	0.05
	(insoluble)	Lung GI (LLI)	84	6×10^{-6}	0.05
$^{73}_{34}$As	(soluble)	GI (LLI) Total body	320	2×10^{-6}	0.01
	(insoluble)	Lung GI (LLI)	20	4×10^{-7}	0.01
$^{74}_{33}$As	(soluble)	GI (LLI)		3×10^{-7}	2×10^{-3}
	(insoluble)	Lung GI (LLI)	2.2	10^{-7}	2×10^{-3}
$^{76}_{33}$As	(soluble)	GI (LLI)		10^{-7}	6×10^{-4}
	(insoluble)	GI (LLI)		10^{-7}	6×10^{-4}
$^{77}_{33}$As	(soluble)	GI (LLI)		5×10^{-7}	2×10^{-3}
	(insoluble)	GI (LLI)		4×10^{-7}	2×10^{-3}
$^{75}_{34}$Se	(soluble)	Kidney Total body	3.5 98	10^{-5} 10^{-6}	9×10^{-3}
	(insoluble)	Lung GI (LLI)	8.9	10^{-7}	8×10^{-3}
$^{82}_{35}$Br	(soluble)	Total body GI (SI)	11	10^{-6}	8×10^{-3} 8×10^{-3}
	(insoluble)	GI (LLI)		2×10^{-7}	10^{-3}
$^{85}_{36}$Kr^m	(submersion)	Total body		6×10^{-6}	
$^{85}_{36}$Kr	(submersion)	Total body		10^{-5}	
$^{87}_{36}$Kr	(submersion)	Total body		10^{-6}	
$^{86}_{37}$Rb	(soluble)	Total body Pancreas Liver	28 0.09 2.2	3×10^{-7} 3×10^{-7}	2×10^{-3} 2×10^{-3}
	(insoluble)	Lung GI (LLI)	1.3	7×10^{-6}	7×10^{-4}

These tables give curies as units of radioactivity; the data for μCi (or μCi/cm^3) should be multiplied by 37 to obtain data in kBq (or kBq/cm^3).

Radionuclide		Critical organ	Organ content giving maximum permissible dose rate (μCi)	Derived air concentration (μCi/cm^3)	Derived water concentration (ingestion) (μCi/cm^3)
$^{87}_{37}Rb$	(soluble)	Pancreas Total body Liver	0.065 220 16	5×10^{-7}	3×10^{-3}
	(insoluble)	Lung GI (LLI)	9.3	7×10^{-8}	5×10^{-3}
$^{85}_{38}Sr^{m}$	(soluble)	GI (SI)		4×10^{-6}	0.2
	(insoluble)	GI (SI)		3×10^{-6}	0.2
$^{85}_{38}Sr$	(soluble)	Total body	59	2×10^{-7}	3×10^{-3}
	(insoluble)	Lung GI (LLI)	5.2	10^{-7}	5×10^{-3}
$^{89}_{38}Sr$	(soluble)	Bone	3.9	3×10^{-8}	3×10^{-4}
	(insoluble)	Lung GI (LLI)	1.5	4×10^{-8}	8×10^{-4}
$^{90}_{38}Sr$	(soluble)	Bone	2	1×10^{-9}	1×10^{-5}
	(insoluble)	Lung GI (LLI)	0.76	5×10^{-9}	10^{-3}
$^{91}_{38}Sr$	(soluble)	GI (LLI)		4×10^{-7}	2×10^{-3}
	(insoluble)	GI (LLI)		3×10^{-7}	10^{-3}
$^{92}_{38}Sr$	(soluble)	GI (ULI)		4×10^{-7}	2×10^{-3}
	(insoluble)	GI (ULI)		3×10^{-7}	2×10^{-3}
$^{90}_{39}Y$	(soluble)	GI (LLI)		10^{-7}	6×10^{-4}
	(insoluble)	GI (LLI)		10^{-7}	6×10^{-4}
$^{91}_{39}Y^{m}$	(soluble)	GI (SI)		2×10^{-5}	0.1
	(insoluble)	GI (SI)		2×10^{-5}	0.1
$^{91}_{39}Y$	(soluble)	GI (LLI) Bone	3.8	4×10^{-8}	3×10^{-4}
	(insoluble)	Lung GI (LLI)	1.4	3×10^{-8}	8×10^{-4}
$^{92}_{39}Y$	(soluble)	GI (ULI)		4×10^{-7}	2×10^{-3}
	(insoluble)	GI (ULI)		3×10^{-7}	2×10^{-3}
$^{93}_{39}Y$	(soluble)	GI (LLI)		2×10^{-7}	8×10^{-4}
	(insoluble)	GI (LLI)		10^{-7}	8×10^{-4}

These tables give curies as units of radioactivity; the data for μCi (or μCi/cm³) should be multiplied by 37 to obtain data in kBq (or kBq/cm³).

Radionuclide		Critical organ	Organ content giving maximum permissible dose rate (μCi)	Derived air concentration (μCi/cm³)	Derived water concentration (ingestion) (μCi/cm³)
$^{93}_{40}Zr$	(soluble)	GI (LLI) Bone	100	10^{-7}	0.02
	(insoluble)	Lung GI (LLI)	43	3×10^{-7}	0.02
$^{95}_{40}Zr$	(soluble)	GI (LLI) Total body	18	10^{-7}	2×10^{-3}
	(insoluble)	Lung GI (LLI)	1.6	3×10^{-8}	2×10^{-3}
$^{97}_{40}Zr$	(soluble)	GI (LLI)		10^{-7}	5×10^{-4}
	(insoluble)	GI (LLI)		9×10^{-8}	5×10^{-4}
$^{93}_{41}Nb^{m}$	(soluble)	GI (LLI) Bone	91	10^{-7}	0.01
	(insoluble)	Lung GI (LLI)	22	2×10^{-7}	0.01
$^{95}_{41}Nb$	(soluble)	GI (LLI) Total body	38	5×10^{-7}	3×10^{-3}
	(insoluble)	Lung GI (LLI)	3.2	10^{-7}	3×10^{-3}
$^{97}_{41}Nb$	(soluble)	GI (ULI)		6×10^{-6}	0.08
	(insoluble)	GI (ULI)		5×10^{-6}	0.03
$^{99}_{42}Mo$	(soluble)	Kidney GI (LLI)	0.56	7×10^{-7}	5×10^{-3}
	(insoluble)	GI (LLI)		2×10^{-7}	10^{-3}
$^{96}_{43}Tc^{m}$	(soluble)	GI (LLI)		8×10^{-5}	0.4
	(insoluble)	Lung GI (LLI)	1.3	3×10^{-5}	0.3
$^{96}_{43}Tc$	(soluble)	GI (LLI)		6×10^{-7}	3×10^{-3}
	(insoluble)	GI (LLI)		2×10^{-7}	10^{-3}
$^{97}_{43}Tc^{m}$	(soluble)	GI (LLI)		2×10^{-6}	0.01
	(insoluble)	Lung GI (LLI)	9.3	2×10^{-7}	5×10^{-3}
$^{97}_{43}Tc$	(soluble)	GI (LLI) Kidney	13	10^{-5} 10^{-5}	0.05
	(insoluble)	Lung GI (LLI)	42	3×10^{-7}	0.02

These tables give curies as units of radioactivity; the data for μCi (or μCi/cm³) should be multiplied by 37 to obtain data in kBq (or kBq/cm³).

Radionuclide		Critical organ	Organ content giving maximum permissible dose rate (μCi)	Derived air concentration (μCi/cm³)	Derived water concentration (ingestion) (μCi/cm³)
$^{99}_{43}Tc^{m}$	(soluble)	GI (ULI)		4×10^{-5}	0.2
	(insoluble)	GI (ULI)		10^{-5}	0.08
$^{99}_{43}Tc$	(soluble)	GI (LLI)		2×10^{-6}	0.01
	(insoluble)	Lung GI (LLI)	8.9	6×10^{-8}	5×10^{-3}
$^{97}_{44}Ru$	(soluble)	GI (LLI)		2×10^{-6}	0.01
	(insoluble)	GI (LLI) Lung		2×10^{-6} 2×10^{-6}	0.01
$^{103}_{44}Ru$	(soluble)	GI (LLI)		5×10^{-7}	2×10^{-3}
	(insoluble)	Lung GI (LLI)	3.1	8×10^{-8}	2×10^{-3}
$^{105}_{44}Ru$	(soluble)	GI (ULI)		7×10^{-7}	3×10^{-3}
	(insoluble)	GI (ULI)		5×10^{-7}	3×10^{-3}
$^{106}_{44}Ru$	(soluble)	GI (LLI)		8×10^{-8}	4×10^{-4}
	(insoluble)	Lung GI (LLI)	0.6	6×10^{-9}	3×10^{-4}
$^{103}_{45}Rh^{m}$	(soluble)	GI (S)		8×10^{-5}	0.4
	(insoluble)	GI (S)		6×10^{-5}	0.3
$^{105}_{45}Rh$	(soluble)	GI (LLI)		8×10^{-7}	4×10^{-3}
	(insoluble)	GI (LLI)		5×10^{-7}	3×10^{-3}
$^{109}_{46}Pd$	(soluble)	GI (LLI) Kidney	4.10	10^{-6}	0.01
	(insoluble)	Lung GI (LLI)	13	7×10^{-7}	8×10^{-3}
$^{109}_{46}Pd$	(soluble)	GI (LLI)		6×10^{-7}	3×10^{-3}
	(insoluble)	GI (LLI)		4×10^{-7}	2×10^{-3}
$^{105}_{47}Ag$	(soluble)	GI (LLI)		6×10^{-7}	3×10^{-3}
	(insoluble)	Lung GI (LLI)	2.9	8×10^{-8}	3×10^{-3}
$^{110}_{47}Ag^{m}$	(soluble)	GI (LLI)		2×10^{-7}	9×10^{-4}
	(insoluble)	Lung GI (LLI)	1	10^{-8}	9×10^{-4}

These tables give curies as units of radioactivity; the data for μCi (or μCi/cm³) should be multiplied by 37 to obtain data in kBq (or kBq/cm³).

Radionuclide		Critical organ	Organ content giving maximum permissible dose rate (μCi)	Derived air concentration (μCi/cm³)	Derived water concentration (ingestion) (μCi/cm³)
$^{111}_{47}Ag$	(soluble)	GI (LLI)		3×10^{-7}	10^{-3}
	(insoluble)	GI (LLI)		2×10^{-7}	10^{-3}
$^{109}_{48}Cd$	(soluble)	GI (LLI) Liver Kidney	 14 2.6	5×10^{-8}	5×10^{-3}
	(insoluble)	Lung GI (LLI)	8.4	7×10^{-8}	5×10^{-3}
$^{115}_{48}Cd^{m}$	(soluble)	GI (LLI) Liver Kidney	 2.3	 4×10^{-8} 4×10^{-8}	7×10^{-4}
	(insoluble)	Lung GI (LLI)	1.4	4×10^{-8}	7×10^{-4}
$^{115}_{48}Cd$	(soluble)	GI (LLI)		2×10^{-7}	10^{-3}
	(insoluble)	GI (LLI)		2×10^{-7}	10^{-3}
$^{113}_{49}In^{m}$	(soluble)	GI (ULI)		8×10^{-6}	0.04
	(insoluble)	GI (ULI)		7×10^{-6}	0.04
$^{114}_{49}In^{m}$	(soluble)	GI (LLI) Kidney Spleen	 0.27 0.14	10^{-7} 10^{-7} 10^{-7}	5×10^{-4}
	(insoluble)	GI (LLI) Lung	 0.89	 2×10^{-8}	5×10^{-4}
$^{115}_{49}In^{m}$	(soluble)	GI (ULI)		2×10^{-6}	0.01
	(insoluble)	GI (ULI)		2×10^{-6}	0.01
$^{113}_{50}Sn$	(soluble)	GI (LLI) Bone	 16	 4×10^{-7}	2×10^{-3}
	(insoluble)	Lung GI (LLI)	3.6	5×10^{-8}	2×10^{-3}
$^{125}_{50}Sn$	(soluble)	GI (LLI)		10^{-7}	5×10^{-4}
	(insoluble)	Lung GI (LLI)	0.87	8×10^{-8}	5×10^{-4}
$^{122}_{51}Sb$	(soluble)	GI (LLI)		2×10^{-7}	8×10^{-4}
	(insoluble)	GI (LLI)		10^{-7}	8×10^{-4}
$^{124}_{51}Sb$	(soluble)	GI (LLI) Total body		2×10^{-7} 2×10^{-7}	7×10^{-4}
	(insoluble)	Lung GI (LLI)	0.91	2×10^{-8}	7×10^{-4}

These tables give curies as units of radioactivity; the data for μCi (or μCi/cm^3) should be multiplied by 37 to obtain data in kBq (or kBq/cm^3).

Radionuclide		Critical organ	Organ content giving maximum permissible dose rate (μCi)	Derived air concentration (μCi/cm^3)	Derived water concentration (ingestion) (μCi/cm^3)
$^{125}_{51}$Sb	(soluble)	GI (LLI) Lung Total body Bone	 3.3 56 18	5×10^{-7}	3×10^{-3}
	(insoluble)	Lung GI (LLI)	3.3	3×10^{-8}	 3×10^{-3}
$^{125}_{52}$Te^m	(soluble)	Kidney GI (LLI) Testis	1.8 0.1	4×10^{-7}	5×10^{-3} 5×10^{-3}
	(insoluble)	Lung GI (LLI)	6	10^{-7}	 3×10^{-3}
$^{127}_{52}$Te^m	(soluble)	Kidney Testis GI (LLI)	0.79 0.063	10^{-7} 10^{-7}	2×10^{-3} 2×10^{-3} 2×10^{-3}
	(insoluble)	Lung GI (LLI)	2.6	4×10^{-8}	 2×10^{-3}
$^{127}_{52}$Te	(soluble)	GI (LLI)		2×10^{-6}	8×10^{-3}
	(insoluble)	GI (LLI)		9×10^{-7}	5×10^{-3}
$^{129}_{52}$Te^m	(soluble)	GI (LLI) Kidney Testis	 0.32 0.016	8×10^{-8}	10^{-3} 10^{-3} 10^{-3}
	(insoluble)	Lung GI (LLI)	1	3×10^{-8}	 6×10^{-4}
$^{129}_{52}$Te	(soluble)	GI (S)		5×10^{-6}	0.02
	(insoluble)	GI (ULI)		4×10^{-8}	0.02
$^{131}_{52}$Te^m	(soluble)	GI (LLI)		4×10^{-7}	2×10^{-3}
	(insoluble)	GI (LLI)		2×10^{-7}	10^{-3}
$^{132}_{52}$Te	(soluble)	GI (LLI)		2×10^{-7}	9×10^{-4}
	(insoluble)	GI (LLI)		10^{-7}	6×10^{-4}
$^{125}_{53}$I	(soluble)	Thyroid	0.21	8×10^{-9}	5×10^{-5}
	(insoluble)	Lung GI (LLI)	4.7	3×10^{-7}	 3×10^{-3}
$^{129}_{53}$I	(soluble)	Thyroid	0.49	2×10^{-9}	10^{-5}
	(insoluble)	Lung GI (LLI)	10	7×10^{-8}	 6×10^{-3}

These tables give curies as units of radioactivity; the data for μCi (or μCi/cm^3) should be multiplied by 37 to obtain data in kBq (or kBq/cm^3).

Radionuclide		Critical organ	Organ content giving maximum permissible dose rate (μCi)	Derived air concentration (μCi/cm^3)	Derived water concentration (ingestion) (μCi/cm^3)
$^{131}_{53}$I	(soluble)	Thyroid	0.15	9×10^{-9}	6×10^{-5}
	(insoluble)	GI (LLI)		3×10^{-7}	2×10^{-3}
		Lung	2.8	3×10^{-7}	
$^{132}_{53}$I	(soluble)	Thyroid	0.052	2×10^{-7}	2×10^{-3}
	(insoluble)	GI (ULI)		9×10^{-7}	5×10^{-3}
$^{133}_{53}$I	(soluble)	Thyroid	0.062	3×10^{-8}	2×10^{-4}
	(insoluble)	GI (LLI)		2×10^{-7}	10^{-3}
$^{134}_{53}$I	(soluble)	Thyroid	0.041	5×10^{-7}	4×10^{-3}
	(insoluble)	GI (S)		3×10^{-6}	0.02
$^{135}_{53}$I	(soluble)	Thyroid	0.065	10^{-7}	7×10^{-4}
	(insoluble)	GI (LLI)		4×10^{-7}	2×10^{-3}
$^{131}_{54}$Xe^m	(submersion)	Total body		2×10^{-6}	
$^{133}_{54}$Xe	(submersion)	Total body		10^{-6}	
$^{135}_{54}$Xe	(submersion)	Total body		4×10^{-6}	
$^{131}_{55}$Cs	(soluble)	Total body	680	10^{-5}	0.07
		Liver	60	10^{-5}	
	(insoluble)	Lung	35	3×10^{-6}	
		GI (LLI)			0.03
$^{134}_{55}$Cs^m	(soluble)	GI (S)		4×10^{-5}	0.2
	(insoluble)	GI (ULI)		6×10^{-6}	0.03
$^{134}_{55}$Cs	(soluble)	Total body	18	4×10^{-8}	3×10^{-4}
	(insoluble)	Lung	1.5	10^{-8}	
		GI (LLI)			10^{-3}
$^{135}_{55}$Cs	(soluble)	Liver	22	5×10^{-7}	3×10^{-3}
		Spleen	1.9	5×10^{-7}	
		Total body	300		
	(insoluble)	GI (LLI)			7×10^{-3}
		Lung	13	9×10^{-8}	
$^{136}_{55}$Cs	(soluble)	Total body	30	4×10^{-7}	2×10^{-3}
	(insoluble)	Lung	2.4	2×10^{-7}	
		GI (LLI)			2×10^{-3}

These tables give curies as units of radioactivity; the data for μCi (or μCi/cm³) should be multiplied by 37 to obtain data in kBq (or kBq/cm³).

Radionuclide		Critical organ	Organ content giving maximum permissible dose rate (μCi)	Derived air concentration (μCi/cm³)	Derived water concentration (ingestion) (μCi/cm³)
$^{137}_{55}Cs$	(soluble)	Total body Liver Spleen Muscle	33 3.5 0.34 14	6×10^{-8}	4×10^{-6}
	(insoluble)	Lung GI (LLI)	2	10^{-8}	10^{-3}
$^{131}_{56}Ba$	(soluble)	GI (LLI)		10^{-6}	5×10^{-3}
	(insoluble)	Lung GI (LLI)	4.4	4×10^{-7}	5×10^{-3}
$^{140}_{56}Ba$	(soluble)	GI (LLI) Bone	2.6	10^{-7}	8×10^{-4}
	(insoluble)	Lung GI (LLI)	0.8	4×10^{-8}	7×10^{-4}
$^{140}_{57}La$	(soluble)	GI (LLI)		2×10^{-7}	7×10^{-4}
	(insoluble)	GI (LLI)		10^{-7}	7×10^{-4}
$^{141}_{58}Ce$	(soluble)	GI (LLI) Liver Bone	7.9 14	4×10^{-7}	3×10^{-3}
	(insoluble)	Lung GI (LLI)	4.7	2×10^{-7}	3×10^{-3}
$^{143}_{58}Ce$	(soluble)	GI (LLI)		3×10^{-7}	10^{-3}
	(insoluble)	GI (LLI)		2×10^{-7}	10^{-3}
$^{144}_{58}Ce$	(soluble)	GI (LLI) Bone Liver	1.7	10^{-8} 10^{-8}	3×10^{-4}
	(insoluble)	Lung GI (LLI)	0.64	6×10^{-9}	3×10^{-4}
$^{142}_{59}Pr$	(soluble)	GI (LLI)		2×10^{-7}	9×10^{-4}
	(insoluble)	GI (LLI)		2×10^{-7}	9×10^{-4}
$^{143}_{59}Pr$	(soluble)	GI (LLI)		3×10^{-7}	10^{-3}
	(insoluble)	Lung GI (LLI)		2×10^{-7}	10^{-3}
$^{147}_{60}Nd$	(soluble)	GI (LLI) Liver	4.5	4×10^{-7} 4×10^{-7}	2×10^{-3}
	(insoluble)	Lung GI (LLI)	2.8	2×10^{-7}	2×10^{-3}

These tables give curies as units of radioactivity; the data for μCi (or μCi/cm³) should be multiplied by 37 to obtain data in kBq (or kBq/cm³).

Radionuclide		Critical organ	Organ content giving maximum permissible dose rate (μCi)	Derived air concentration (μCi/cm³)	Derived water concentration (ingestion) (μCi/cm³)
$^{149}_{60}$Nd	(soluble)	GI (LLI)		2×10^{-6}	8×10^{-3}
	(insoluble)	GI (ULI)		10^{-6}	8×10^{-3}
$^{147}_{61}$Pm	(soluble)	GI (LLI) Bone	 31	 6×10^{-9}	6×10^{-3}
	(insoluble)	Lung GI (LLI)	12	10^{-7}	 6×10^{-3}
$^{149}_{61}$Pm	(soluble)	GI (LLI)		3×10^{-7}	10^{-3}
	(Insoluble)	GI (LLI)		2×10^{-7}	10^{-3}
$^{147}_{62}$Sm	(soluble)	Bone GI (LLI)	9.5×10^{-2}	7×10^{-11}	2×10^{-3} 2×10^{-3}
	(insoluble)	Lung GI (LLI)	0.036	3×10^{-10}	 2×10^{-3}
$^{151}_{62}$Sm	(soluble)	GI (LLI) Bone	 84	 6×10^{-8}	0.01
	(insoluble)	Lung GI (LLI)	20	10^{-7}	 0.01
$^{153}_{62}$Sm	(soluble)	GI (LLI)		5×10^{-7}	2×10^{-3}
	(insoluble)	GI (LLI)		4×10^{-7}	2×10^{-3}
$^{152}_{63}$Eu (9.2 h)	(soluble)	GI (LLI)		4×10^{-7}	2×10^{-3}
	(insoluble)	GI (LLI)		3×10^{-7}	2×10^{-3}
$^{152}_{63}$Eu (13 yr)	(soluble)	GI (LLI) Kidney	 1	 10^{-8}	2×10^{-3}
	(insoluble)	Lung GI (LLI)	2.5	2×10^{-8}	 2×10^{-3}
$^{154}_{63}$Eu	(soluble)	GI (LLI) Kidney Bone	 0.33 4.1	 4×10^{-9} 4×10^{-9}	6×10^{-4}
	(insoluble)	Lung GI (LLI)	0.97	7×10^{-9}	 6×10^{-4}
$^{155}_{63}$Eu	(soluble)	GI (LLI) Kidney Bone	 3 39	 9×10^{-8}	6×10^{-3}
	(insoluble)	Lung GI (LLI)	8.8	7×10^{-8}	 6×10^{-3}

These tables give curies as units of radioactivity; the data for μCi (or μCi/cm^3) should be multiplied by 37 to obtain data in kBq (or kBq/cm^3).

Radionuclide		Critical organ	Organ content giving maximum permissible dose rate (μCi)	Derived air concentration (μCi/cm^3)	Derived water concentration (ingestion) (μCi/cm^3)
$^{153}_{64}$Gd	(soluble)	GI (LLI) Bone	47	2×10^{-7}	6×10^{-3}
	(insoluble)	Lung GI (LLI)	8.5	9×10^{-8}	6×10^{-3}
$^{159}_{64}$Gd	(soluble)	GI (LLI)		5×10^{-7}	2×10^{-3}
	(insoluble)	GI (LLI)		4×10^{-7}	2×10^{-3}
$^{160}_{65}$Tb	(soluble)	GI (LLI) Bone Kidney Total body	10	 10^{-7} 10^{-7} 10^{-7}	10^{-3}
	(insoluble)	Lung GI (LLI)	1.7	3×10^{-8}	10^{-3}
$^{165}_{66}$Dy	(soluble)	GI (ULI)		3×10^{-6}	0.01
	(insoluble)	GI (ULI)		2×10^{-6}	0.01
$^{166}_{66}$Dy	(soluble)	GI (LLI)		2×10^{-7}	10^{-3}
	(insoluble)	GI (LLI)		2×10^{-7}	10^{-3}
$^{166}_{67}$Ho	(soluble)	GI (LLI)		2×10^{-7}	9×10^{-4}
	(insoluble)	GI (LLI)		2×10^{-7}	9×10^{-4}
$^{169}_{68}$Er	(soluble)	GI (LLI)		6×10^{-7}	3×10^{-3}
	(insoluble)	Lung GI (LLI)	3.8	4×10^{-7}	3×10^{-3}
$^{171}_{68}$Er	(soluble)	GI (ULI)		7×10^{-7}	3×10^{-3}
	(insoluble)	GI (ULI)		6×10^{-7}	3×10^{-3}
$^{170}_{69}$Tm	(soluble)	GI (LLI) Bone	6.5	 4×10^{-8}	10^{-3}
	(insoluble)	Lung GI (LLI)	2.5	3×10^{-8}	10^{-3}
$^{171}_{69}$Tm	(soluble)	GI (LLI) Bone	73	 10^{-7}	0.01
	(insoluble)	Lung GI (LLI)	28	2×10^{-7}	0.01
$^{175}_{70}$Yb	(soluble)	GI (LLI)		7×10^{-7}	3×10^{-3}
	(insoluble)	GI (LLI)		6×10^{-7}	3×10^{-3}

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Radionuclide		Critical organ	Organ content giving maximum permissible dose rate (μCi)	Derived air concentration (μCi/cm^3)	Derived water concentration (ingestion) (μCi/cm^3)
$^{177}_{71}$Lu	(soluble)	GI (LLI)		6×10^{-7}	3×10^{-3}
	(insoluble)	GI (LLI) Lung	5.2	5×10^{-7}	3×10^{-3}
$^{181}_{72}$Hf	(soluble)	GI (LLI) Spleen	0.50	4×10^{-8}	2×10^{-3}
	(insoluble)	Lung GI (LLI)	2.9	7×10^{-8}	2×10^{-3}
$^{182}_{73}$Ta	(soluble)	GI (LLI) Liver	2.6	4×10^{-8}	10^{-3}
	(insoluble)	Lung GI (LLI)	1.5	2×10^{-8}	10^{-3}
$^{181}_{74}$W	(soluble)	GI (LLI)		2×10^{-6}	0.01
	(insoluble)	Lung GI (LLI)	9.6	10^{-7}	0.01
$^{185}_{74}$W	(soluble)	GI (LLI)		8×10^{-7}	4×10^{-3}
	(insoluble)	Lung GI (LLI)	6	10^{-7}	3×10^{-3}
$^{187}_{74}$W	(soluble)	GI (LLI)		4×10^{-7}	2×10^{-3}
	(insoluble)	GI (LLI)		3×10^{-7}	2×10^{-3}
$^{183}_{75}$Re	(soluble)	GI (LLI) Total body	82	3×10^{-6}	0.02 0.02
	(insoluble)	Lung GI (LLI)	8.4	2×10^{-7}	8×10^{-3}
$^{186}_{75}$Re	(soluble)	GI (LLI)		6×10^{-7}	3×10^{-3}
	(insoluble)	GI (LLI)		2×10^{-7}	10^{-3}
$^{187}_{75}$Re	(soluble)	GI (LLI) Skin	280	9×10^{-6}	0.07
	(insoluble)	Lung GI (LLI)	70	5×10^{-7}	0.04
$^{188}_{75}$Re	(soluble)	GI (LLI)		4×10^{-7}	2×10^{-3}
	(insoluble)	GI (LLI)		2×10^{-7}	9×10^{-4}
$^{195}_{76}$Os	(soluble)	GI (LLI)		5×10^{-7}	2×10^{-3}
	(insoluble)	Lung GI (LLI)	2.9	5×10^{-8}	2×10^{-3}

These tables give curies as units of radioactivity; the data for μCi (or μCi/cm^3) should be multiplied by 37 to obtain data in kBq (or kBq/cm^3).

Radionuclide		Critical organ	Organ content giving maximum permissible dose rate (μCi)	Derived air concentration (μCi/cm^3)	Derived water concentration (ingestion) (μCi/cm^3)
$^{191}_{76}Os^m$	(soluble)	GI (LLI)		2×10^{-5}	0.07
	(insoluble)	Lung GI (LLI)	6.4	9×10^{-6}	0.07
$^{191}_{76}Os$	(soluble)	GI (LLI)		10^{-6}	5×10^{-3}
	(insoluble)	Lung GI (LLI)	7	4×10^{-7}	5×10^{-3}
$^{193}_{76}Os$	(soluble)	GI (LLI)		4×10^{-7}	2×10^{-3}
	(insoluble)	GI (LLI)		3×10^{-7}	2×10^{-3}
$^{190}_{77}Ir$	(soluble)	GI (LLI)		10^{-5}	6×10^{-3}
	(insoluble)	Lung GI (LLI)	5.2	4×10^{-7}	5×10^{-3}
$^{192}_{77}Ir$	(soluble)	GI (LLI) Kidney Spleen	0.5	 10^{-7} 10^{-7}	10^{-3}
	(insoluble)	Lung GI (LLI)	1.4	3×10^{-8}	10^{-3}
$^{194}_{77}Ir$	(soluble)	GI (LLI)		2×10^{-7}	10^{-3}
	(insoluble)	GI (LLI)		2×10^{-7}	9×10^{-4}
$^{191}_{78}Pt$	(soluble)	GI (LLI)		8×10^{-7}	4×10^{-3}
	(insoluble)	GI (LLI)		6×10^{-7}	3×10^{-3}
$^{193}_{78}Pt^m$	(soluble)	GI (LLI)		7×10^{-6}	0.03
	(insoluble)	GI (LLI) Lung	28	5×10^{-6}	0.03
$^{193}_{78}Pt$	(soluble)	Kidney	18	10^{-6}	0.03
	(insoluble)	Lung GI (LLI)	44	3×10^{-7}	0.05
$^{197}_{78}Pt^m$	(soluble)	GI (ULI)		6×10^{-6}	0.03
	(insoluble)	GI (ULI)		5×10^{-6}	0.03
$^{197}_{78}Pt$	(soluble)	GI (LLI)		8×10^{-7}	4×10^{-3}
	(insoluble)	GI (LLI)		6×10^{-7}	3×10^{-3}
$^{195}_{79}Au$	(soluble)	GI (LLI)		10^{-6}	5×10^{-3}
	(insoluble)	Lung GI (LLI)	4	6×10^{-7}	4×10^{-3}

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Radionuclide		Critical organ	Organ content giving maximum permissible dose rate (μCi)	Derived air concentration (μCi/cm³)	Derived water concentration (ingestion) (μCi/cm³)
$^{196}_{79}Au$	(soluble)	GI (LLI)		3×10^{-7}	2×10^{-3}
	(insoluble)	GI (LLI)		2×10^{-7}	10^{-3}
$^{199}_{79}Au$	(soluble)	GI (LLI)		10^{-6}	5×10^{-3}
	(insoluble)	GI (LLI)		8×10^{-7}	4×10^{-3}
$^{197}_{80}Hg^{m}$	(soluble)	Kidney	1.4	7×10^{-7}	6×10^{-3}
	(insoluble)	GI (LLI)		8×10^{-7}	5×10^{-3}
$^{197}_{80}Hg$	(soluble)	Kidney	5.9	10^{-6}	9×10^{-3}
	(insoluble)	GI (LLI)		3×10^{-6}	0.01
$^{203}_{80}Hg$	(soluble)	Kidney	1.7	7×10^{-8}	5×10^{-4}
	(insoluble)	Lung GI (LLI)	4.9	10^{-7}	3×10^{-3}
$^{200}_{81}Tl$	(soluble)	GI (LLI)		3×10^{-6}	0.01
	(insoluble)	GI (LLI)		10^{-6}	7×10^{-3}
$^{201}_{81}Tl$	(soluble)	GI (LLI)		2×10^{-6}	9×10^{-3}
	(insoluble)	GI (LLI)		9×10^{-7}	5×10^{-3}
$^{202}_{81}Tl$	(soluble)	GI (LLI)		8×10^{-7}	4×10^{-3}
	(insoluble)	Lung GI (LLI)	3.1	2×10^{-7}	2×10^{-3}
$^{204}_{81}Tl$	(soluble)	GI (LLI) Kidney	1	6×10^{-7}	3×10^{-3}
	(insoluble)	Lung GI (LLI)	3.4	3×10^{-6}	2×10^{-3}
$^{203}_{82}Pb$	(soluble)	GI (LLI)		3×10^{-6}	0.01
	(insoluble)	GI (LLI)		2×10^{-6}	0.01
$^{210}_{82}Pb$	(soluble)	Kidney Total body	0.025	10^{-10}	4×10^{-6} 4×10^{-6}
	(insoluble)	Lung GI (LLI)	0.034	2×10^{-10}	5×10^{-3}
$^{212}_{82}Pb$	(soluble)	Kidney GI (LLI)	0.0031	2×10^{-8}	6×10^{-4} 6×10^{-4}
	(insoluble)	Lung GI (LLI)	0.010	2×10^{-8}	5×10^{-4}
$^{206}_{83}Bi$	(soluble)	GI (LLI) Kidney	0.43	2×10^{-7} 2×10^{-7}	10^{-3}
	(insoluble)	Lung GI (LLI)	1	10^{-7}	10^{-3}

These tables give curies as units of radioactivity; the data for μCi (or μCi/cm^3) should be multiplied by 37 to obtain data in kBq (or kBq/cm^3).

Radionuclide		Critical organ	Organ content giving maximum permissible dose rate (μCi)	Derived air concentration (μCi/cm^3)	Derived water concentration (ingestion) (μCi/cm^3)
$^{207}_{83}$Bi	(soluble)	GI (LLI) Kidney	0.76	2×10^{-7}	2×10^{-3}
	(insoluble)	Lung GI (LLI)	1.9	10^{-8}	2×10^{-3}
$^{210}_{83}$Bi	(soluble)	GI (LLI) Kidney	0.013	6×10^{-9}	10^{-3}
	(insoluble)	Lung GI (LLI)	0.032	6×10^{-9}	10^{-3}
$^{212}_{83}$Bi	(soluble)	GI (S) Kidney	0.0030	10^{-7}	0.01
	(insoluble)	Lung GI (S)	0.010	2×10^{-7}	0.01
$^{210}_{84}$Po	(soluble)	Spleen Kidney	0.002 0.0045	5×10^{-10} 5×10^{-10}	2×10^{-5} 2×10^{-5}
	(insoluble)	Lung GI (LLI)	0.015	2×10^{-10}	8×10^{-4}
$^{211}_{85}$At	(soluble)	Thyroid Ovary	0.00047 0.000031	7×10^{-9} 7×10^{-9}	5×10^{-5} 5×10^{-5}
	(insoluble)	Lung GI (ULI)	0.11	3×10^{-8}	2×10^{-3}
$^{220}_{86}$Rn[a]		Lung		3×10^{-7} [a]	
$^{222}_{86}$Rn[a]		Lung		3×10^{-8} [a]	
$^{223}_{88}$Ra	(soluble)	Bone	0.039	2×10^{-9}	2×10^{-5}
	(insoluble)	Lung GI (LLI)	3×10^{-3}	2×10^{-10}	10^{-4}
$^{224}_{88}$Ra	(soluble)	Bone	0.039	5×10^{-9}	7×10^{-5}
	(insoluble)	Lung GI (LLI)	0.0029	7×10^{-10}	2×10^{-4}
$^{226}_{88}$Ra	(soluble)	Bone	0.1	3×10^{-11}	4×10^{-7}
	(insoluble)	GI (LLI)		2×10^{-7}	9×10^{-4}
$^{228}_{88}$Ra	(soluble)	Bone	0.058	7×10^{-11}	8×10^{-7}
	(insoluble)	Lung GI (LLI)	0.0052	4×10^{-11}	7×10^{-4}

[a] The daughter elements of ^{220}Rn and ^{222}Rn are assumed to be present to the extent that they occur in unfiltered air. For all other radionuclides, the daughter elements are not considered as part of the intake; if daughter elements are present, they must be considered on the basis of the rules for mixtures.

These tables give curies as units of radioactivity; the data for μCi (or μCi/cm³) should be multiplied by 37 to obtain data in kBq (or kBq/cm³).

Radionuclide		Critical organ	Organ content giving maximum permissible dose rate (μCi)	Derived air concentration (μCi/cm³)	Derived water concentration (ingestion) (μCi/cm³)
$^{227}_{89}$Ac	(soluble)	Bone	0.011	2×10^{-12}	6×10^{-5}
	(insoluble)	Lung GI (LLI)	0.0036	3×10^{-11}	9×10^{-3}
$^{228}_{89}$Ac	(soluble)	GI (ULI) Bone Liver	 0.011 0.026	 8×10^{-8}	3×10^{-3}
	(insoluble)	Lung GI (ULI)	0.0052	2×10^{-8}	3×10^{-3}
$^{227}_{90}$Th	(soluble)	GI (LLI) Bone	0.03 0.011	 3×10^{-10}	5×10^{-4}
	(insoluble)	Lung GI (LLI)	0.0036	2×10^{-10}	5×10^{-4}
$^{228}_{90}$Th	(soluble)	Bone	0.011	9×10^{-12}	2×10^{-4}
	(insoluble)	Lung GI (LLI)	3.5×10^{-3}	6×10^{-12}	4×10^{-4}
$^{230}_{90}$Th	(soluble)	Bone	0.046	2×10^{-12}	5×10^{-5}
	(insoluble)	Lung GI (LLI)	0.017	10^{-11}	9×10^{-4}
$^{231}_{90}$Th	(soluble)	GI (LLI)		10^{-6}	7×10^{-3}
	(insoluble)	GI (LLI)		10^{-6}	7×10^{-3}
$^{232}_{90}$Th [a]	(soluble)	Bone	0.041	2×10^{-12} [a]	5×10^{-5}
	(insoluble)	Lung GI (LLI)	0.018	10^{-11}	10^{-3}
$^{234}_{90}$Th	(soluble)	GI (LLI) Bone	 2.4	 6×10^{-8}	5×10^{-4}
	(insoluble)	Lung GI (LLI)	0.93	3×10^{-8}	5×10^{-4}
$_{90}$Th (natural) [a]	(soluble)	Bone		2×10^{-12} [a]	3×10^{-5}
	(insoluble)	Lung GI (LLI)		4×10^{-12}	3×10^{-4}

[a] Provisional values for ^{232}Th and natural thorium. Although calculations and animal experiments suggest that natural thorium, if injected intravenously, is perhaps as hazardous as plutonium and therefore the above-listed values are indicated, experience to date has suggested that in industry the hazard of natural thorium is not much greater than that of natural uranium. Therefore, pending further investigation, the value of $(DAC)_a = 3 \times 10^{-11}$ μCi/cm³ for occupational exposure (40 h/week) is recommended as a provisional level, permissible for exposure to inhaled natural thorium or ^{232}Th. However, the above values are listed to indicate the possibility that further evidence may require lower values and to urge especially that exposure levels for these radionuclides be kept as low as is operationally readily achievable. It may be possible to show that similar considerations apply to other inhaled long-lived thorium isotopes under conditions in which the physical characteristics of the airborne particulates are much the same as in the case of natural thorium and where there is a large amount of airborne material serving as an effective carrier for the thorium.

These tables give curies as units of radioactivity; the data for μCi (or μCi/cm^3) should be multiplied by 37 to obtain data in kBq (or kBq/cm^3).

Radionuclide		Critical organ	Organ content giving maximum permissible dose rate (μCi)	Derived air concentration (μCi/cm^3)	Derived water concentration (ingestion) (μCi/cm^3)
$^{230}_{91}$Pa	(soluble)	GI (LLI) Bone	0.034	2×10^{-9}	7×10^{-3}
	(insoluble)	Lung GI (LLI)	0.014	8×10^{-10}	7×10^{-3}
$^{231}_{91}$Pa	(soluble)	Bone	0.015	10^{-12}	3×10^{-5}
	(insoluble)	Lung GI (LLI)	0.016	10^{-10}	8×10^{-4}
$^{233}_{91}$Pa	(soluble)	GI (LLI) Kidney	1.7	6×10^{-7}	4×10^{-3}
	(insoluble)	Lung GI (LLI)	4.7	2×10^{-7}	3×10^{-3}
$^{230}_{92}$U [a]	(soluble)	Kidney	0.00072	3×10^{-10}	7×10^{-5}
	(insoluble)	Lung GI (LLI)	0.0024	10^{-10}	10^{-4}
$^{232}_{92}$U [a]	(soluble)	Bone	0.0091	10^{-10}	2×10^{-5}
	(insoluble)	Lung GI (LLI)	0.004	3×10^{-11}	8×10^{-4}
$^{233}_{92}$U [a]	(soluble)	Bone	0.044	5×10^{-10}	10^{-4}
	(insoluble)	Lung GI (LLI)	0.017	10^{-10}	9×10^{-4}
$^{234}_{92}$U [a]	(soluble)	Bone	0.046	6×10^{-10}	10^{-4}
	(insoluble)	Lung GI (LLI)	0.017	10^{-10}	9×10^{-4}
$^{235}_{92}$U [a]	(soluble)	Kidney Bone	1.9×10^{-3} 0.048	5×10^{-10}	10^{-4} 10^{-4}
	(insoluble)	Lung GI (LLI)	0.018	10^{-10}	8×10^{-4}
$^{236}_{92}$U [a]	(soluble)	Bone	0.047	6×10^{-10}	10^{-4}
	(insoluble)	Lung GI (LLI)	0.018	10^{-10}	10^{-3}
$^{238}_{92}$U [a]	(soluble)	Kidney	3.1×10^{-4}	7×10^{-11}	2×10^{-5}
	(insoluble)	Lung GI (LLI)	0.02	10^{-10}	10^{-3}

[a] Because of the chemical toxicity of natural uranium, ^{238}U, ^{236}U, and ^{235}U in soluble form, inhalation of uranium of any isotopic composition should not exceed 2.5 mg of soluble uranium per day; ingestion of soluble uranium should not exceed 150 mg per day.

These tables give curies as units of radioactivity; the data for μCi (or μCi/cm^3) should be multiplied by 37 to obtain data in kBq (or kBq/cm^3).

Radionuclide		Critical organ	Organ content giving maximum permissible dose rate (μCi)	Derived air concentration (μCi/cm^3)	Derived water concentration (ingestion) (μCi/cm^3)
^{92}U (natural)[a]	(soluble)	Kidney		7×10^{-11}	2×10^{-6}
	(insoluble)	Lung GI (LLI)		6×10^{-11}	5×10^{-4}
$^{240}_{92}$U + ^{240}Np	(soluble)	GI (LLI)		2×10^{-7}	10^{-3}
	(insoluble)	GI (LLI)		2×10^{-7}	10^{-3}
$^{237}_{93}$Np	(soluble)	Bone	0.044	4×10^{-12}	9×10^{-5}
	(insoluble)	Lung GI (LLI)	0.017	10^{-10}	9×10^{-4}
$^{239}_{93}$Np	(soluble)	GI (LLI)		8×10^{-7}	4×10^{-3}
	(insoluble)	GI (LLI)		7×10^{-7}	4×10^{-3}
$^{238}_{94}$Pu	(soluble)	Bone	0.039	2×10^{-12}	10^{-4}
	(insoluble)	Lung GI (LLI)	0.015	3×10^{-11}	8×10^{-4}
$^{239}_{94}$Pu	(soluble)	Bone	0.041	2×10^{-12}	10^{-4}
	(insoluble)	Lung GI (LLI)	0.016	4×10^{-11}	8×10^{-4}
$^{240}_{94}$Pu	(soluble)	Bone	0.041	2×10^{-12}	10^{-4}
	(insoluble)	Lung GI (LLI)	0.016	4×10^{-11}	8×10^{-4}
$^{241}_{94}$Pu	(soluble)	Bone	0.78	9×10^{-11}	7×10^{-3}
	(insoluble)	Lung GI (LLI)	16	4×10^{-8}	0.04
$^{242}_{94}$Pu	(soluble)	Bone	0.044	2×10^{-12}	10^{-4}
	(insoluble)	Lung GI (LLI)	0.016	4×10^{-11}	9×10^{-4}
$^{243}_{94}$Pu	(soluble)	GI (ULI)		2×10^{-6}	0.01
	(insoluble)	GI (ULI)		2×10^{-6}	0.01
$^{244}_{94}$Pu	(soluble)	Bone	0.045	2×10^{-12}	10^{-4}
	(insoluble)	Lung GI (LLI)	0.017	3×10^{-11}	3×10^{-4}

[a] Because of the chemical toxicity of natural uranium, ^{238}U, ^{236}U, and ^{235}U in soluble form, inhalation of uranium of any isotopic composition should not exceed 2.5 mg of soluble uranium per day; ingestion of soluble uranium should not exceed 150 mg per day.

These tables give curies as units of radioactivity; the data for μCi (or μCi/cm^3) should be multiplied by 37 to obtain data in kBq (or kBq/cm^3).

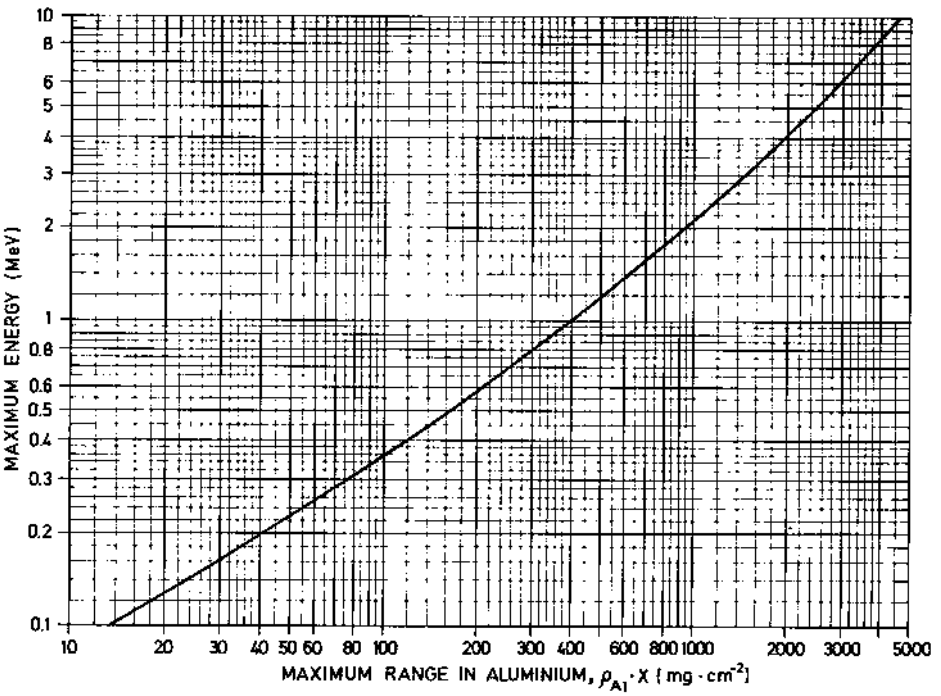
Radionuclide		Critical organ	Organ content giving maximum permissible dose rate (μCi)	Derived air concentration (μCi/cm^3)	Derived water concentration (ingestion) (μCi/cm^3)
$^{241}_{95}$Am	(soluble)	Kidney Bone	0.0044 0.039	6×10^{-12} 6×10^{-12}	10^{-4} 10^{-4}
	(insoluble)	Lung GI (LLI)	0.015	10^{-10}	8×10^{-4}
$^{242}_{95}$Am^m	(soluble)	Bone Kidney	0.036	6×10^{-12} 6×10^{-12}	10^{-4} 10^{-4}
	(insoluble)	Lung GI (LLI)	0.037	3×10^{-10}	9×10^{-4}
$^{242}_{95}$Am	(soluble)	GI (LLI) Liver	0.098 0.023	4×10^{-8}	4×10^{-3}
	(insoluble)	Lung GI (LLI)	0.037	5×10^{-8}	4×10^{-3}
$^{244}_{95}$Am	(soluble)	GI (SI) Bone Kidney	 0.044 0.044	 4×10^{-6} 4×10^{-6}	0.1
	(insoluble)	Lung GI (SI)	0.52	2×10^{-5} 2×10^{-5}	0.1
$^{242}_{96}$Cm	(soluble)	GI (LLI) Liver	 0.018	 10^{-10}	7×10^{-4}
	(insoluble)	Lung GI (LLI)	0.013	2×10^{-10}	7×10^{-4}
$^{243}_{96}$Cm	(soluble)	Bone	0.037	6×10^{-12}	10^{-4}
	(insoluble)	Lung GI (LLI)	0.014	10^{-10}	7×10^{-4}
$^{244}_{96}$Cm	(soluble)	Bone	0.037	9×10^{-12}	2×10^{-4}
	(insoluble)	Lung GI (LLI)	0.014	10^{-10}	8×10^{-4}
$^{245}_{96}$Cm	(soluble)	Bone	0.039	5×10^{-12}	10^{-4}
	(insoluble)	Lung GI (LLI)	0.015	10^{-10}	8×10^{-4}
$^{246}_{96}$Cm	(soluble)	Bone	0.039	5×10^{-12}	10^{-4}
	(insoluble)	Lung GI (LLI)	0.015	10^{-10}	8×10^{-4}
$^{247}_{96}$Cm	(soluble)	Bone	0.041	5×10^{-12}	10^{-4}
	(insoluble)	Lung GI (LLI)	0.015	10^{-10}	6×10^{-4}
$^{248}_{96}$Cm	(soluble)	Bone	0.0048	8×10^{-13}	4×10^{-5}
	(insoluble)	Lung GI (LLI)	0.0018	10^{-11}	4×10^{-5}

These tables give curies as units of radioactivity; the data for μCi (or μCi/cm^3) should be multiplied by 37 to obtain data in kBq (or kBq/cm^3).

Radionuclide		Critical organ	Organ content giving maximum permissible dose rate (μCi)	Derived air concentration (μCi/cm^3)	Derived water concentration (ingestion) (μCi/cm^3)
$^{248}_{96}$Cm	(soluble)	GI (S) Bone	0.41	10^{-5} 10^{-8}	0.06
	(insoluble)	GI (S)		10^{-5}	0.06
$^{249}_{97}$Bk	(soluble)	GI (LLI) Bone	0.55	9×10^{-10}	0.02
	(insoluble)	Lung GI (LLI)	12	10^{-7}	0.02
$^{250}_{97}$Bk	(soluble)	GI (ULI) Bone	0.038	10^{-7}	6×10^{-3}
	(insoluble)	GI (ULI)		10^{-5}	6×10^{-3}
$^{249}_{98}$Cf	(soluble)	Bone	0.037	2×10^{-12}	10^{-4}
	(insoluble)	Lung GI (LLI)	0.014	10^{-10}	7×10^{-4}
$^{250}_{98}$Cf	(soluble)	Bone	0.035	5×10^{-12}	4×10^{-4}
	(insoluble)	Lung GI (LLI)	0.014	10^{-10}	7×10^{-4}
$^{251}_{98}$Cf	(soluble)	Bone	0.038	2×10^{-12}	10^{-4}
	(insoluble)	Lung GI (LLI)	0.014	10^{-10}	8×10^{-4}
$^{252}_{98}$Cf	(soluble)	GI (LLI) Bone	0.01	6×10^{-12}	2×10^{-4}
Spontaneous fission	(insoluble)	Lung GI (LLI)	0.004	3×10^{-11}	2×10^{-4}

Appendix VI–4

BETA-PARTICLE RANGE AS A FUNCTION OF ENERGY (E_{max})



Appendix VI–5

INTEGRATION OF EQUATION (I–1). THE RADIOACTIVE DECAY LAW

Given Eq.(I–1) in Part I:

$$\frac{dN}{dt} = -\lambda N \qquad \text{(VI–5.1)}$$

Separating variables for the indefinite integration:

$$\frac{dN}{N} = -\lambda \int dt + \text{constant} \qquad \text{(VI–5.2)}$$

Now integrating

$$\ln N = -\lambda t + C \qquad \text{(VI–5.3)}$$

where C is the constant of integration. C is evaluated at any initial conditions which are:

$$N = N_0 \text{ at } t = t_0$$

That is, N_0 is the number of atoms present at any initial time, t_0. Therefore, at $t = t_0$:

$$C = \ln N_0 + \lambda t_0 \qquad \text{(VI–5.4)}$$

Substituting Eq.(VI–5.4) into (VI–5.3):

$$\ln N - \ln N_0 = -\lambda t + \lambda t_0 \qquad \text{(VI–5.5)}$$

or

$$\ln\left(\frac{N}{N_0}\right) = -\lambda(t - t_0) \qquad \text{(VI–5.5')}$$

Now taking the antilogarithm:

$$\frac{N}{N_0} = e^{-\lambda(t - t_0)} \qquad \text{(VI–5.6)}$$

or

$$N = N_0\, e^{-\lambda(t - t_0)} \qquad \text{(VI–5.7)}$$

In the special case of $t_0 = 0$, Eq.(VI–5.7) becomes:

$$N = N_0\, e^{-\lambda t}$$

giving the desired equation (I–3).

Appendix VI–6

DERIVATION OF EQUATION (I–31)

From Eq. (I-30) in Part I:

$$\sigma_{nat,R_s} = \left(\frac{R}{T} + \frac{R_b}{T_b}\right)^{\frac{1}{2}} \qquad \text{(VI–6.1)}$$

where σ_{nat,R_s} is the natural standard deviation of the net sample count-rate.

By squaring and taking the time derivative of both sides

$$2\sigma_{nat,R_s} \cdot d\sigma_{nat,R_s} = -\frac{R}{T^2}\,dT - \frac{R_b}{T_b^2}\,dT_b \qquad \text{(VI–6.2)}$$

To obtain the minimum value, set $d\sigma_{nat,R_s} = 0$; and since the given total period counting time, T_{tot}, has to be apportioned between T and T_b:

$$T_{tot} = T + T_b \qquad \text{(VI–6.3)}$$

Since T_{tot} is constant:

$$dT_{tot} = dT + dT_b = 0 \qquad \text{(VI–6.4)}$$

or

$$dT = -\,dT_b \qquad \text{(VI–6.5)}$$

Therefore, rearranging Eq.(VI–6.2) for $d\sigma_{nat,R_s} = 0$:

$$\frac{T_b^2}{T^2} = -\frac{R_b}{R}\frac{dT_b}{dT} \qquad \text{(VI–6.6)}$$

and introducing Eq.(VI–6.5):

$$\frac{T_b}{T} = \left(\frac{R_b}{R}\right)^{\frac{1}{2}} \qquad \text{(VI–6.7)}$$

which is the desired equation (I–31).

The partition of T_{tot} (between T and T_b) in conformity with Eq.(VI–6.7) corresponds mathematically to the smallest, i.e. best-partition, σ_{nat,R_s} value which is obtainable under the given conditions. If we call the fractional natural standard deviation of the net count-rate of the sample f, then, from formula (I-30):

$$f = \frac{\sigma_{nat,R_s}}{R_s} = \frac{1}{R_s}\left(\frac{R}{T} + \frac{R_b}{T_b}\right)^{\frac{1}{2}} \qquad \text{(VI–6.8)}$$

and using $T_b + T = T_{tot}$ and Eq.(VI–6.7) to substitute in Eq.(VI–6.8) for T_b and T, one obtains for the best partition value, f_{bpv}:

$$f_{bpv} = \frac{1}{T_{tot}^{\frac{1}{2}}\,(R^{\frac{1}{2}} - R_b^{\frac{1}{2}})} \qquad \text{(VI–6.9)}$$

For a given sample activity in a proportional counter (e.g. gas-flow or scintillation), the values of R and R_b may be altered independently by variation of the high voltage and/or the input-bias voltage. For a certain setting of these two variables, the so-called optimum setting, $(\sqrt{R} - \sqrt{R_b})$ will attain its maximum value, and the natural uncertainty (for the best partition of the given T_{tot}) will, according to Eq.(VI–6.7), attain its minimum value, $f_{bpv,min}$. For another sample containing a different activity the optimum setting corresponding to $f_{bpv,min}$ will in general be different.

Theoretically, the choice of operating conditions (high voltage and input-bias voltage) on the basis of minimum natural uncertainty is thus a complex problem. However, natural counting uncertainty, at least in biological experimentation, is usually not critical in comparison with technical uncertainty, except when *low* activity samples ($R \triangleq R_b$) are to be measured. But when R is not much greater than R_b, the difference $R^{\frac{1}{2}} - R_b^{\frac{1}{2}}$ may be approximated[5] by $[(R - R_b)/2R_b^{\frac{1}{2}}]$, so that the optimum setting (corresponding to $f_{bpv,min}$) may be approximated by that for which $[(R - R_b)/2R_b^{\frac{1}{2}}]$ or $(R - R_b)^2/R_b$ attains its maximum value. Since this approximate optimum criterion for low activities is equivalent to ϵ^2/R_b attaining its maximum value, it is *independent* of sample activity (ϵ is the overall counting yield).

In tracer work (non-GM counter) operating conditions are usually chosen as optimum on the basis of minimum natural uncertainty for very low-activity samples; i.e. the maximum of $(R - R_b)^2/R_b$ or ϵ^2/R_b is taken as the criterion. However, for expediency, a medium or high-activity source is normally used in finding the operating conditions that give the maximum value of $(R - R_b)^2/R_b$, which is permissible because this maximum, as mentioned above, is independent of sample activity.

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[5] $R^{\frac{1}{2}} - R_b^{\frac{1}{2}} = [R_b + (R - R_b)]^{\frac{1}{2}} - R_b^{\frac{1}{2}} = R_b^{\frac{1}{2}}\{[1 + (R - R_b)/R_b]^{\frac{1}{2}} - 1\}$

so, for $R \triangleq R_b$, using the binomial expansion:

$$R^{\frac{1}{2}} - R_b^{\frac{1}{2}} \triangleq R_b^{\frac{1}{2}}\,[1 + \tfrac{1}{2}(R - R_b)/R_b - 1] = R_b^{\frac{1}{2}}\,(R - R_b)/2R_b$$

or

$$R^{\frac{1}{2}} - R_b^{\frac{1}{2}} \triangleq (R - R_b)/2R_b^{\frac{1}{2}}.$$

Appendix VI–7

DERIVATION OF EQUATIONS (I–56) AND (I–57)

If the rate of exchange or *turnover* is proportional only to the amount of the substance present at any time, the process is random and any single particle may have a lifetime varying from 0 to ∞. The average lifetime ($\bar{t}$) will be the sum of the existence times of all the particles divided by the initial number. Therefore, if A_0 represents the initial number of tracer particles present:

$$\bar{t} = -\frac{1}{A_0} \int_{t=0}^{t=\infty} t \, dA \qquad \text{(VI–7.1)}$$

However:

$$A = A_0 \, e^{-kt} \qquad \text{(VI–7.2)}$$

when the loss is by a first-order process, and thus:

$$dA = -kA_0 \, e^{-kt} \, dt \qquad \text{(VI–7.3)}$$

Substituting in Eq.(VI–7.1):

$$\bar{t} = \frac{1}{A_0} \int_0^{\infty} tkA_0 \, e^{-kt} \, dt = k \int_0^{\infty} t \, e^{-kt} \, dt \qquad \text{(VI–7.4)}$$

Integrating:

$$\bar{t} = k \left[-\frac{e^{-kt}}{k} \left(t + \frac{1}{A} \right) \right]_0^{\infty} = k \left(0 + \frac{1}{k^2} \right) = \frac{1}{k} \qquad \text{(VI–7.5)}$$

which is Eq.(I–56), and since:

$$k = \frac{0.693}{T_{\frac{1}{2},\,biol}} \qquad \text{(see I–51)}$$

then:

$$\bar{t} = \frac{1}{0.693/T_{\frac{1}{2},biol}} = 1.44 \, T_{\frac{1}{2},biol} \qquad \text{(VI–7.6)}$$

which is the desired equation (I–57).

Appendix VI–8

DERIVATION OF EQUATION (I–69)

When ^{14}N and ^{15}N atoms combine randomly to form N_2-molecules, the latter will be binomially distributed, i.e. with fractional abundances of $^{14}N^{14}N$, $^{14}N^{15}N$ and $^{15}N^{15}N$ given by

$$(p + q)^2 \qquad \text{(VI–8.1)}$$

or

$$p^2 + 2pq + q^2 \qquad \text{(VI–8.1')}$$

where p = fractional abundance of ^{14}N, q = fractional abundance of ^{15}N, and $p + q = 1$.

By definition:

$$K = \frac{\text{abundance of } ^{14}N^{14}N}{\text{abundance of } ^{14}N^{15}N} \left\{ = \frac{H_{28\text{-}N_2}}{H_{29\text{-}N_2}} \text{ (see Eq.(I–69))} \right\} \qquad \text{(VI–8.2)}$$

Therefore, according to Eq.(VI–8.1'):

$$K = \frac{p^2}{2pq} = \frac{p}{2q} = \frac{1-q}{2q} \qquad \text{(VI–8.3)}$$

Solving for q in Eq.(VI–8.3), we find:

$$q = \frac{1}{2K+1} \qquad \text{(VI–8.4)}$$

Finally,

$$Ab_{N\text{-}15}\ (\text{at.}\%) = 100q \qquad \text{(VI–8.5)}$$

Thus, by introduction of Eq.(VI–8.4) into Eq.(VI–8.5):

$$Ab_{N\text{-}15}\ (\text{at.}\%) = \frac{100}{2K+1} \qquad \text{(VI–8.6)}$$

which is the desired equation (I–69).

PART VII. GLOSSARY OF SOME BASIC TERMS AND CONCEPTS

PART VII. GLOSSARY OF SOME BASIC TERMS AND CONCEPTS

Absorbed dose. The mean radiation energy imparted by ionizing radiation to unit mass of the irradiated material (see ICRU Report 19). Expressed in rads, grays or joules per kilogram (see **units**).

Absorber. Layer of matter inserted between source and detector, thereby causing a reduction in the radiation transmitted.

Absorbing event. An interaction, such as photoelectric absorption, the Compton effect or pair-production, by which photon energy is transferred to matter.

Abundance. Relative concentration of the individual isotope in a mixture of isotopes of the same chemical element.

Activation analysis, neutron. A method for determining trace amounts of a chemical element, based on the assay of one or more radioisotopes produced by neutron bombardment (see also **neutron activation equation**).

Activity. The amount of a radioactive substance expressed as the number of disintegrations taking place per unit time, expressed in becquerels or curies (see **units**). *Count-rate is a non-absolute measure of activity dependent on counting efficiency.*

Alpha particle, α-particle. An alpha particle comprises two neutrons and two protons (a helium nucleus), ejected at high speed from a disintegrating alpha-active nucleus.

Amplifier. A device whose output is a magnified function of its input signal, drawing its power from a source other than the signal. In counting contexts, usually an electronic unit.

Annihilation. The reaction of a particle and its antiparticle whereby they both cease to exist as such. For instance, a positron is annihilated together with an electron, and two photons of 0.51 MeV energy are created in their place.

Anti-coincidence circuit. An electronic circuit that rejects pulses arriving at two detectors *in coincidence*, i.e. within a very short interval of time, typically 1 μs.

Atomic number, Z. An integer that expresses the positive charge on the nucleus of an atom and hence defines the position of a chemical element in the periodic table. **Z** is equal to the number of protons in the nucleus of any atom that belongs to element number **Z**.

Attenuation coefficient, total. Fractional decrease in number of particles or photons per unit distance traversed in a medium as a result of interactions in that medium (see §I–1.6). This concept includes absorption and scattering.

Attenuation factor. Ratio of the photon intensity after traversing a layer of matter to its intensity before, for example matter placed in the path of a radiation beam for radiation protection purposes (a reduction factor).

Auger electron. Fast electron ejected as the result of interaction between an X-ray photon and an orbital (valence) electron, resulting in a non-radiative transition of an atom to a lower excited electronic energy state (see **isomeric transition**).

Autoradiography. Method for recording the two-dimensional distribution of radioactive material in an object (or a thin section thereof). Performed by placing the surface of the object in contact with photographic emulsion, which, by blackening on development, indicates where particles were emitted by radioactive nuclei.

Average lifetime, see **turnover time.**

Background. Signals not emanating from the tracer but from the surroundings, sample holder, etc. which the measuring system does not discriminate from the tracer signals. Background count-rates may be determined with a blank (zero-tracer) sample in place. The net count-rate of tracer in any sample is obtained by subtracting the background from the gross-sample count-rate.

Bandhead. The optical spectra of molecules appear as groups of closely spaced and partly overlapping lines. These groups are called bands. At one end the band rises to a relatively high-intensity peak that falls off sharply to zero, and this is called the bandhead. Synonym is band edge.

Becquerel (Bq). The SI derived unit of activity, being one radioactive disintegration per second.

Beta-particle, β-particle. A β^--particle is a high-speed (negatively charged) electron ejected from a nucleus during radioactive decay. A β^+-particle is a high-speed positron (positively charged electron) ejected from a nucleus during radioactive decay.

Biological half-life, see **half-life, biological.**

Body burden, permissible. The maximum acceptable amount of a given radionuclide that may be continuously contained in the human body.

Bremsstrahlung. Photon radiation emitted by fast moving charged particles that are sharply decelerated or deflected by an electric or magnetic field. (The originally German word means, literally, braking radiation.)

Carrier. (1) A given substance added in ponderable amount to a trace amount of test substance to facilitate chemical or physical separation or manipulation. (2) A ponderable amount of stable element mixed with a radioisotopic tracer of that element.

Carrier-free. Designation for a radioisotope of a given element prepared by transmutation and which is free or *practically* free of any stable isotope of that element.

Cerenkov radiation. Light emitted when a charged particle travels through a medium at a speed that exceeds the speed of light in that medium.

Channels-ratio method. A method of correcting for quench in liquid scintillation counting by establishing a relationship between (1) the increased ratio of lower to higher energy pulses due to quenching, and (2) the reduced counting efficiency due to quenching.

Closed system. A kinetic system in which no transfer of matter (or energy) to or from its surroundings takes place.

Coincidence circuit. An electronic circuit that rejects single pulses and passes a one-pulse signal only when it receives two pulses (one from each of two detectors) within a specified short interval of time, e.g. 10^{-6} seconds (i.e. *simultaneously* or in coincidence).

Compton effect. Interaction (elastic, incoherent scattering) between a (high-energy) photon and a free or loosely bound electron, whereby the photon suffers a change in direction and a loss in energy, and the electron gains an amount of energy equivalent to that lost by the photon.

Compton region. The lower-energy part of a gamma spectrum due to photons that undergo Compton interaction in the detector and then escape total absorption.

Contamination. The dirtying or soiling of objects (e.g. the body, clothes, samples, equipment) with undesired (radioactive) material.

Continuous spectrum. A spectrum that exhibits no detailed structure (lines or bands) but represents an unbroken sweep of intensity or activity over the wavelength or energy range considered.

Cosmic ray. Radiation originating outside the earth and its atmosphere, capable of producing ionizing events in interactions with matter.

Counting efficiency, counting yield, ϵ. The fraction of disintegrations counted. Expressed as a percentage, it is equal to the number of counts registered by the counter per 100 disintegrations in the source (see §I–2.7).

Count-rate. The number of counts registered per unit of time, e.g. counts per second (counts/s).

Cross-section. A value expressing the probability of a given reaction occurring, for example, between a thermal neutron and a specified atomic nucleus. The dimensions of cross-section are area, expressed in units of the order of 10^{-28} m^2 (10^{-24} cm^2); a special unit, the barn (b), is often used (see **units**).

Curie (Ci). The special unit of activity which is being superseded by the becquerel. The curie is defined as: 1 Ci = 3.7×10^{10} disintegrations/s = 3.7×10^{10} Bq.

Daughter nucleus. The nucleus to which a given radioactive nucleus is transformed when it decays.

Dead time. The period following a discharge during which an ionizing particle entering the sensitive volume of a GM tube will not initiate a new pulse (see also **resolving time**).

Decay constant. The fraction of the (large) number of radioactive atoms of a given radionuclide that decays in unit time.

Decay scheme. A diagram depicting the process(es) involved in the decay of a given radionuclide. The half-life, the branching ratios, the energies, and the type of particle(s) and/or photon(s) as well as the daughter nuclide(s) involved in the transition(s) are shown in schematic form (see §I–1.5).

Density thickness, see **mass per unit area.**

Differential discriminator. Electronic circuit, used in pulse-height analysers, which only passes pulses above a certain minimum (voltage) value and below a higher value. Pulses above and below the limits are rejected.

Discrete (energy) spectrum. A spectrum consisting of a number of well resolved lines.

Discriminator, pulse-height. Electronic circuit designed to pass all pulses above a certain minimum (voltage) value and reject all those below.

Dose equivalent. Is the product of absorbed dose, D, quality factor, Q, and N, the product of other modifying factors, i.e.:

$$H = DQN$$

Is only used for radiation protection purposes (see §I–3.1 and ICRU Supplement to Report 19).

Effective half-life, see under **half-life, effective.**

Einstein's equation. A quantitative relationship expressing the transformation of mass to energy and vice versa (§I–1.2).

Electron. A small particle having a rest mass of 9.107×10^{-28} g, an atomic mass of $\frac{1}{1837}$ of a hydrogen atom, a diameter of 10^{-12} cm, and carrying one elementary unit of positive or negative charge. The positively-charged electron is called the positron, while the negatively-charged electron is usually just termed electron (the term negatron is rarely used). (See also **beta particle.**)

Electron capture (EC). Mode of radioactive decay of an atom in which its nucleus captures one of its orbital electrons, whereby a proton in the nucleus is transformed to a neutron and a neutrino is emitted.

Electronvolt (eV). A unit of energy equal to the energy acquired by a singly charged particle when it is accelerated in a vacuum through a potential difference of 1 volt (see **units**).

Enrichment (atom per cent excess). The abundance of a given stable isotope in a labelled sample minus the abundance of that isotope in nature.

Epicadmium neutrons. Neutrons with energies greater than those readily absorbed by cadmium (greater than approximately 0.5 eV).

Excitation. The transition of a nucleus, an atom or a molecule to an energy level above that of its ground-state.

Exposure. The absolute value of the total charge of ions of one sign produced in air when all the electrons (positrons and negatrons) liberated by photons (usually X-rays or gamma rays) in a volume element of air of unit mass are completely stopped in air (see ICRU Report 19). Expressed in units of coulombs per kilogram (C/kg) or, more commonly, roentgens (see **units**).

External standard. (i) A radioactive source permanently installed in a liquid scintillation counter in such a way that by unshielding it the source can be brought to irradiate the sample for the purpose of evaluating counting efficiency. (ii) Any suitable radioactive source that is accurately defined and can be used as a standard to calibrate a measuring system.

Film badge. Small photographic film in light-tight envelope worn by personnel to register exposure to ionizing radiation.

First-order kinetics. A process in which the rate of change of the variable is proportional to the magnitude of the variable present at any time. If the variable is P, then: $dP/dt \propto P$, i.e. $dP/dt = kP$, where k is termed the *first-order rate constant.* This behaviour is common to many processes, e.g. the radioactive decay law, many biological and open-compartment processes (see Eqs (I–1), (I–13), (I–49) in Part I). The integrated equation is of the form $P = P_0 e^{-kt}$, giving the basis for constants of the type **half-life** (q.v.), **half-value thickness** (q.v.).

Fission. Splitting of a heavy nucleus into two (or rarely more) lighter nuclei of about equal masses, whereby one or two neutrons and a relatively large amount of energy (including some gamma radiation) are released. Fission is usually preceded by neutron capture.

Fluence, particle. The time integral of particle flux, being the number of particles incident per unit area during a given time interval (for a full definition, see ISO-921).

Fluorescence. Luminescence that exists only as long as the energy source is exciting the phosphor. (Phosphorescence is luminescence that continues after the exciting source is removed.)

Flux, particle. The number of particles incident per unit area and per unit time. It is identical with the product of the particle density and the average speed (for a full definition, see ISO-921).

Gamma photon (γ-photon). A gamma photon is an electromagnetic energy packet emitted at the speed of light from an atomic nucleus.

Gamma radiation. Electromagnetic radiation emitted in the process of nuclear transition or particle annihilation.

Gas amplification, gas multiplication. The production of secondary ionization by primary ions (electrons) produced in a gas-filled detector across which the voltage is sufficiently high (regions III and IV in Fig. I–9, §I–2.2), thus adding to the total charge collected.

Geiger-Müller tube, GM tube. A gas-filled detector across which the voltage is so high that gas amplification has reached saturation (region IV in Fig. I–9, §I–2.2).

Geometry. A term used loosely to designate the arrangement in space of the various components of an experimental or measuring system. This designation includes positions of source, detector and any intervening absorber. The solid angle around the source that is irradiated or measured is also sometimes indicated, e.g. '2π geometry'.

Glove box. An area in which one can handle small quantities of radioisotopes and avoid contamination of the hands. It comprises a dust-tight box, fitted with windows and gloves, in which the manipulations with hazardous alpha or beta active material may be carried out.

Gray (Gy). The SI derived unit of absorbed dose of ionizing radiation. It is defined as being equal to one joule of energy absorbed per kilogram of matter undergoing irradiation.

Ground-state. The lowest energy level of an atom, molecule or nucleus (with respect to the emission of photons); is used to designate the normal stable state in some contexts.

Half-life, biological. The period of time during which a given biological organism physiologically eliminates half the amount of a given substance that has been introduced into it when the rate of elimination is approximately exponential (see **first-order kinetics**, also §I–3.2.2, §I–4.3.1).

Half-life, effective. In a biological organism, the time taken for the activity of a radioisotope to decrease to half its value due to both radioactive decay and physiological elimination when the rate of removal is approximately exponential (see **first-order kinetics**, also §I–3.2.2, §I–4.3.1).

Half-life, radioactive. For a single radioactive decay process, the time required for the (radio)activity to decrease to half its value by that process.

Half-value layer, half-value thickness. The thickness of a given material which, when introduced into the path of a given beam of radiation, reduces the value of a specified radiation quantity by one half. (In general, the thickness that reduces by half the intensity of a beam of photons of given energy.)

ICRP. International Commission on Radiological Protection, Sutton (Surrey, UK).

ICRU. International Commission of Radiation Units and Measurements, Washington, DC (USA).

Infinite thickness. Saturation thickness of solid samples with respect to self-absorption of beta particles from a given isotopic tracer. The count-rate of a given sample material of *infinite thickness* is independent of variations in thickness and is proportional to the activity concentration in that material.

Integrated dose. The dose obtained by summing up all individual dose contributions over a period of time.

Internal conversion (IC). A process whereby an atomic nucleus, that would otherwise emit a gamma photon, de-excites by interacting with one of its own orbital electrons (usually in the K, L or M shell), the electron being ejected at high velocity. The ejected electron (termed conversion electron) has a kinetic energy which is the difference between the transition (de-excitation) energy and the binding energy.

Internal standard. A known amount of tracer activity (in becquerels, i.e. disintegrations per second) added in a non-quenching form to a liquid scintillation sample in order to determine the counting efficiency of the tracer in that sample.

Inverse-square law. A law stating that the intensity of radiation emanating uniformly over the full solid angle (4π) from a source in a vacuum *decreases* proportionally and monotonically with the square of the distance from the source, i.e. is inversely proportional to the square of the distance.

Inverse tracer dilution. A method of isotopic dilution analysis in which a known amount of tracee of natural isotopic composition is added to a sample containing tracee which is labelled.

Ion-exchange resin. An artificial solid material that can adsorb ions and in solution exchanges these with other ions of the same sign.

Ionization. The production of ion pairs (of which one may be an electron).

Ionization chamber. A device which detects ionizing radiations (particles which produce primary or secondary ionization) by means of the ionization produced in a volume of gas. The sensitive volume of the instrument is filled with air or other suitable gas and the voltage across the electrodes is high enough for all ions formed to be collected on the electrodes but not so high as to cause gas amplification (q.v.). It can measure high and low dose rates and, for biological measurements, can be designed to give *tissue equivalent* measurements.

Ionizing radiation. Particles or photons of sufficient energy to produce ion pairs (of which one may be an electron) on passing through matter.

Ion pair. Most often an ion pair is formed by division of a neutral atom into a free electron (negative ion) and an atomic residue (positive ion).

ISO. International Organization for Standardization, Geneva (Switzerland).

Isomer, nuclear. Nuclides having the same mass number and atomic number, but occupying different nuclear energy states.

Isomeric transition (IT). Decay with a measurable half-life of an isomer (in a metastable state) to an isomer of lower energy. De-excitation may occur by emission of a gamma photon or an internal conversion electron, with emissions of X-rays and/or Auger electrons.

Isotope dilution. A method of analysis in which a known amount of tracer is added carrier-free or together with a known amount of tracee (carrier) to a sample containing tracee of natural isotopic composition.

Isotopes. Nuclides having the same atomic number (i.e. the same chemical element) but having different mass numbers (i.e. same Z, different N).

K-capture, see **electron capture.**

LET (Linear Energy Transfer), **restricted linear stopping power**, L_Δ. The LET of charged particles in a medium is the energy loss due to collisions with energy transfers less than some specified value Δ per unit distance traversed by the particle. It is recommended that Δ be expressed in electronvolts (see ICRU Report 19). (L_∞ = linear collision stopping power.)

Liquid scintillation counting. A method of counting radiation, especially beta particles of low energy, by mixing the sample with an organic solvent containing an organic scintillator (i.e. an organic compound that transforms part of the energy dissipated into a flash of light). The light flashes emitted are registered by photomultiplier tubes.

Luminescence. The property of some substances of emitting light in response to excitation (excluding incandescence). Radiofluorescence, i.e. luminescence in a phosphor due to prompt release of energy absorbed from ionizing radiation, is termed scintillation (q.v.). Absorption of such energy with subsequent release only after illuminating or heating the phosphor is termed radiophotoluminescence or radiothermoluminescence, respectively, and forms the principle underlying the operation of certain types of dose meters.

Mass number. The total number of protons and neutrons (Z + N) in the nucleus of any given nuclide. The mass number, A, is the nearest whole number to the atomic weight (mass) of the nuclide.

Mass per unit area. A parameter used for specifying thickness of absorber that, for a given radiation, is independent of the absorbing material itself over a wide range. It is obtained by multiplying the absorber thickness, i.e. path length through the absorbing medium, by the density of the medium. There is no agreement on a name for this, it being called variously surface density, density thickness, area density, mass thickness and, simply, thickness. The dimensionally descriptive title is used in this manual.

Mass-spectrometer. An instrument that separates (ionized) atoms or molecules according to mass (actually mass divided by ionic charge), these being focussed onto electrodes and registered electrically. The record is a spectrum indicating relative intensity as a function of mass divided by charge of each species.

Mass-thickness, see **mass per unit area.**

Monoenergetic radiation. Radiation comprising photons or particles that all have the same energy.

Natural isotope. Naturally occurring nuclide of any given chemical element. A natural isotope is either (1) stable, (2) of very long half-life, or (3) the descendant of a very long-lived precursor.

Natural uncertainty. Statistical deviations from a mean value of activity due to the natural randomness in time inherent in the decay of radioactive nuclei (see §I–2.8).

Net count-rate. The count-rate of a radioactive sample minus the count-rate of the background (with or without a blank sample in place for the background measurement, as appropriate).

Neutron. A nuclear particle with no electric charge and a mass approximately the same as that of a proton. Free neutrons are unstable, and decay with a lifetime of about 13 min to ^{1}H (i.e. a proton) and a β^- particle.

Neutron activation analysis, see **activation analysis.**

Neutron activation equation. An equation relating activity produced to neutron flux, cross-section, number of target nuclei and duration of neutron bombardment.

Neutron number, N. The number of neutrons incorporated in the nucleus of a given nuclide.

Normal distribution, Gaussian distribution. Symmetrical arrangement of replicate values that deviate randomly on either side of a mean value. This *bell-shaped* distribution is described mathematically by the Gaussian equation.

Nucleon. One of the types of particles, comprising protons and neutrons, constituting an atomic nucleus. Hence a commonly used name for protons or neutrons belonging to a nucleus.

Nuclide. Any given atomic species characterized by (1) the number of protons, Z, in the nucleus, (2) the number of neutrons, N, in the nucleus, and (3) the energy state of the nucleus (in the case of an isomer).

Open compartment. A kinetic compartment that is free to exchange matter (or energy) with its surroundings.

Open system. A system of kinetic compartments containing at least one open compartment.

Pair production. The transformation of a high-energy photon into an electron and a positron.

Photocathode. A device that liberates electrons when struck by photons of light.

Photoelectric absorption. A process by which an atom completely absorbs a photon. The energy of the photon is transferred to an (inner) orbital electron that is liberated from the atom.

Photospectrometer (emission spectrometer). An instrument that disperses the light from a source and produces a spectrum indicating relative intensity as a function of wavelength.

Pocket dose meter. A robust electrometer-type instrument designed to be worn in the pocket to register the integrated dose of penetrating (photon) radiation to which a person has been exposed (see §I–2.2.1).

Point source. A source of radiation with dimensions that are small compared with the distance from the source to the point of observation (see §I–2.6).

Poisson distribution. (Asymmetrical) statistical arrangement of replicate observations of the (relatively small) number of events (for example, radioactive disintegrations) that occur when the number of 'tries' included in the single observation is extremely great and the probability of the event occurring in any one 'try' is small but constant. In the case of a Poisson distribution, the standard deviation of the observed number of events can be mathematically predicted; it is equal to the square root of the mean number of events observed.

Primary ionization. Ionization produced by primary particles on interaction with matter. In counting tubes, the ionization produced by the incident radiation, without gas amplification.

Proportional region. Pertaining to a gas-filled detector, the high-voltage region in which the size of the output pulse is proportional to the amount of input energy, i.e. the region in which the amplification is linear (see §I–2.2.4).

Proton. A nuclear (elementary) particle with an atomic weight (mass) of approximately one unified atomic mass unit, carrying one elementary unit of positive charge.

Pulse height. The magnitude, usually measured in terms of the maximum voltage, of an electronic pulse.

Pulse-height analyser. An instrument that uses voltage-level discriminators to sort electronic pulses according to height (i.e. maximum voltage).

Pulse size. A more general term, sometimes synonymous with pulse height and sometimes referring to the area under a pulse.

Quality factor, Q. A factor, dependent on LET (i.e. linear collision stopping power), by which absorbed dose of a given type of radiation is multiplied in order to obtain a quantity that expresses dose equivalent, H, used for *radiation protection* (see §I–3 and ICRU Report 19 and its Supplement). Q is defined by the ICRP, and is formulated currently as a continuous function of the linear collision stopping power (see also under **LET**).

Quenching gas. Gas that is added to a Geiger-Müller tube to prevent multiple or continuous discharge.

Quenching of light. In liquid scintillation counting, the reduction of light output from the scintillator due to chemical inhibition (chemical quenching) and/or chromatic absorption (colour quenching).

rad. The special unit of absorbed dose of ionizing radiation which is being superseded by the gray. The rad is defined as:

$$100 \text{ rad} = 1 \text{ J/kg} = 1 \text{ Gy}.$$

Radioactive decay law. A law of nature that may be stated as follows: the probability of a radioactive atom decaying within a unit of time is a constant, characteristic of the particular radionuclide, but independent of its age and surroundings (see §I–1.3).

Radioactivity, see **activity.**

Radiocarbon dating. A method of dating objects whose life terminated hundreds or thousands of years ago. The method is based on (1) the natural abundance of ^{14}C in the past, (2) the known radioactive half-life of ^{14}C, and (3) the fact that on death an organism ceases to exchange carbon with its surroundings (see §I–1.4).

Radiotoxicity (as opposed to chemical or biological toxicity). The quality of a radionuclide of being poisonous on entry into the body of a person, an animal or other living organism due to the ionizing radiation emitted by the nuclide.

Range. The distance that a particle will penetrate a given substance before its kinetic energy is reduced to such a level that it will no longer cause ionization. As used of alpha and beta particles of given (maximum) energy, the maximum distance they will penetrate a given substance in a specified direction (since β^- particle tracks, in particular, are tortuous).

Rate constant. The fractional rate of reaction in **first-order kinetics** (q.v.).

rem. The unit of dose equivalent used for radiation protection purposes only (see §I–3 and ICRU Report 19 and its Supplement).

Resolving time. Relating to a detector and counting system, in particular a GM counter, the short period of time that elapses after the detector has been activated by a particle or photon, before the counting system is capable of registering another particle or photon. It comprises the dead time of the detector and any further delay due to gradual pulse-size recovery and delays in the electronics (see §I–2.2.5 and **dead time**). Since the latter in a well designed system is relatively very small, the resolving time usually approaches the dead time.

Roentgen (R). The special unit of exposure. There is no derived SI unit for this quantity, and the interrelationship is given in terms of coulombs per kilogram of air: $1\ R = 2.58 \times 10^{-4}\ C \cdot kg^{-1}$ (exactly). The definition and a discussion thereof is given in §I–3.1.

Scattering (see also **Compton effect**). Change in direction of a particle or photon due to a collision or interaction with another particle, an atom or a system. (i) If momentum and kinetic energy of the incident and scattering particle are conserved, the process is termed **elastic scattering.** (ii) If some of the total kinetic energy raises a target atom or nucleus to a higher energy state, the process is termed **inelastic scattering.** (iii) If the scattering centres act such that the scattered particles bear a phase relationship to one another, **coherent scattering** results; for example, a crystal lattice will scatter particles in a way that results in a diffraction pattern. (iv) If no phase relationship exists, the scattering is **incoherent**; for example, Compton scattering (see Compton effect). (v) If the scattering angle is less than 90°, the term **forward scattering** is used; is it greater than 90°, the term **back-scattering** is used. (vi) A photon or particle may undergo **single** or **multiple scattering.**

Scintillation. A flash of light produced in a phosphor by an ionizing event.

Scintillation crystal. A crystal in which a *significant* fraction of the energy absorbed from ionizing radiation is re-emitted as light in the visible or near ultra-violet region.

Scintillator. Any phosphor used for detection or measurement of ionizing radiation by the scintillation produced.

Secondary ionization. Ionization produced by ions or particles resulting from primary ionization. For example, in counting tubes, the primary ions formed by incident radiation are accelerated at high voltages to cause *secondary ionization* in a filling gas – the effect being termed gas amplification.

Self-absorption. The absorption by a radioactive sample of some of its own radiation, whereby the number of externally detectable particles or photons is reduced.

Semi-conductor radiation detector. A solid-state detector in which an ionizing ray produces electron—'hole' pairs (the functioning thus being analogous to that of a gas-filled ionization chamber). When cooled in liquid nitrogen (to reduce thermal noise), a semi-conductor detector has a high power of photon-energy resolution.

Sievert (Sv). See footnote 15 to §I–3.1.

Specific activity. Pertaining to a radioactively labelled sample, the amount of tracer activity per unit amount of tracee. Expressed in curies per mole, becquerels per mole, curies per gram, etc.

Stable isotope. Naturally occurring isotope with no observable radioactivity.

Steady state. With respect to a specified substance, the characterization of a dynamic system in which the input rate and the output rate of the substance are equal.

Technical uncertainty. Statistical deviations in replicate observations as a result of instrumental and operational variations and/or errors.

Thermal neutrons. Neutrons that are in thermal equilibrium with their surroundings, i.e. having kinetic energies corresponding to the temperature of the surroundings.

Total-absorption peak (as opposed to Compton region). A peak in a photon energy spectrum arising from events in which the detector completely absorbs the energy of the photon.

Total attenuation coefficient, see **attenuation coefficient.**

Tracee. The test object, element or compound, that the investigator is endeavouring to trace.

Tracer. A substance (e.g. an isotope) that is mixed with or fixed to the tracee in order to follow its translocation or identify its location.

Tracer equilibrium. The stage (real or extrapolated) at which the tracer has become completely and uniformly mixed with the tracee, so that the specific activity has become the same throughout the system.

Transfer rate of exchange. The amount of a given substance transferred per unit time both ways during the process of exchange between two compartments (see §I–4.3.3).

Turnover time. Pertaining to a given substance in a steady-state system, the time needed for the input (and thus the output) to equal the total amount of the substance in the system (see §I–4.3.2).

Units. To facilitate conversion of units that may be met with into SI equivalents, the following table has been appended to aid the reader. (Factors are given exactly or to a maximum of 4 significant figures.)

SI base units are the metre (m), kilogram (kg), second (s), ampere (A), kelvin (K), candela (cd) and mole (mol).

Multiply		*by*		*to obtain*
Radiation units				
becquerel	1 Bq	(= 2.7027	$\times 10^{-11}$	Ci)
disintegrations per second	(1 dis/s)	= 1.00	$\times 10^{0}$	Bq
curie (= 3.7×10^{10} dis/s)	1 Ci	= 3.70	$\times 10^{10}$	Bq
roentgen	1 R	= 2.58	$\times 10^{-4}$	C/kg
gray	1 Gy	(= 1.00	$\times 10^{0}$	J/kg)
rad	1 rad	= 1.00	$\times 10^{-2}$	Gy
		(= 1.00	$\times 10^{-2}$	J/kg)
rem (in radiation protection only)		dimensions of J/kg		
Mass				
unified atomic mass unit ($\frac{1}{12}$ of the mass of ^{12}C)	1 u	= 1.661	$\times 10^{-27}$	kg
pound mass (avoirdupois)	1 lbm	= 4.536	$\times 10^{-1}$	kg
ounce mass (avoirdupois)	1 ozm	= 2.835	$\times 10^{1}$	g
ton (long) (= 2240 lbm)	1 ton	= 1.016	$\times 10^{3}$	kg
ton (short) (= 2000 lbm)	1 short ton	= 9.072	$\times 10^{2}$	kg
tonne (=metric ton)	1 t	= 1.00	$\times 10^{3}$	kg
Length				
statute mile	1 mile	= 1.609	$\times 10^{0}$	km
yard	1 yd	= 9.144	$\times 10^{-1}$	m
foot	1 ft	= 3.048	$\times 10^{-1}$	m
inch	1 in	= 2.54	$\times 10^{-2}$	m
mil (= 10^{-3} in)	1 mil	= 2.54	$\times 10^{-2}$	mm
Area				
hectare	1 ha	= 1.00	$\times 10^{4}$	m^2
(statute mile)^2	1 mile^2	= 2.590	$\times 10^{0}$	km^2
acre	1 acre	= 4.047	$\times 10^{3}$	m^2
yard^2	1 yd^2	= 8.361	$\times 10^{-1}$	m^2
foot^2	1 ft^2	= 9.290	$\times 10^{-2}$	m^2
inch^2	1 in^2	= 6.452	$\times 10^{2}$	mm^2
barn	1 b	= 1.00	$\times 10^{-28}$	m^2

Multiply		*by*		*to obtain*
Volume				
yard^3	1 yd^3	= 7.646	$\times 10^{-1}$	m^3
foot^3	1 ft^3	= 2.832	$\times 10^{-2}$	m^3
inch^3	1 in^3	= 1.639	$\times 10^{4}$	mm^3
gallon (Brit. or Imp.)	1 gal (Brit)	= 4.546	$\times 10^{-3}$	m^3
gallon (US liquid)	1 gal (US)	= 3.785	$\times 10^{-3}$	m^3
litre	1 l	= 1.00	$\times 10^{-3}$	m^3
Force				
dyne	1 dyn	= 1.00	$\times 10^{-5}$	N
kilogram force	1 kgf	= 9.807	$\times 10^{0}$	N
poundal	1 pdl	= 1.383	$\times 10^{-1}$	N
pound force (avoirdupois)	1 lbf	= 4.448	$\times 10^{0}$	N
Density				
pound mass/inch^3	1 lbm/in^3	= 2.768	$\times 10^{4}$	kg/m^3
pound mass/foot^3	1 lbm/ft^3	= 1.602	$\times 10^{1}$	kg/m^3
Energy				
British thermal unit	1 Btu	= 1.054	$\times 10^{3}$	J
calorie	1 cal	= 4.184	$\times 10^{0}$	J
electronvolt	1 eV	$\cong$ 1.602	$\times 10^{-19}$	J
erg	1 erg	= 1.00	$\times 10^{-7}$	J
foot-pound force	1 ft·lbf	= 1.356	$\times 10^{0}$	J
kilowatt-hour	1 kW·h	= 3.60	$\times 10^{6}$	J
Temperature, energy/area· time				
Fahrenheit, degrees−32 Rankine	°F−32 °R	$\frac{5}{9}$		°C K
1 Btu/ft^2·s		= 1.135	$\times 10^{4}$	W/m^2
1 cal/cm^2·min		= 6.973	$\times 10^{2}$	W/m^2

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